

## Genetic associations between tuberculosis and economically important performance traits in Irish Holstein Friesian dairy cows

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**Introduction** *Mycobacterium bovis* is the principal agent of bovine tuberculosis (bTB). A recent study has demonstrated genetic variation exists among Holstein-Friesian dairy cattle for resistance to *M. bovis* infection (Bermingham *et al.* 2009). The objective of this study was to estimate the genetic associations between susceptibility to *M. bovis* infection and economically important traits.

**Materials and methods** The single intradermal comparative tuberculin test (SICTT) is used as a measure of susceptibility of cows to *M. bovis* infection. The test involves injecting *M. bovis*-purified protein derivative (PPD) into the neck of each animal, and comparing the reaction induced to that produced by *M. avium*-PPD (a measure of sensitisation to environmental mycobacteria). Susceptibility to *M. bovis*-PPD responsiveness was dichotomised as standard reactor (a *M. bovis*-PPD reaction 4 mm or greater than the *M. avium*-PPD reaction) or nonreactor (a *M. bovis*-PPD reaction equal to the *M. avium*-PPD reaction). National SICTT records between November 2000 and December 2007 were available for inclusion in the analysis. Cows that calved outside the normal age for a given parity, that had inconclusive SICTT results, or that moved into the herd within six weeks of the SICTT (it takes three to six weeks to develop a positive reaction to the test post infection) were discarded. Following edits, only episodes (herd restrictions initiated by two or more standard reactors [one of which was home bred], and terminated by two consecutive clear herd tests) with at least one standard reactor and ten or more tested cows were retained; 17,178 *M. bovis*-PPD responsiveness records from 598 episodes remained. Data on first to third parity 305-day milk, fat, and protein yield, somatic cell score (SCS), calving interval (CI), first parity body condition score (BCS), as well as survival from parity 1 to 2, 2 to 3 and 3 to 4 were extracted from the Irish Cattle Breeding Federation database for cows calving between 1985 and 2007. Cows with an age at calving more than 22 months from the parity median, and herd-year-season and paternal half sib groups with less than five records were removed. Following edits, 105,064 (with 2,185 *M. bovis* infection records) cows had information on production, 112,337 (with 2,389 bTB records) had information on CI, 104, 044 (with 2,895 bTB records) had information on survival, and 57,250 (with 354 bTB records) had information on BCS. Genetic and residual (co)variance components between susceptibility to *M. bovis*-PPD responsiveness and performance were estimated using bivariate linear-linear (LLSM) and threshold-linear (TLSM) sire models in ASREML (Gilmour *et al.* 2009). The likelihood ratio test of nested models was used to determine whether the correlations differed significantly from zero. The performance data were randomly split by herd-year-season contemporary group and correlations re-estimated, to assess the repeatability of correlations.

**Results** Heritability (standard errors in parentheses) for susceptibility to *M. bovis*-PPD responsiveness estimated from the threshold model was 0.12 (0.024). Susceptibility to *M. bovis*-PPD responsiveness was positively genetically correlated with second parity fat production and BCS, and negatively genetically correlated with first parity SCS and survival from parity 3 to 4. Similar correlations were obtained from the LLSM and TLSM and from both sub-sets of data.

**Table 1** Genetic correlations (standard errors in parentheses) from linear-linear models between susceptibility to *M. bovis*-PPD responsiveness and economically performance

Trait	Parity 1	Parity 2	Parity 3
Milk yield	0.23 (0.14)	0.24 (0.14)	0.13 (0.16)
Fat yield	0.32 (0.14)	0.39 (0.13)*	0.23 (0.15)
Protein yield	0.16 (0.15)	0.32 (0.14)	0.06 (0.16)
Somatic cell score	-0.34 (0.14)*	-0.11 (0.15)	-0.14 (0.17)
Calving interval	-0.07 (0.18)	0.00 (0.22)	-0.18 (0.30)
Survival	-0.08 (0.22)	-0.17 (0.23)	-0.62 (0.22)*
Body condition score	0.36 (0.14)*		

\*Genetic correlations significantly different from zero.

**Conclusions** This is the first study to estimate genetic correlations between susceptibility to *M. bovis* infection and performance. The results from this study suggest that selection for increased survival may indirectly reduce, while selection for reduced SCS and increased fat production and BCS may increase susceptibility to *M. bovis* infection within the national Holstein Friesian dairy herd. However, future work on independent data that corroborates these finding is required before definitive conclusions can be drawn.

**Acknowledgements** Financial support from ERAD is gratefully acknowledged

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## Determining host genetic susceptibility or resistance to bovine digital dermatitis in cattle

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**Introduction** Bovine digital dermatitis (BDD) is a bacterial infection of the hoof, causing painful lesion formation and progressive lameness. Treponemes have been implicated as the causative organism [1]. Primarily affecting commercial dairy herds, the disease presents agriculture with a huge economic and animal welfare problem. Within any herd there appears to be great variation in the way cattle are affected; some are recurrently and severely affected, where as others are relatively untouched by digital dermatitis, suggesting an underlying genetic susceptibility. Using a candidate gene approach we are investigating whether there is any genetic pre-disposition to the disease, by examining variations in the DNA known as single nucleotide polymorphisms (SNPs), in key genes with immune function. This study examines what SNP differences exist in BDD affected cattle, BDD unaffected cattle and in different breeds of cattle with differing BDD susceptibilities. Alongside this, we have conducted a genome wide association study using the Illumina bovine SNP-50 bead chip, to investigate SNP differences across the genome of BDD affected and BDD unaffected animals. Ultimately this study aims to identify key SNPs that may be associated with susceptibility or resistance to bacterial treponeme infection, and thus development of digital dermatitis, in cattle.

**Materials and methods** Cattle from three commercial pedigree Holstein Friesian farms were screened over a period of several months to identify BDD affected and BDD un-affected animals. DNA samples were obtained from BDD affected cattle (n=88) and BDD unaffected cattle (n=114). In addition DNA samples were collected from eight various other breeds of beef and dairy cattle (total n=178). Fifteen immune-related candidate genes were identified by literature search including CD14, iCAM1, IL1B, IL6, S10A8, MBL, VDR, iNOS, NRAMP1, TNFa, TIMP2, MMP9, TLR4, TLR2, IFN- $\gamma$ . In this group of genes there were 60 publicly available SNPs that were of interest to the study. Eleven candidate genes had few SNPs available so SNP discovery was undertaken. The DNA of 10 Holstein-Friesian, 10 Guernsey, and 7 British Whites was screened for novel SNPs using Transgenomic WAVE technology. The screened fragments yielded 70 novel SNPs. Genotyping assays (Sequenom mass-array technology) were designed to validate the panel of novel and public SNPs, and determine allele frequencies in the cohort of BDD unaffected animals, BDD affected animals and in the different cattle breeds. In addition the Illumina bovine SNP-50 bead chip was used to screen DNA samples from 24 of the BDD affected animals and 24 of the BDD unaffected animals.

**Results** A preliminary analysis of the genotyping data suggests several of the candidate gene SNPs are significantly associated with BDD susceptibility/resistance. A preliminary analysis of the whole genome screening with the illumina SNP-50 bead chip suggests there are several regions on the genome where SNPs are associated with BDD resistance/susceptibility. Figure 1 shows a plot of the significance of SNP associations after population stratification was taken into account and out liars removed for the BDD affected group (n=19) and the BDD unaffected group (n=21). The most associated SNP (chromosome 26) had a p value of 2.23E-5 (corrected using 1000 permutations). Currently a confirmation study is being performed on the most associated SNPs in a larger cohort of affected and unaffected animals.

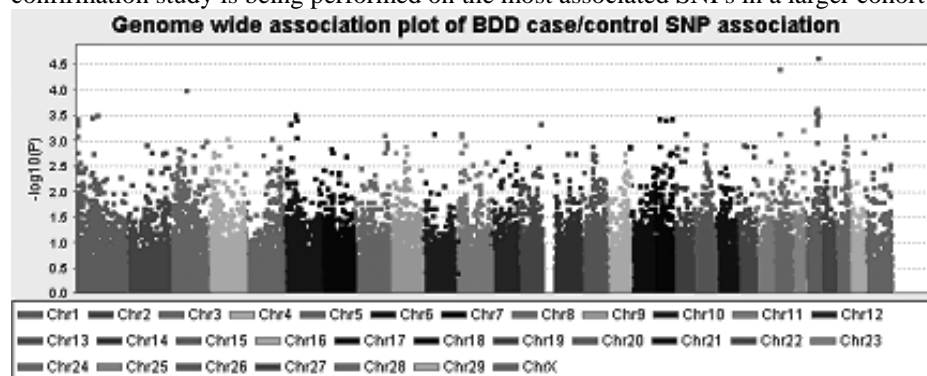


Figure 1

**Conclusion** This study has found some potentially interesting SNPs that are associated with susceptibility/resistance to BDD and therefore also bacterial treponeme infection. Once susceptibility/resistance SNPs are identified, populations can be screened for alleles associated with the disease. The results may help reduce digital dermatitis in dairy cattle by better informing breeding strategies. The principles of this project can be applied to a range of cattle infectious diseases, and will be useful in addressing the ever increasing consumer demands for improved animal welfare and food safety.

**Acknowledgements** This work is funded by the veterinary training research initiative (VTRI) scheme and the BBSRC.

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## Genetic association between direct and indirect measures of body energy in dairy cows

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**Introduction** Body energy refers to the amount of internal energy a cow has at her disposal to support physiological functions (milk production, growth, reproduction, maintenance, activity). Ideally, body energy would be directly measured using individual feed intake and physiological function records. In field conditions, however, such information is not available. For this reason, indirect measures of body energy have been suggested based on the body condition score and live weight of a cow. The usefulness of these traits depends on their true association with direct body energy. The objective of this study was to derive the correlation between direct and indirect measures of body energy in dairy cows.

**Materials and methods** Individual records for milk yield and composition, dry matter intake, live weight, and body condition score (BCS) were extracted for 1<sup>st</sup> lactation Holstein cows from the Scottish Agricultural College database of records gathered at the research station at Langhill Farm. Cows had calved between 1990 and 2005, and participated in feed and selection experiments. Milk yield records were available almost daily (5-7 days/week), dry matter intake was recorded 3 times/week, and milk composition, live weight and BCS were available on a weekly basis. Daily phenotypic records were calculated for each cow and trait using a mixed model that included the fixed effects of feeding and genetic group, year-by-month of record, year-by-season of calving, age at calving, and 3<sup>rd</sup> order orthogonal polynomial of days in milk; an interaction of the latter with cow was also fitted as a random effect. Solutions were used to calculate daily records for each trait. At the end of this step, there were 246,708 daily records for 801 cows. Subsequently, a direct measure of body energy was calculated based on all trait records plus data on the chemical composition of feed. The effective energy system proposed by Emmans (1994) was used. In addition, 3 indirect measures of body energy were considered: BCS, energy content (EC) and cumulative effective energy (CEE), the last two being combinations of BCS with live weight. Banos and Coffey ( ) describe these direct and indirect traits in detail. The genetic correlation of each indirect trait with direct body energy was calculated using a bivariate random regression model that included the same fixed effects as the previous model, in addition to a cow genetic and a permanent environment random effect. This analysis yielded genetic correlation estimates for each day of lactation. Finally, the possibility to predict direct body energy from each indirect measure was assessed with a model including the same fixed effects plus a regression on each indirect measure. For this reason, the dataset was randomly split into 3 equally sized subsets and, in each of 3 permutations, 2 subsets were used to calculate the regression coefficients while the 3<sup>rd</sup> subset was used to implement and compare predicted and observed direct body energy on independent data. Comparison criteria were the mean absolute difference, root mean square error, product moment correlation and an accuracy indicator based on the prediction error variance.

**Results** Significant ( $P < 0.05$ ) genetic correlations between direct body energy and BCS were observed on days 21-107 of lactation and ranged from 0.41 to 0.60. For EC and CEE, corresponding results were days 18-101 and 32-122 of lactation and correlation estimates of 0.38-0.54 and 0.45-0.70, respectively. Strongest genetic correlations with direct body energy were found on days 61, 60 and 71 for BCS, EC and CEE, respectively. Comparisons of observed and predicted direct body energy from indirect traits (BCS, EC and CEE) are shown in Table 1. These results are averages of the 3 permutations and pertain to days of lactation with a significant genetic correlation between direct and indirect body energy measures. All estimates in Table 1 were significantly greater than zero ( $P < 0.05$ ). No statistically significant differences were found in the predictive ability of the three indirect measures.

**Table 1** Comparison between observed direct body energy and predicted direct body energy from 3 indirect traits

Predictor trait	Mean absolute difference (MJ)	Root mean square error (MJ)	Correlation (%)	Accuracy (%)
Body condition score	14.84	18.97	89.02	82.74
Energy content	15.26	19.49	88.52	82.14
Cumulative effective energy	14.48	18.51	89.22	82.99

**Conclusions** This study revealed significant positive genetic correlations between direct and indirect body energy measures, especially in the first 2-3 months of lactation. These indirect body energy measures may be used to predict, with relative accuracy, direct body energy. Differences among the three indirect body energy traits were negligible. Because of its simplicity in recording at field conditions, body condition scored at the time of the 2<sup>nd</sup> or 3<sup>rd</sup> milk test is recommended as a proxy to direct body energy at that stage of lactation.

**Acknowledgements** This work was financed by a grant from the Scottish government. Data were made available by the Langhill Farm, Scotland. Ross McGinn is acknowledged for maintaining the database and managing the process.

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## Genetic and non-genetic factors influencing *Ostertagia ostertagi* antibodies in UK Holstein Friesian cattle

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**Introduction** Today's Holstein-Friesian dairy cattle selection indices are much broader than their predecessors; however there are still areas, concerning health and welfare that require investigation. Sub-clinical gastrointestinal (GI) nematode infections are one such area, *O. ostertagi* (*Oo*) being one of the most widespread and important GI nematode parasites of cattle in temperate regions. It is known to cause severe clinical disease in young cattle and causes reduced yield, weight and body condition score in adults. Although sheep have been selected for resistance and/or resilience to nematode infection, knowledge of protective immune responses in cattle and thus genetic selection opportunities to date are limited. The predominant immunoglobulin involved in the humoral immune response to GI nematodes is IgG (Sanchez *et al.*, 2004b). In sheep, IgA is considered to regulate *O. circumcincta* worm fecundity (Stear *et al.*, 1996) but it has been rarely studied in cattle and is barely detectable in serum or milk, thus its role is unresolved. However, total IgG levels in serum have been related to acquired and protective immunity to *Oo* in cattle (Kloosterman *et al.*, 1984). The aim of this work was to estimate the genetic variation, heritability and effects of other non-genetic factors on *Oo* specific total IgG concentration in milk of Holstein Friesian cattle.

**Materials and methods** Anti- *Oo* antibody level (total IgG<sub>1</sub> and G<sub>2</sub>) was determined by a solid-phase indirect enzyme linked immunosorbent assay kit (ELISA; Charlier *et al.*, 2005) and measured using optical density ratio ( $ODR = \frac{OD_{\text{sample}} - OD_{\text{negative control}}}{OD_{\text{positive control}} - OD_{\text{negative control}}}$ ) from milk samples collected from 1,303 Holstein-Friesian cattle in 255 commercial dairy farms between 2002 and 2004 during their first (82%) and other (2-12) lactations. Various fixed, random and nested effects (herd, area of country, year, month and season of sample, time of sample, parity and days postpartum) were systematically investigated in the model. The final model included the fixed effects of herd ( $n = 229$ ), season of sample ( $n = 4$ ) and the random effect of sire of animal ( $n = 461$ ; mean  $\pm$  standard deviation;  $2.76 \pm 2.99$  daughters per sire, range 1 to 27). Analysis of the data with a full animal model was not possible due to data limitations (lack of dam identification and some sire pedigree information). Thus, only the most simple sire model fitted to the ODR data using ASREML software and variance components estimated. Caution must therefore be used when interpreting the variance component estimates in terms of reduced accuracy and potential bias.

**Results** The association with days postpartum, area of country, parity, sample time (AM or PM), sample month and year on ODR were not significant and therefore were not included in the final univariate model. Season had a significant ( $P < 0.0001$ ) effect with ODR largest during summer months (June to August) and lowest in winter (December to February). This pattern is thought to reflect the ingestion of infective larvae, which are present on pasture and whose concentrations typically increase throughout the grazing season. Antibody levels typically decline during housing over winter because of the cessation of larval ingestion. ODR was heritable and the estimate was significantly different from zero ( $0.13 \pm 0.12$ ;  $h^2 \pm \text{s.e.}$ ;  $P < 0.05$ ). This is comparable to estimates of antibody response to other conditions in serum (Wagter *et al.*, 2000; Gonda *et al.*, 2006). Furthermore, the present study confirms the significant effects of sire and herd on *Oo* antibody level also found in a smaller study ( $n = 9$  sires) using pooled milk samples per sire in New Zealand dairy cattle (Morris *et al.*, 2002).

**Conclusion** This study found that anti-*Oo* total IgG antibody response is under genetic control and highlights the significant effect of season of sample and herd. These sources of environmental variation would need to be considered in future investigations into the potential use of antibody response in genetic selection and also in parasite control programs. The possibility of anthelmintic resistance may mean that in the future, producers may use a combination of management and genetic selection to control parasitic infection and susceptibility. Because of the relatively small dataset available and the very simplified genetic model applied, further large scale genetic studies need to be carried out to fully dissect the relationship between parasitic susceptibility and antibody response and the genetic and non-genetic factors affecting them.

**Acknowledgements** The authors are grateful to BSAS/Genesis Faraday for a vacation scholarship and Merial for their funding.

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## Nutritional sensitivity of correlations between estimated breeding values for faecal egg counts and resistance to parasites in periparturient ewes

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**Introduction** Only at times of metabolisable protein (MP) scarcity, resistance to gastrointestinal parasites was lower in highly productive Mules than in the less productive Blackface ewes (Kidane *et al* 2009). Thus, genetic differences in parasite resistance may be more pronounced when MP is scarce. Estimated breeding values (EBV) for faecal egg counts (FEC) may describe within-breed variation in parasite resistance. Here we tested whether FEC are positively correlated with EBV for FEC at times of MP scarcity only.

**Materials and methods** Single (-1) and twin (-2) bearing pure-bred Suffolk ewes (n=16) with an EBV for FEC ranging from -0.37 to +0.91, were trickle infected with 10,000 *Teladorsagia circumcincta* larvae from day<sub>-44</sub> onwards (day<sub>0</sub> is parturition). Their body weight (kg) and condition score on day<sub>-22</sub> were 61.1±1.9 and 1.9±0.1, and 66.0±1.4 kg and 1.9±0.1, respectively. From day<sub>-22</sub>, ewes were fed at 0.9 times metabolizable energy requirement and at either 0.8 (LP-) or 1.3 (HP-) times MP requirements (AFRC, 1993). Ewes and lambs were weighed weekly and within 12h post lambing. Ewe FEC (in eggs per gram (epg) faeces) were assessed twice weekly, and log(FEC+1) was used for statistical analyses. Ewe body weight gain (g/day) and relative litter daily gain (g/day/kg) was estimated by linear regression. FEC were analysed using a repeated measures 2 x 2 factorial ANOVA (REML). Ewe weight gain, post parturition ewe body weight, litter birth weight and relative litter weight gain were analysed using a 2 x 2 factorial ANOVA (REML). Pearson's correlations were calculated between EBV and mean FEC during late pregnancy and during lactation.

**Results** Feeding treatment and litter size did not significantly interact for any performance measures taken. Treatments did not affect late pregnancy body weight gain and ewe body weight at parturition, which averaged at 206±23 g/day and 58.1±1.0 kg, respectively. However, during lactation, ewe body weight gain averaged at -56 and +59 g/day for single- and twin-rearing ewes (s.e.d. 42.4 g/day; P<0.01) and -81.2 and +83.1 g/d for LP and HP ewes, respectively (s.e.d. 42.4 g/day; P<0.001). Lactational weight gain of twin-rearing HP ewes (HP2) was different from zero (P<0.05). Feeding treatment did not affect litter birth weight, which averaged 5.1 and 6.9 kg for single and twin litters, respectively (s.e.d. 0.34 kg; P<0.001). Litter size did not affect relative litter weight gain, which averaged 36.7 and 47.0 g/d/kg for LP and HP ewes, respectively (s.e.d. 4.95 g/day/kg; P<0.05).

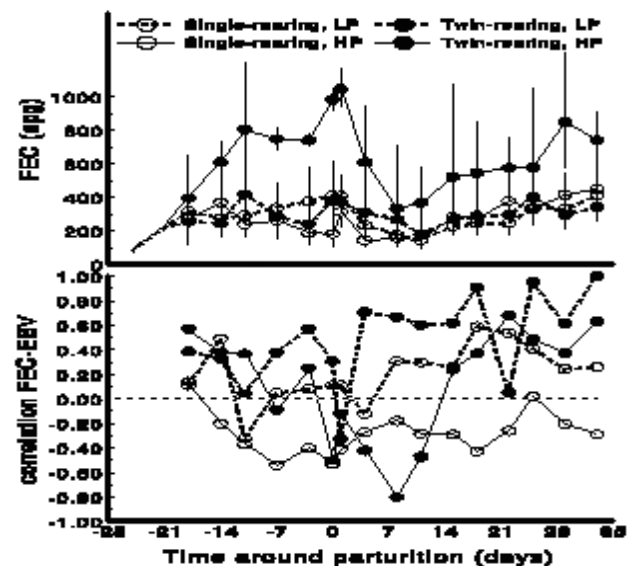
Figure 1 show the backtransformed FEC of the ewes and their correlation with EBV for FEC on each time point. Time did not interact with feeding treatment and litter size for FEC. During late pregnancy, FEC tended to increase (P=0.06), whilst during lactation, FEC first reduced and then gradually increased until the end of the experiment (P<0.001). During late pregnancy and lactation, single-rearing ewes had lower FEC than twin-rearing ewes (P<0.01). However, feeding treatment and litter size interacted (P<0.05); MP feeding did not affect FEC in single-rearing ewes but HP2 ewes had higher FEC than LP2 ewes. Ewe EBV for FEC correlated significantly with observed FEC during lactation only, and when calculated across lactation only for LP ewes (LP1: r=0.70; LP2: r=0.81; P<0.05) but not for HP ewes (HP1: r=-0.39; HP2: r=0.16; P>0.35).

**Conclusion** Response in litter gain to MP supply suggests that MP was limiting for LP ewes. However, in contrast to the expectation (Coop and Kyriazakis, 1999), MP supplementation did not reduce FEC but increased ewe body weight gain in twin-rearing HP ewes, which also had a lower than expected litter birth weight. This could suggest that at times of low body condition score, live weight gain may be prioritised over immunity to parasites. The presence of significant correlations between FEC and EBV for FEC in LP ewes only supports the view that genetic superiority in terms of resistance to parasites may only be observed at times of protein scarcity.

### Acknowledgements

A. Kidane received a post graduate scholarship from Quality Meat Scotland, Meat Promotion Wales and English Beef and Lamb Executive, and P. Sakkas from Hellenic State Scholarship Foundation. SAC receives support from Scottish Government.

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**Figure 1** FEC and their correlation with EBV for FEC of LP and HP fed single- and twin rearing ewes over time

## Performance of Suffolk and Texel sheep grazing pastures that presented contrasting levels of parasite challenge

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**Introduction** Suffolk sheep have substantially lower faecal egg counts for gastrointestinal nematode parasites than co-grazed Texel sheep and these breeds also have different nematode burdens in the abomasum and small intestine (Hanrahan and Crowley, 1999; Good *et al.*, 2006). The impact of these differences in resistance to parasite infection on animal performance needs to be established in order to determine whether there are breed differences in resilience to gastrointestinal parasite infection. The objective of this study was to evaluate differences in breed performance when grazed under contrasting level of parasite challenge. The null hypothesis was that breed differences in lamb growth rate are independent of level of parasite challenge.

**Materials and methods** The study was repeated over two grazing seasons and the animals involved (143 Suffolk and 151 Texel lambs) were from the purebred flocks of Suffolk and Texel sheep maintained at this research centre. The ewes in these flocks are housed between December and lambing (early to mid March) and are turned out, with their lambs, to pasture within 2 to 3 days after parturition. Routine animal performance records include ewe weight and condition score post mating, 5 weeks post lambing and at weaning and ewe condition score immediately post lambing; lambs were weighed at birth and at 5, 10 and 14 weeks (weaning) of age. In present study all lambs were also weighed at 18 weeks of age. Level of nematode infection was monitored by faecal egg counts at regular intervals up to 18 weeks of age and the number of infective larvae on pastures was evaluated weekly in year 1. Two pastures with different grazing histories were used: 'Clean' = sward established in the autumn prior to year 1 of this study and not grazed by sheep prior to turnout in spring of year 1, 'Dirty' = permanent old pasture that had been grazed by sheep only for at least 10 years prior to year 1. The management of the 'Clean' sward during the late summer and autumn of year 1 was designed to minimise any accumulation of infective larvae so that herbage larval challenge in year 2 was maintained at a low level. Ewes were assigned at random, within breed and lambing date, to treatment and were managed as a single group within treatment. Anthelmintic treatment of lambs during the period to 18 weeks of age was as follows: all lambs were dosed at 5 weeks of age and no further anthelmintic was administered until after 18 weeks of age. All ewes were dosed post lambing, prior to turnout, to minimise larval output onto the 'Clean' pasture. Data were analysed using mixed model procedures.

**Results** The objective of providing contrasting levels of parasite challenge between the 'Clean' and 'Dirty' pastures was achieved as shown by contrasting faecal egg counts (40/g for Clean v 400/g for Dirty at 18 weeks; the 'clean' group had essentially zero counts at 14 weeks whereas the dirty group averaged 300 eggs/g) and the number of infective larvae per 1 kg herbage dry matter (near zero for 'Clean'; over 500 for 'Dirty' between mid June and mid August). Data on lamb growth are summarised in Table 1. There was a highly significant breed x pasture type interaction for weights at 14 and 18 weeks of age and growth rate from 5 weeks to weaning. These interactions reflected the much greater impact of "Dirty" grazing on Suffolk lambs. At 18 weeks of age Suffolk lambs on 'Dirty' pasture were 4.6 kg lighter than Suffolk lambs on "Clean" pasture whereas the corresponding difference for Texel lambs was only 1 kg. There was no evidence for an effect of grazing treatment on lamb growth rate between birth and 5 weeks and it is evident that the negative impact of dirty grazing on Suffolk lambs increased as the season progressed (Table 1). There was also evidence for a breed x grazing treatment interaction for some aspects of ewe performance. Thus, live weight of Texel ewes at weaning was unaffected by grazing system whereas Suffolk ewes on the "Dirty" pasture were 4.7 kg lighter than those on 'Clean' pasture ( $P < 0.05$ ).

**Table 1** Lamb growth as a function of breed and level of parasite challenge

Breed	Pasture type	Live weight (kg) at			Growth rate g/day	
		14 weeks	18 weeks	0 to 5 weeks	5 to 14 weeks	14 to 18 weeks
Texel	Clean	34.6	39.6	322	302	149
	Dirty	34.3	38.6	334	271	129
Suffolk	Clean	36.7	41.7	318	332	140
	Dirty	34.5	37.1	330	268	91
s.e.		0.49	0.51	5.1	7.3	10.1
Breed x Pasture type interaction		$P < 0.02$	$P < 0.001$	$P = 0.9$	$P < 0.02$	$P = 0.4$

**Conclusions** Suffolk lambs grow faster than Texel lambs when parasite infection is minimised. The interaction between breed and level of parasite challenge shows that the breed differences in lamb growth depend on level of parasite challenge. The impact of parasite infection on Suffolk lambs increases as the grazing season progresses.

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## Changes in the Irish *Phytophthora infestans* population affect potato late blight resistances

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**Introduction** Currently potato late blight, caused by the oomycete pathogen *Phytophthora infestans*, is the most devastating disease of potato crops worldwide. With yield losses of up to 100%, fungicides are relied upon to provide protection. This is both economically and environmentally undesirable. The development of integrated control programmes utilising host resistance, forecasting and fungicides will provide a more sustainable method of disease control. Changes in *P. infestans* populations can quickly render host resistances ineffective and undermine such a strategy. Using data from the Teagasc Oak Park potato breeding programme screening trials we investigate how the recent changes in the Irish *P. infestans* population (Kildea *et al.*, 2009) have affected foliage blight resistance.

**Materials and methods** Plots (20 tubers) of seven potato varieties with varying levels of foliage blight resistance (Table 1) were planted as part of the Oak Park potato breeding programmes foliage blight trials in 2006 (old *P. infestans* population) and 2009 ('new' *P. infestans* population). In both seasons the trial was planted in late April as a completely randomised block design with six replicates. The trial site received no fungicide treatments and natural late blight infections were allowed develop. Disease assessments commenced in mid June (prior to first infection) and continued at seven day intervals until all plots were completely infected or were naturally senescing. Levels of infection were determined using the British Mycological Society foliage blight key (Cox and Large, 1969). Using this data the development of the disease on each variety was calculated as the relative area under the disease progress curve (RAUDPC) (Shtienberg *et al.* 1990), from which differences between varieties in the individual years were analysed by ANOVA.

**Results** In 2006 late blight was first detected on the 27<sup>th</sup> of July, while in 2009 it was first detected on the 29<sup>th</sup> of June. In both seasons plots of the susceptible varieties Bintje, British Queen and Eersterling became completely infected within three weeks from the first detection. Disease development on the more resistant varieties Cara, Robijn and Setanta was significantly greater in 2009 than in 2006.

**Table 1** Effect of season on the development of late blight on seven potato varieties

Variety	Resistance Rating <sup>‡</sup>	rAUDPC <sup>†</sup>	
		2006	2009
Alpha	3	0.43 <sup>A</sup>	0.54 <sup>AB</sup>
Bintje	2	0.61 <sup>A</sup>	0.62 <sup>A</sup>
British Queen	2	0.57 <sup>A</sup>	0.65 <sup>A</sup>
Cara*	5	0.23 <sup>B</sup>	0.4 <sup>B</sup>
Eersterling	2	0.69 <sup>A</sup>	0.61 <sup>A</sup>
Robijn	6	0.17 <sup>BC</sup>	0.42 <sup>BC</sup>
Setanta*	8	0.09 <sup>C</sup>	0.57 <sup>AC</sup>

\*Bred at Oak Park; <sup>‡</sup>Resistance rating 2006; <sup>†</sup>Relative Area under disease progress curve

**Conclusions** The change in resistance of the variety Setanta between 2006 in 2009 is a worrying development and suggests the presence of a single resistance gene overcome by the 'new' *P. infestans* population. Although the levels and speed of disease on both Cara and Robijn increased compared to 2006 it was not as dramatic and is likely due to the high disease pressure experienced in 2009. Similar changes in disease development has been observed on Lady Balfore and Sterling (Lees *et al.* 2008) which suggests re-evaluation of resistances among commercial varieties maybe required.

**Acknowledgements** This research has been funded by Teagasc, Irish Potato Marketing and the Department of Agriculture under the Research Stimulus Fund.

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## Monitoring of organic and inorganic nitrogen sources for the nitrogen nutrition of winter wheat using a chlorophyll meter (N-Tester®)

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**Introduction** Biomass production of crops is a function of nitrogen (N) content in the plant (Lemaire *et al.*, 2005). The N content of plants is highest at early growth stages and decreases continually up to the stage of senescence (Mistele & Schmidhalter, 2008). Nitrogen is applied to crops in two main forms, organic manures (e.g. pig manures) or inorganic manures (e.g. calcium ammonium nitrate (CAN)). It is difficult for farmers to assess the available N in organic manures and their perception of its value is poor. Simple hand-held optical analysis methods like the Yara N-Tester® which measures leaf chlorophyll content could be used to quickly and accurately assess the N% of a crop. The N-tester gives a dimensionless value which is proportional to the total value of chlorophyll, which can then be correlated to the N content of the plant. In this trial the N-Tester® method was used to monitor crop nitrogen uptake in trials comparing chemical fertiliser (CF) and separated liquid pig manure (LPM) as crop N sources for winter wheat.

**Materials and methods** Field experiments were carried out on a winter wheat crop cv. Einstein in 2009 at two sites Newcastle and Lyons. The Newcastle site was a clay loam N index 1 soil (continuous cereals), while Lyons was a clay loam, N index 2 soil (after 3 years ley). The crops were sown in mid-October with mid-September harvest dates. The trials were laid out in a randomised complete block design with four replicates in a factorial arrangement with two factors – N source and N application timing (Table 1). Pig manure treatments received 30,000 l/ha (120kgN/ha) of LPM and CF treatments received 120kgN/ha supplied as calcium ammonium nitrate (CAN 27% N). Post N application N-Tester® measurements were taken at 10 day intervals until natural senescence occurred consisting of 30 leaf readings per plot (1.6m\*12m). The N-tester uses light transmission at two wavelengths (650nm and 960nm) to quickly assess chlorophyll content. Optical analysis methods such as the N-Tester® offers advantages over traditional plant sampling techniques as it is a rapid in-crop method which is non-destructive.

**Table 1** Trial treatments and N-Tester® chlorophyll unit readings (average site 1 & 2 ANOVA).

Days (d) post Application	N Source				Application Timing			
	CF	LPM	Untreated	L.S.D	GS 30-31	GS 35	GS 37-39	L.S.D
+10d	554 <sup>b</sup>	572 <sup>a</sup>	471 <sup>c</sup>	11.56	557 <sup>a</sup>	516 <sup>b</sup>	524 <sup>b</sup>	11.56
+20d	612 <sup>a</sup>	607 <sup>a</sup>	443 <sup>b</sup>	14.25	567 <sup>a</sup>	551 <sup>b</sup>	544 <sup>b</sup>	14.24
+30d	613 <sup>a</sup>	598 <sup>a</sup>	428 <sup>b</sup>	24.42	562 <sup>a</sup>	551 <sup>a</sup>	526 <sup>b</sup>	24.42
+40d	616 <sup>a</sup>	578 <sup>b</sup>	371 <sup>c</sup>	22.05	532 <sup>a</sup>	528 <sup>a</sup>	504 <sup>b</sup>	22.05
+50d	575 <sup>a</sup>	520 <sup>b</sup>	313 <sup>c</sup>	19.46	512 <sup>a</sup>	507 <sup>a</sup>	389 <sup>b</sup>	19.46
+60d	480 <sup>a</sup>	405 <sup>b</sup>	225 <sup>c</sup>	22.04	499 <sup>a</sup>	372 <sup>b</sup>	239 <sup>c</sup>	22.04
+70d	330 <sup>a</sup>	296 <sup>b</sup>	129 <sup>c</sup>	19.48	384 <sup>a</sup>	233 <sup>b</sup>	138 <sup>c</sup>	19.48
Yield (t/ha)	9.57 <sup>a</sup>	8.76 <sup>b</sup>	5.94 <sup>c</sup>	0.44	8.26 <sup>a</sup>	8.14 <sup>a</sup>	7.88 <sup>a</sup>	0.44
GNU (kgN/ha)	85.00 <sup>a</sup>	78.66 <sup>b</sup>	52.91 <sup>c</sup>	5.84	73.51 <sup>a</sup>	71.82 <sup>a</sup>	71.23 <sup>a</sup>	5.84

\*Means with a common superscript are not significantly ( $P < 0.05$ ) different. LSD = Least significant difference

**Results** Differences between N source treatments were visible within 10 days of application ( $P < 0.0001$ ) with the LPM treatment having higher chlorophyll unit readings (CU) than the CF treatment. As the LPM was applied in liquid form compared to granular CF, N was more freely available for uptake in the days immediately after application. However, once CF became available for plant uptake CU increased. Until 30d post manure application the LPM and CF treatments were similar with the untreated treatments consistently showing reduced CU ( $P < 0.0001$ ). However, from day 40-70 the CF treatment appeared greener and had higher CU ( $P < 0.05$ ). The same trend is evident in grain yield and grain N uptake data with the CF significantly higher than both the LPM and control treatments ( $P < 0.0001$ ). Treatments receiving N at the earliest application timing (GS 30-31) had consistently higher CU than timings 2 and 3. At 30d post application date 1 and 2 were similar, however timing 3 was lower ( $P < 0.02$ ). Later applied N at GS 37-39 was not used to its full potential as evident from CU from d50-70 where a dramatic fall in CU indicates N deficiency in the crop and subsequent early senescence. This 100 unit fall occurred at d60 with timing 2 and not until d70 in timing 1 showing that a higher level of chlorophyll was present in the crop receiving N at an earlier growth stage.

**Conclusion** N application at earlier growth stages (GS 30-31) is more effective at increasing leaf chlorophyll content and therefore has a greater ability to influence crop yield. The N-Tester® data indicates that N applied as LPM is more available to the crop the 10-20 days after application. However, the data from this study also indicates that the N in LPM is less utilised than CF 40-60 days after the initial application date. The N-Tester® is an effective tool in monitoring crop N status and as a research tool has the ability to separate subtle colour differences not visually apparent as observed between LPM and CF treatments in this study.

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## Crop establishment systems for winter oilseed rape

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**Introduction** Oilseed rape, although a minority crop in Ireland, offers potential benefits as a break crop for cereal growers who find it increasingly difficult to find a market for other break crops. While its use in the production of biodiesel or pure-plant-oil fuel increases the potential market for rapeseed, the crop economics are not favourable, with frequently volatile gross margins achieved. Low-cost production methods must be sought. In Ireland the winter oilseed rape crop is traditionally established with plough-based cultivation and sowing systems. Research from other countries indicates that lower cost minimum tillage (min-till) establishment systems can be successful with oilseed rape (Freer, 2002). The aim of this research was to determine the impact of crop cultivation system, in particular minimum tillage systems, on crop establishment, development and yield.

**Materials and methods** Eight crop establishment systems were evaluated in each of the years 2007, 2008 and 2009 on different sites. All sites were located close to Oak Park Research centre in the south east of Ireland. The establishment systems were: Plough, press/roll, power harrow/drill, roll (A); Plough, press/roll, cultivator drill, roll (B); Min-till 75-100mm 1 run, roll, broadcast sow, roll (C); Min-till 75 – 100mm 1 run, roll, cultivator drill, roll (D); Min-till 75 – 100mm 2 runs, roll, cultivator drill, roll (E); Min-till 75- 100mm 2 runs, roll, broadcast sow, roll (F); Min-till 150 – 200mm 2 runs, cultivator drill, roll (G); Broadcast sow (30% extra seed), roll (H). The extra seed rate was used in treatment H as it mimicked commercial practice where poorer establishment rate was expected. The treatments were applied to 30m x 6m plots laid out in a randomised block design with 5 replications. A second factor (seed rate) was incorporated by splitting the cultivation plots but these results are not presented. The crops were sown on Aug 29, Sept 7 and Sept 17 in 2007, 2008 and 2009 respectively, with a mean seeding rate of 65 seeds/m<sup>2</sup> for all treatments except H. The cultivation treatments were applied to the cereal stubble of the previous crop on the day prior to sowing and the day of sowing. Crop agronomy practices such as fertiliser, herbicide and fungicide application were applied uniformly across all cultivation treatment plots following standard production guidelines. Plant establishment was determined approximately five weeks post sowing. Approximately 6 weeks prior to harvest, the oilseed rape canopy structure was assessed by taking 10 full plant samples from each plot and quantifying branching and pod distribution. The plots were harvested following desiccation using a direct-cut plot combine. All data was analysed by ANOVA for split-plot design (Genstat).

**Results** In each of the trial years, all of the cultivation treatments allowed the crop to be established, grown and harvested successfully. There were considerable year to year differences caused by site differences and particularly weather and its effect on sowing date (Table 1). Late sowing in 2009 resulted in slower establishment, bird damage, and weed control difficulties impacting on yield. There was a significant difference ( $P < 0.05$ ) in the numbers of plants established each year. Plant structure varied considerably with cultivation system having a significant effect. Generally lower crop establishment resulted in greater levels of stem branching and greater numbers of pods per plant. In 2007 and 2008 this resulted in similar yields being achieved with all crop establishment systems except H in 2007. In 2009, A, B and H cultivation treatments gave lower yields.

**Table 1** Cultivation system effect on plant structure and yields of winter oilseed rape over three seasons.

	2007				2008				2009			
	Plants (n/m <sup>2</sup> )	Branches (n/plant)	Pods (n/plant)	Yield (t/ha)	Plants (n/m <sup>2</sup> )	Branches (n/plant)	Pods (n/plant)	Yield (t/ha)	Plants (n/m <sup>2</sup> )	Branches (n/plant)	Pods (n/plant)	Yield (t/ha)
A	103.6	11.1	209	5.30	79.3	69.9	374	4.65	70.4	12.0	210	3.54
B	53.4	33.4	336	5.25	56.2	69.9	355	4.56	54.2	28.4	283	3.28
C	88.8	23.4	260	5.52	54.9	97.8	439	4.84	60.8	39.3	314	4.46
D	80.2	27.8	272	5.30	55.9	69.2	344	4.65	60.3	35.5	305	4.36
E	79.0	22.0	257	5.55	58.7	101.6	358	4.76	61.1	39.0	351	4.23
F	75.4	43.5	304	5.42	56.3	69.4	358	5.00	68.0	42.5	333	4.63
G	69.3	36.0	309	5.48	57.4	116.6	446	4.66	65.4	33.0	273	4.17
H	76.3	40.8	313	5.05	26.1	115.8	533	4.57	51.8	75.0	428	2.97
s.e.d	9.0	9.9	34	0.11	4.3	23.7	76	0.15	2.8	7.6	42	0.27
P	<0.001	0.05	0.02	0.004	<0.001	0.166	0.206	0.116	<0.001	<0.001	0.002	<0.001

**Conclusions** Minimum tillage crop establishment systems can effectively establish winter oilseed rape, with most of the cultivation systems evaluated producing consistent yields that compare favourably with plough-based establishment systems. While the cultivation system used impacted on plant structure in some seasons, this generally did not impact on seed yield. The simple broadcast sowing method used without cultivation did not perform consistently however.

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## Evaluation of separated solid pig manure as a nitrogen source for spring barley (*Hordeum vulgare* L.)

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**Introduction** Spring barley is the most widely grown cereal crop in Ireland. In recent years cereal farmers have relied almost totally on chemical fertilisers as their main crop nitrogen (N) source. However, with low grain prices and high chemical fertiliser prices the use of a cheaper fertiliser alternative in the form of pig manure must be examined. Recent research studies have shown that organic manures including pig manure can make a significant contribution to the N nutrition of cereal crops (Jackson and Smith, 1997), while Petersen (1996) indicated that satisfactory yields could be achieved from spring barley grown with pig manure alone. Due to the location of many pig farms and the logistics of moving pig manure from pig to tillage farms, manure separation is a potentially viable means of decreasing manure volume for transportation. Techniques for pig manure separation into solid and liquid components have been developed and this process provides a solid pig manure (SPM) product with a high dry matter content containing significant quantities of both nitrogen (N) and phosphorus (P). In this current study SPM was evaluated as a N source for spring barley.

**Materials and methods** This trial was carried out at Lyons Research Farm on a clay loam soil (N index 2) in the 2008 and 2009 growing seasons on spring barley cv. Wicket (2008) and cv. Magaly (2009). Trials were sown in early May both years with mid-September harvest dates. Each trial was a randomised split plot design in a factorial arrangement with 3 replicates. Four rates of the SPM (Factor 1) were combined with three rates of chemical fertiliser (Factor 2) (Table 1) and examined for their effect on crop nitrogen uptake (CNU), grain yield and quality. The SPM applied consisted of 26.5% DM, 0.6% P and 0.8% N. The SPM was applied to the stubble of the previous crop prior to sowing and ploughed to a depth of 16-18cm. The trial area was cultivated and ploughed in one operation. The chemical N (CN) rates were then applied immediately after sowing with a tractor mounted SISIS fertiliser spreader. The site had a high P status (index 4) so no supplemental P was applied to trials while trace elements were foliar applied. Main plot size (SPM) measured 10m\*15m, with sub-plot size measuring 1.6m\*15m. Crop nitrogen uptake was measured at crop growth stage (G.S) 37-39. Two 1m lengths of the crop were cut to ground level in each plot, weighed, dried, ground and analysed by use of a combustion analysis device to measure N content by use of the Dumas method. Harvest index samples were taken pre-harvest to calculate straw nitrogen content and grains/m<sup>2</sup>. A visual crop lodging score was also noted. During harvest a sub-sample of grain from each plot was collected for grain quality analysis such as hectolitre weight (HL), thousand grain weight (TGW) and grain protein percentage (GP). Statistical analysis was carried out by analysis of variance using the SAS statistical package.

**Table 1** SPM Treatments and Results (2008 & 2009)

2008	SPM (t/ha) (kgN/ha)					Chemical N (kgN/ha)			
	0 (0)	4 (32)	8 (64)	16 (128)	L.S.D	0	60	120	L.S.D
Grain Yield (t/ha)	4.51 <sup>b</sup>	4.94 <sup>a</sup>	5.06 <sup>a</sup>	4.86 <sup>a</sup>	0.28	4.88 <sup>a</sup>	4.91 <sup>a</sup>	4.73 <sup>a</sup>	0.25
CNU (kg N/ha)	98.29 <sup>b</sup>	108.8 <sup>ab</sup>	116.56 <sup>a</sup>	112.03 <sup>a</sup>	11.35	100.2 <sup>b</sup>	106.72 <sup>b</sup>	119.83 <sup>a</sup>	9.83
Lodging (%)	25.89 <sup>b</sup>	30.89 <sup>b</sup>	46.67 <sup>a</sup>	52.11 <sup>a</sup>	13.00	27.25 <sup>b</sup>	40.83 <sup>a</sup>	48.58 <sup>a</sup>	11.26
2009									
Grain Yield (t/ha)	8.61 <sup>a</sup>	8.81 <sup>a</sup>	8.71 <sup>a</sup>	8.79 <sup>a</sup>	0.46	7.83 <sup>b</sup>	9.13 <sup>a</sup>	9.19 <sup>a</sup>	0.42
CNU (kg N/ha)	168.91 <sup>b</sup>	172.18 <sup>b</sup>	167.22 <sup>b</sup>	185.36 <sup>a</sup>	11.01	143.42 <sup>b</sup>	190.70 <sup>a</sup>	186.13 <sup>a</sup>	9.54
Lodging (%)	6.46 <sup>b</sup>	11.57 <sup>b</sup>	14.22 <sup>ab</sup>	22.78 <sup>a</sup>	9.54	2.6 <sup>c</sup>	13.75 <sup>b</sup>	24.92 <sup>a</sup>	8.26

\*Means with a common superscript are not significantly ( $P < 0.05$ ) different. LSD = Least significant difference

**Results** The 2008 and 2009 growing seasons had a high rainfall period during the summer months (June and July). In the absence of a SPM by CN interaction, the results of main effects are presented. In both years increasing levels of SPM ( $P < 0.0008$ ) and CN ( $P < 0.002$ ) significantly increased lodging. The wet weather led to early crop lodging in June 2008 and July 2009 and reduced grain yield and CNU. CNU levels were sub-optimal in 2008 with no treatment exceeding 120kgN/ha. CNU levels increased in both years with higher SPM rates ( $P < 0.02$ ). The zero SPM treatment had lower yields ( $P < 0.0039$ ) in 2008 than treatments receiving SPM, however there is no yield benefit from high vs. low SPM rates. SPM treatments gave no significant yield response in 2009.

### Conclusion

Due to adverse climatic conditions during the summer months crop utilisation of both organic N and inorganic N was below expected levels in 2008. The higher rate SPM treatments significantly increased CNU in both years indicating useful N utilisation but the average NUE was still relatively low being  $< 20\%$  in both years. The high incidence of crop lodging in response to SPM use in both years is a serious negative factor which may adversely affect the interest of cereal farmers in using this slurry-based product in spring barley production in the future.

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## Evaluation of late blight control in potato cultivars for organic or reduced input production in Northern Ireland

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**Introduction** Late blight, caused by *Phytophthora infestans*, may result in total loss of organic potato crops in Northern Ireland. Trials in Belfast (Cooke and Little, 2004) showed that cultivars with partial blight resistance could withstand high infection pressure. In this study, performance was evaluated against the current *P. infestans* population, containing A1 and A2 mating types (Cooke *et al.*, 2009).

**Materials and methods** The trials were planted (6 May 2008, 5 June 2009) at the Agri-Food & Biosciences Institute, Belfast in a split-plot design with fungicide regimes as main plots, four cultivars as sub-plots and four replicate blocks. The cultivars/clones (rated on a 1-9 scale for foliage and tuber blight resistance, respectively, where 9 is maximum resistance), were, in both years, Santé (7, 6) and Sárpo Mira (7, 9), with, in 2008 Galactica (7, 7) and Setanta (8, 9), and in 2009 Sunset (7, 5) and AFBI Loughgall clone L5937/2 (7, 7). Ratings were from the British Potato Variety Database except for L5937/2 (breeder's estimates). Each sub-plot comprised two drills x 10 tubers of each cultivar (3 x 1.5 m). Main plots were separated by unsprayed drills of cv. Désirée. In 2008 these were inoculated (early July) with N. Ireland *P. infestans* isolates (A1 and A2 types) to provide an infection source. In 2009, because natural infection was observed in early July, the trial was not inoculated. The treatment regimes included no fungicide and a programme based on the non-systemic fluazinam (150 g a.i./ha as 'Shirlan', Syngenta, 300 ml/ha) applied at extended intervals. In 2008 four fluazinam applications were made (2, 28 July, 8, 27 August) and in 2009 two (22 July, 12 August). Foliage blight was assessed twice weekly on all drills after blight was seen until haulm destruction (29 August 2008; 4 September 2009). The trials were lifted on 4 November 2008 and 1 October 2009. The yield from each plot was graded and the number and weight of blighted tubers recorded. The remaining healthy tubers were stored until January when they were re-assessed for tuber blight. Data were subjected to analyses of variance.

**Results** In both years, foliage infection built up rapidly favoured by wet weather. In 2008, the unsprayed Santé and Galactica were almost dead by 8 August (Table 1), Setanta survived a week longer, but Mira had only 23% infection on 29 August. Fluazinam application delayed blight development as indicated by the smaller Area Under the Disease Progress Curve (AUDPC, Table 1); after mid-August build up was slower in Setanta than in Santé and Galactica, while Mira had few blight lesions by 29 August. The presence of other rots (notably pink rot, *P. erythrosetptica*) favoured by very wet soil complicated tuber blight and yield assessments; Mira had the greatest yield of healthy tubers for both untreated and fluazinam-treated plots, but differences were not significant. In 2009, Mira again developed significantly less foliage blight than any other cultivar in both untreated and fluazinam-treated plots, while foliar infection was slower to build up in Sunset and L5937/2 than in Santé. Yield and tuber blight data for 2009 are not yet available.

**Table 1** Foliage blight, tuber blight and yield of potato cultivars, 2008 and 2009 trials

Year/ cultivar	Foliage blight (%) <sup>a</sup>		AUDPC <sup>b</sup>		Tuber blight and rots (%)		Yield (kg/plot)	
	U	F	U	F	U	F	U	F
<u>2008</u>								
Santé	95.0	7.8	2417	1400	1.73	2.30	16.74	20.23
Galactica	95.0	11.5	2456	1485	8.33	5.92	14.82	18.42
Setanta	37.5	7.8	1977	1042	1.98	2.31	15.21	20.69
Mira	1.1	0.0	281	13	6.18	5.80	18.86	23.09
L.S.D. ( <i>P</i> <0.05)	5.99		211.8		11.73		6.551	
<u>2009</u>								
Santé	95.0	27.5	3345	2064	-	-	-	-
Sunset	71.2	20.1	2656	1203	-	-	-	-
L5937/2	85.0	21.0	3019	1254	-	-	-	-
Mira	3.9	0.0	247	86	-	-	-	-
L.S.D. ( <i>P</i> <0.05)	26.01		816.6					

<sup>a</sup> at early August assessments: 8 August 2008, 6 August 2009; <sup>b</sup> Area Under Disease Progress Curve; U untreated, fluazinam-treated

**Conclusions** Despite the presence of *P. infestans* genotypes including the aggressive 'blue 13' A2 (data not presented), in 2008 and 2009 Mira proved very resistant to foliage blight. Cvs. Santé and Galactica proved too susceptible to be grown without fungicide, but inputs may be reduced with cv. Setanta. Sunset and L5937/2 also appear promising, but results of the yield and tuber blight assessments are needed before it can be ascertained if they should be evaluated further.

**Acknowledgements** The authors thank P. Watts (AFBI Loughgall) for providing L5937/2, Mark Wilson (AFBI) and Queen's University students for their technical assistance and DARD for funding.

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## The effects of rhizome size, planting density and plastic mulch on the growth and dry matter yield of miscanthus over three seasons

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**Introduction** *Miscanthus* (*Miscanthus x giganteus*), a C4 perennial grass from the Far East, is being increasingly widely grown in the British Isles as a biomass crop with an annual harvest. Following establishment by planting rhizome sections into cultivated ground it can take four to five years for the crop to reach its maximum level of productivity. An experiment is in progress in Northern Ireland into the use of degradable plastic mulch to accelerate the development of the crop, and its interaction with rhizome size and density at planting. The first three years of the study are described in this paper.

**Materials and methods** Rhizomes from miscanthus planted in 2003 were harvested in April 2007 and stored at 3°C. The rhizome pieces were sorted into three size fractions which averaged weights of 26g, 74g and 204g. Experimental sites at Hillsborough and Loughgall were prepared by conventional cultivation and rhizomes planted by hand in early May in shallow furrows in plots 10m x 7.5m at densities equivalent to 450 kg, 1350 kg and 4050 kg per hectare for each size fraction. The rhizomes were covered with soil to a depth of about 7.5cm and the herbicide pendimethalin applied at 2.5 litres/ha. The four randomised blocks at each site comprised main plots with and without bio-degradable plastic mulch, applied shortly after planting with a single row mulch layer supplied by SAMCO Agricultural Manufacturing Ltd, and nine size x density sub-plots. Regular counts were taken of the number of shoots, and of shoot height, over the 2007, 2008 and 2009 seasons. DM yield above ground was assessed by sampling in the late autumn and again in the spring before the plots were harvested. Data was statistically analysed as a split-split-plot randomised block design.

**Results and discussion** The increase in the number of shoots as the rhizome size was reduced from 204 g to 26 g reported by Easson *et al* (2008) for the first year of this study was maintained into the 2<sup>nd</sup> and 3<sup>rd</sup> years (Table 1), along with an overall doubling of the number of shoots in the 3<sup>rd</sup> season compared with the first season. The increased number of shoots resulted in significantly higher DM yields with decreasing rhizome size at planting at all harvest dates.

**Table 1** Main treatment effects of rhizome size, planting density and plastic mulch treatment at planting on the growth and yield of miscanthus in the first three seasons (mean of two sites)

		Shoot numbers per hectare ('000s)			Crop height (cm)		DM yield (t/ha)		
		Oct '07	Oct '08	Oct '09	Oct '08	Oct '09	Mar '08	Nov '08	Mar '09
Rhizome size	26g	140	222	309	187	234	0.81	9.28	6.07
	74g	99	166	234	184	228	0.65	7.79	5.27
	204g	69	99	130	182	221	0.44	5.03	2.95
l.s.d. (P=0.05, 94 df)		12.6	14.8	22.5	3.3	9.7	0.126	1.636	1.215
Density kg/ha	450	39	63	90	160	201	0.19	3.59	2.09
	1350	80	151	215	179	227	0.43	6.51	3.94
	4050	189	273	368	214	256	1.27	12.00	8.25
l.s.d. (P=0.05, 94 df)		12.6	14.8	22.5	3.3	9.7	0.126	1.636	1.215
Mulch	without	67	134	196	180	227	0.47	5.98	3.97
	with	131	191	253	189	229	0.79	8.75	5.55
l.s.d. (P=0.05, 6 df)		12.9	23.2	35.0	5.7	25.6	0.108	1.571	1.422
Mean		103	162	224	185	228	0.63	7.37	4.76

The number of shoots also increased significantly at higher planting densities so that the number of shoots and the DM yield almost doubled with each threefold increase in planting rate, the ratio remaining similar into the 3<sup>rd</sup> season. Crop height increased significantly with increased density. The use of mulch increased the number of shoots in the first season by 96%, and this effect carried on into the 2<sup>nd</sup> and 3<sup>rd</sup> seasons in which shoot numbers were higher by 48% and 38% respectively. Crop height was significantly increased in the 2<sup>nd</sup> season, but not the 3<sup>rd</sup>. Crop yield increased by 70%, 46% and 40% at the March and November 2008 and March 2009 sampling dates respectively. Interaction effects between the use of mulch, rhizome size and planting density were relatively small. The highest yields were therefore from the 24g rhizome size planted at high density with the use of plastic mulch with yields of 17.8 tDM/ha and 10.7 tDM/ha in November 2008 and March 2009 respectively. The results from these harvest dates reveal over-winter losses from leaf fall and the loss of upper internodes of 30% to 40%. Under Northern Ireland conditions little drying of the crop took place over winter and at the March harvest date in both 2008 and 2009 the stem was about 50% DM.

**Conclusions** The use of degradable plastic mulch at planting accelerated the early development of miscanthus, with the benefits continuing into subsequent years. Within the ranges of weights used in this experiment dividing rhizomes into smaller sections for planting achieved higher shoot numbers and yields over the first two seasons.

**Acknowledgement** This work was funded by the Department of Agriculture and Rural Development

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## Improvements and validation of milk fatty acid predictions using mid-infrared spectrometry

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**Introduction** The requirement for gas chromatography (GC) to quantify milk FA concentration is expensive to be undertaken on large number of samples. The recent development of equations based on mid-infrared spectrometry (MIR) for the prediction of milk FA content (Soyeurt *et al.*, 2006) offers a solution. The first objective was to improve the predictions of FA by using different approaches. The second objective was to validate these new equations using an independent sample set.

**Materials and methods** The calibration set contained 239 Belgian milk samples collected between March 2005 and December 2007 from several cows and breeds. These samples were selected based on their spectral variability. The MIR spectrum from each sample was obtained (Foss MilkoScan FT6000) and the FA content of each sample was quantified by GC. The equations were built by Foss WINISI software using partial least squares (PLS) and/or first derivation and/or repeatability file. Using a derivative applied to the spectra permits to normalize the spectral data. The repeatability file contained spectra generated from the same samples but from five different spectrometers. A cross-validation using 20 groups from the calibration set was used to estimate the accuracy of the FA predictions. The methods used were 1) just PLS, 2) PLS and first derivative, 3) PLS and repeatability and 4) PLS, first derivative and repeatability (\*). These methods were compared using the ratio of the standard deviation of GC results (SD) to the standard error of cross-validation (RPD). An external validation was done using 362 GC independent milk samples collected between April 2008 and August 2009 from several breeds and cows in Belgium, Ireland, and Scotland to confirm the results obtained by cross-validation. The validation coefficient of determination ( $R^2v$ ) was calculated.

**Results** If RPD is superior to 3, the predictions given by the equation can be considered as good. Table 1 presents results for equations showing RPD superior or equal to 3 and summarises the descriptive statistics of the GC results. As expected, the equations with the higher values of RPD were globally from groups of FA, rather than the individual FA. The different approaches used to develop the equations showed generally different RPD values. All equations were not better using a calibration equation built from first derivative and repeatability file even if the results were generally better with this approach. These results suggest adapting the methodology used to develop the equation in function of the studied FA.  $R^2v$  shown in Table 1 confirms it. The highest  $R^2v$  were observed for the same equations, which showed the highest RPD values except for C18:0.

**Table 1** Descriptive statistics of the calibration set, RPD values, and  $R^2v$  obtained from the developed equations using the 4 methods.

Constituent	N=239		RPD (N=239)				$R^2v$ (N=362)			
	Mean	SD	1 (*)	2 (*)	3 (*)	4 (*)	1 (*)	2 (*)	3 (*)	4 (*)
C6:0	0.08	0.02	3.95	4.02	3.89	3.95	0.88	0.90	0.87	0.90
C8:0	0.05	0.02	3.21	3.27	3.21	3.33	0.84	0.88	0.86	0.81
C10:0	0.12	0.04	3.03	2.99	3.07	3.07	0.82	0.87	0.80	0.73
C14:0	0.48	0.14	3.51	3.62	3.66	3.70	0.90	0.90	0.90	0.90
C16:0	1.29	0.42	3.07	3.12	3.17	3.16	0.91	0.90	0.90	0.90
C18:0	0.49	0.23	2.89	2.93	2.90	3.01	0.73	0.62	0.74	0.72
Total C18:1 trans	0.15	0.09	3.16	3.09	3.05	3.09	0.46	0.46	0.52	0.49
C18:1 cis-9	0.89	0.36	4.61	4.68	4.35	4.60	0.86	0.92	0.93	0.91
Total C18:1 cis	0.96	0.37	4.62	4.71	4.50	4.73	0.85	0.93	0.94	0.93
Saturated	2.98	0.85	9.34	10.01	9.55	9.95	0.98	0.98	0.98	0.98
Monounsaturated	1.26	0.43	5.47	5.85	5.41	5.88	0.93	0.95	0.95	0.95
Unsaturated	1.46	0.48	5.82	6.24	5.77	6.26	0.93	0.95	0.96	0.96
Short chain (C4-C10)	0.39	0.11	3.85	3.96	3.90	3.97	0.89	0.91	0.91	0.93
Medium chain (C12-C16)	2.19	0.64	4.10	4.19	4.14	4.27	0.92	0.94	0.92	0.94
Long chain (C17-C22)	1.86	0.69	4.56	4.86	4.64	4.93	0.94	0.92	0.95	0.95

**Conclusions** MIR is a good technology to predict the contents of major FA in milk especially saturated fatty acids. Results presented here are superior to those presented by Soyeurt *et al.* (2006). The 3<sup>rd</sup> and 4<sup>th</sup> proposed methodologies give globally the best results.

**Acknowledgement** The authors gratefully acknowledge funding from the FP7 KBBE-2007-1 project, RobustMilk. The National Fund for Scientific Research is also acknowledged for its financial support through the FRFC 2.4.623.08.F.

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## Effects of feeding *Chrysanthemum coronarium* flowers to lactating dairy cows on milk fatty acid composition

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**Introduction** The presence of *Chrysanthemum coronarium* plants at 34% of pasture dry matter (DM) consumed by sheep increased milk fat *cis*-9, *trans*-11 conjugated linoleic acid (CLA) concentration (Cabiddu *et al.*, 2006). This was associated with an increase in milk fat *trans*-11 18:1 concentration, suggesting effects on rumen biohydrogenation. The *Chrysanthemum coronarium* plant is rich in 18:2 n-6 and 18:3 n-3 compared with other *Chrysanthemum* species (Cabiddu *et al.*, 2006), but other factors may be involved (e.g. coronaric acid; Earle, 1970). The objective of the present study was to determine the effect of feeding dehydrated *Chrysanthemum coronarium* flowers on milk fatty acid composition in dairy cows fed a typical commercial ration based on conserved forages.

**Materials and methods** Six multiparous lactating Holstein-Friesian cows averaging 39.5 litres milk/d at the start of the study were used in a simple cross-over design experiment with 2 treatments and 21 day periods that were separated by a 14 day 'washout' period. Treatments were a control diet (Con) and the same diet with dried, ground *Chrysanthemum coronarium* flowers added at 5% of ration DM (Chr), diluting other ingredients. The Con diet was a total-mixed ration containing on a DM basis: 24.5% grass silage, 18.5% maize silage, 4% grass hay, and 53% concentrate blend with minerals. Measurements of DM intake and milk yield and composition were obtained in the last week of each period. Data were statistically analyzed using Mixed Models procedures and a model testing fixed effects of diet and period and random effects of cow.

**Results** Fatty acid analysis indicated little difference between the diets fed, with the Con and Chr diets containing 8.4 and 8.0 g/kg DM 18:2 n-6 and 2.9 and 2.7 g/kg DM 18:3 n-3, respectively. Feed DM intakes increased ( $P < 0.04$ ) when Chr was fed (Table 1), in part due to a higher concentration of crude protein and lower concentration of neutral detergent fibre in the Chr diet. There was no effect on milk yield, milk fat concentration, or milk component yield, but milk protein concentration was higher when Chr was fed (29.8 vs 30.9 g/kg;  $P < 0.05$ ). Feeding Chr had little effect on milk saturated fatty acid (SFA) concentration (Table 1) apart from increases ( $P < 0.05$ ) in 4:0 and 6:0 and a tendency for a decrease ( $P < 0.10$ ) in 18:0. The concentration of milk fat *trans*-monounsaturated fatty acids (MUFA) increased ( $P < 0.05$ ) after feeding Chr (Table 1), mainly due to tendencies for increases ( $P < 0.09$ ) in concentrations of specific *trans*-18:1 isomers (6-8, 10, 12, 13-14 and 16 18:1, data not shown), but there was no difference in *trans*-11 18:1 concentration. Likewise there was no difference ( $P > 0.05$ ) between treatments in total and *cis*-9, *trans*-11 CLA concentration (Table 1). However, increases ( $P < 0.01$ ) in milk fat concentration of other CLA isomers were observed (e.g. 17.7 vs 27.6 mg *trans*-11, *trans*-13 CLA/100 g fatty acids and 7.4 vs 12.4 mg *trans*-12, *trans*-14 CLA/100 g fatty acids) when Chr was fed.

**Table 1** Effects of feeding diets containing 5 % *Chrysanthemum coronarium* flowers on feed intake, milk yield, and milk fatty acid composition (g/100 g fatty acids) in lactating dairy cows.

	Con	Chr	s.e.	P<
DM intake, kg/d	20.3	21.3	0.75	0.036
Milk yield, kg/d	33.3	33.9	0.99	0.630
Σ SFA	71.6	71.8	0.57	0.489
Σ <i>trans</i> MUFA	3.69	3.97	0.162	0.042
Σ <i>trans</i> 18:1	3.13	3.41	0.146	0.048
<i>trans</i> -11 C18:1	0.69	0.67	0.050	0.240
Σ CLA	0.52	0.55	0.030	0.127
<i>cis</i> -9, <i>trans</i> -11 CLA	0.37	0.38	0.027	0.602

**Conclusions** The results of the present study demonstrate that feeding dried *Chrysanthemum coronarium* flowers at 5% of ration DM (> 1 kg/d) had some effects on *trans*-monoene fatty acid concentrations in milk fat suggesting that rumen biohydrogenation had been modified, but in contrast to previous studies in grazing sheep, consumption of *Chrysanthemum coronarium* had no effect on milk fat *cis*-9, *trans*-11 CLA or *trans*-11 18:1 content. This may reflect the lower inclusion level in the present study, differences in the composition of the *Chrysanthemum* consumed, variations in the rumen environment and dynamics between cattle and sheep, or differences in the basal diets. Palatability was not a problem and in fact intake was increased by 1 kg of DM daily when dried *Chrysanthemum* flowers were added to the ration fed to these lactating dairy cows, and milk protein concentration was increased.

**Acknowledgements** The authors gratefully acknowledge funding from the EU Framework 6 programme (REPLACE).

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## Effect of omitting one milking weekly on milk production and quality characteristics

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**Introduction** Milking cows twice a day is a time-constraining task for dairy farmers (Clark *et al.*, 2006). Omitting one milking weekly, particularly a week-end afternoon milking may offer an opportunity to reduce labour costs if the farm employs hired labour, or alternatively to improve lifestyle in a family operated farm situation. The objective of the current study was to determine the effect of thirteen times per week milking (13TWM) at different stages of lactation compared to normal twice per day (TAD) milking every day, in terms of milk yield, composition and quality.

**Materials and methods** Thirty-six spring-calving, pluriparous Holstein-Friesian cows were assigned to one of three treatments after calving (12 cows per treatment); normal TAD milking (TAD); 13TWM commencing at approximately 50 days in milk (DIM) (13TWM 50); 13TWM commencing at approximately 180 DIM (13TWM 180), balanced for calving date, cow breed, somatic cell count (SCC), lactation number and milk yield in previous lactation. In the 13TWM treatments one milking each week was eliminated from the milking routine; cows were not milked on Wednesday afternoons. Mean calving date for all cows was 20<sup>th</sup> February. The trial extended to the end of lactation when cows were dried off at 7 kg milk/cow per day. Cows were allocated grass daily within a rotational grazing system and grazed to an average post-grazing sward surface height of 50 mm. Cows received 384 kg concentrate during the course of the lactation. When concentrate meals were being fed, the weekly meal fed was the same for all cow groups. Individual cow milk yield was recorded daily. The milk fat, protein and lactose concentrations were determined weekly. Cow live weight and body condition score (BCS) were recorded weekly and fortnightly, respectively. Bulk milk SCC was measured weekly. All data were analysed according to a factorial design using the PROC Mixed procedure in SAS.

**Results** Cumulative milk yield, yields of milk solids (MS) and fat, protein and lactose concentrations were not different for the three treatments (Table 1). Cow live weight and BCS were also similar for the three treatments. Average milk SCC of the treatment groups TAD, 13TWM 50 and 13TWM 180 were  $163 \times 10^3$ ,  $164 \times 10^3$  and  $147 \times 10^3$  cells/ml, respectively. Only minor changes in SCC were observed on the day after the omitted milking. The relatively low SCC levels may be influenced by the fact that only cows with SCC  $< 200 \times 10^3$  cells/ml in the previous lactation and during the pre-trial period were used.

**Table 1** Effect of omitting one milking weekly (13TWM) commencing at 50 and 180 days in milk compared to normal twice a day (TAD) milking on milk production characteristics

	TAD	13TWM 50	13TWM 180	SE	Significance
Cummulative milk yield (kg/cow)	6128	6498	6352	274.6	NS
Cummulative milk solids yield (kg/cow)	477	502	481	20.6	NS
Mean milk fat (g/100g)	4.16	4.11	4.00	0.115	NS
Mean milk protein (g/100g)	3.61	3.64	3.60	0.040	NS
Live weight at end of trial (kg)	655	655	642	16.8	NS

**Conclusion** In conclusion, the data indicates that omitting milking on one consistent occasion per week does not adversely affect overall milk yield, composition or quality.

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## Effect of rumen protected choline supplementation on milk production and composition of lactating Friesian cows

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**Introduction:** Rumen-protected choline (RPC) products have been fed to periparturient dairy cows to increase the supply of choline to the small intestine with the goal of increasing milk or component yields or alleviating the development of fatty liver syndrome (Hartwell *et al.*, 2000; Piepenbrink and Overton, 2003; Pinotti *et al.*, 2003; Overton and Waldron, 2004). Increasing the postruminal supply of choline by an infusion of choline into the abomasum has increased milk production and milk fat yield (Erdman and Sharma 1991).

**Materials and methods** Twelve lactating Friesian cows were used in a complete switch-back design (Lucas, 1956). The cows were fed the basal ration consisted on DM basis from 40% concentrate feed mixture + 40% fresh berseem + 20% rice straw without supplement (G<sub>1</sub>) or supplemented with 15 and 30 g choline chloride / head / day for G<sub>2</sub> and G<sub>3</sub>, respectively.

**Results** Results in Table (1) showed that the digestibility coefficients of all nutrients and nutritive values increased significantly ( $P<0.05$ ) with rumen protected choline supplementation. Rumen protected choline supplementation increased significantly ( $P<0.05$ ) the intake of TDN and DCP. The pH values and NH<sub>3</sub>-N concentration decreased significantly ( $P<0.05$ ) and TVFA's concentration increased significantly ( $P<0.05$ ) in rumen liquor with rumen protected choline supplementation. Rumen protected choline supplementation led to significant decrease ( $P<0.05$ ) in the concentration of plasma cholesterol and significant increase ( $P<0.05$ ) in triglycerides. However, the concentrations of glucose, total protein, albumin, globulin and urea-N and the activity of AST and ALT were nearly similar for the different groups. Rumen protected choline supplementation led to significant increase ( $P<0.05$ ) in actual milk and 4% FCM yield. The contents of fat and total solids (TS) and the yield of all milk constituents except ash increased significantly ( $P<0.05$ ) with rumen protected choline supplementation. Results in Table (2) revealed that rumen protected choline supplementation improved feed conversion, which led to significant decrease ( $P<0.05$ ) in the quantities of DM, TDN and DCP per kg 4% FCM. Average daily feed cost were nearly similar for the different groups. While, the feed cost per one kg 4% FCM decreased significantly ( $P<0.05$ ), the average income of milk yield increased significantly ( $P<0.05$ ) with rumen protected choline supplementation.

**Table 1** Effect of rumen protected choline supplementation on nutritive values, feed intake, rumen parameters, milk yield and composition.

Item	Nutritive values %		Intake kg/day		Rumen parameters			Milk yield kg/day		Milk composition %				
	TDN	DCP	DM	TDN	DCP	pH	NH <sub>3</sub> -N	TVFA's	Actual	FCM	Fat	Protein	Lactose	TS
control	60.87 <sup>b</sup>	8.57 <sup>b</sup>	16.08	9.79 <sup>b</sup>	1.38 <sup>b</sup>	6.95 <sup>a</sup>	14.81 <sup>b</sup>	18.95 <sup>a</sup>	15.22 <sup>b</sup>	14.22 <sup>b</sup>	3.56 <sup>b</sup>	3.12	4.39	11.78 <sup>b</sup>
15 g RPC	63.98 <sup>a</sup>	8.96 <sup>a</sup>	16.17	10.35 <sup>a</sup>	1.45 <sup>a</sup>	6.72 <sup>b</sup>	17.14 <sup>a</sup>	16.63 <sup>b</sup>	16.54 <sup>a</sup>	15.77 <sup>a</sup>	3.69 <sup>a</sup>	3.15	4.39	11.94 <sup>a</sup>
30 g RPC	64.62 <sup>a</sup>	9.05 <sup>a</sup>	16.20	10.47 <sup>a</sup>	1.47 <sup>a</sup>	6.67 <sup>b</sup>	17.43 <sup>a</sup>	16.25 <sup>b</sup>	17.46 <sup>a</sup>	16.82 <sup>a</sup>	3.75 <sup>a</sup>	3.16	4.43	12.05 <sup>a</sup>

a, b: Values and means in the same row with different superscripts differ significantly at 5% level.

**Table 2** Effect of rumen protected choline supplementation on feed conversion and economic efficiency.

Item	Feed conversion kg / kg FCM			Economic efficiency LE			
	DM	TDN	DCP	Cost	Cost/ kg FCM	Income	Income %
control	1.14 <sup>a</sup>	0.69 <sup>a</sup>	0.098 <sup>a</sup>	19.18	1.36 <sup>a</sup>	28.44 <sup>b</sup>	100.00 <sup>c</sup>
15 g RPC	1.03 <sup>b</sup>	0.66 <sup>ab</sup>	0.093 <sup>ab</sup>	19.37	1.24 <sup>b</sup>	31.55 <sup>a</sup>	110.94 <sup>b</sup>
30 g RPC	0.97 <sup>b</sup>	0.62 <sup>b</sup>	0.088 <sup>b</sup>	19.50	1.17 <sup>b</sup>	33.64 <sup>a</sup>	118.28 <sup>a</sup>

a, b: Values and means in the same row with different superscripts differ significantly at 5% level.

**Conclusions** It could be concluded that rumen protected choline supplementation to lactating Friesian cows improved nutrients digestibility, milk yield and composition, feed conversion and economic efficiency.

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## Effect of calving difficulty on the saleable milk yield of UK Holstein Friesian dairy cattle at different stages of lactation

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**Introduction** Calving difficulty, or dystocia, results in assistance being provided at delivery, thus increasing farm labour. In the dairy cow, dystocia is associated with stillbirth, but also leads to impaired fertility, reduced milk production and increased risks for peripartum diseases and culling (Mee, 2008). Thus, calving difficulty raises animal welfare and economic issues. Considering milk yield, it is not clear how long the adverse effect on production lasts. Studies usually consider the milk produced by animals with full lactations but the saleable milk production of the whole herd, regardless of each cow having achieved a full lactation, might be more representative of the real losses that producers incur. The objective of this study was to investigate how various degrees of calving difficulty would alter the production of saleable milk in UK dairy cattle over different stages of their subsequent lactation.

**Materials and methods** The calving difficulty scores and the subsequent milk production of Holstein Friesian cattle having calved on the SAC experimental herd (Edinburgh, UK) between 1990 and 2000 inclusive were extracted from the farm database. The calving difficulty was scored as follows: no assistance (N), Farm assistance without and with malpresentation (FN/FM), Veterinarian assistance without/with malpresentation (VN/VM) and caesarean section (VC). Cows were conventionally milked twice a day and their individual daily milk yields were recorded automatically at milking conditional on the milk being sent to the tank for sale. Cumulative saleable milk yield (l) was calculated at 30, 60, 90 and 300 days in milk (DIM) unconditional on the animal having achieved the lactation stage of interest. Lactation cumulative yields were obtained on the basis of the real lactation length achieved by the animal. Animals were from two genetic groups (S: animals selected toward greater milk solids production; C: animals selected to be UK average) and split over 3 diet types (H: high forage diet; L: low forage diet; NT: standard high concentrate diet type). Linear mixed models were used following a REML procedure in Genstat to analyse the various cumulative milk yields. In the fixed effects, parity of the cow (primiparous vs multiparous), genetic group\*diet, calving season (Summer: April to September; Winter: October to March) and calving ease were fitted as factors and calving year as a covariate. The random model included the cow identity nested within its sire.

**Results** Cows experiencing FN and VN scores had decreased cumulative saleable milk production throughout their lactation compared to non assisted animals ( $P<0.05$ ; Table 1). Losses occurred as early as 30 DIM (FN: -5.2%; VN: -8.8%) and persisted until the end of the lactation (FN: -8.1%; VN: -12.5%). No losses were found for FM, VM and VC dams. As shown by previous research, parity, genetic group, diet, season and calving year affected the saleable yields. Those effects were found at all stages of the lactation except at 30 DIM for calving season and at both 300 DIM and over the lactation for genetic group\*diet.

**Table 1** Estimated means of the cumulative marketable milk yields (l) over subsequent lactation of dairy cattle following different degrees of calving difficulty. Number of lactations available for analysis for each calving ease score is given in brackets.

Cumulative Yield (l)	Calving difficulty						s.e	P value
	N (n=1855)	FN (n=227)	FM (n=74)	VN (n=38)	VM (n=30)	VC (n=15)		
30 DIM	647 <sup>a</sup>	613 <sup>b</sup>	635 <sup>a,b</sup>	590 <sup>b</sup>	606 <sup>a,b</sup>	572 <sup>a,b</sup>	22.5	**
60 DIM	1 502 <sup>a</sup>	1 430 <sup>b</sup>	1 479 <sup>a,b</sup>	1 384 <sup>a,b</sup>	1 425 <sup>a,b</sup>	1 377 <sup>a,b</sup>	50.3	*
90 DIM	2 320 <sup>a</sup>	2 218 <sup>b</sup>	2 304 <sup>a,b</sup>	2 110 <sup>b</sup>	2 222 <sup>a,b</sup>	2 165 <sup>a,b</sup>	78.5	*
300 DIM	6 375 <sup>a</sup>	5 859 <sup>b,c</sup>	6 377 <sup>a,c</sup>	5 520 <sup>b</sup>	6 003 <sup>a,b</sup>	5 722 <sup>a,b</sup>	264.0	***
lactation	6 857 <sup>a</sup>	6 305 <sup>b,c</sup>	6 931 <sup>a,c</sup>	5 999 <sup>b</sup>	6 578 <sup>a,b</sup>	6 221 <sup>a,b</sup>	320.0	**

Within a row, means without a common letter differ. s.e : pooled standard error of the mean. \* :  $P<0.05$ ; \*\* :  $P<0.01$ ; \*\*\* :  $P<0.001$ .

**Conclusion** Decreased cumulative saleable milk production was found for cows needing farm staff or veterinarian assistance but only if the calf was not malpresented (FN and VN scores). Not only did these losses occur in the early stages of the lactation but they persisted over time and appeared to be higher by the end of the lactation. Therefore, our results support the idea that calving assistance triggers long-term saleable milk production losses for the dairy producer. The lack of effect seen for VM and VC scores is probably due to the low number of animals available whereas failure to find an effect for FM might be due to the farm staff assisting more quickly when malpresentation becomes obvious. Lower saleable milk production may be the result of decreased milk yields by the cow herself as well as higher milk wastage due to a subsequent poorer health. Further analyses into the health of the cow during the relevant lactation would be needed to investigate that hypothesis.

**Acknowledgements** The authors are grateful to Defra, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR for funding as well as to farm and technical staff for data collection over those years.

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## The effect of adding stinging nettle (*urtica dioica*) haylage to a total mixed ration on performance and rumen function of lactating dairy cows

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**Introduction** *In-vitro* studies have identified that the inclusion of dried Stinging Nettle (*Urtica dioica*) at 100mg/g increased rumen pH of a fermentation medium by 30% (Kliem *et al.*, 2005a) and that the effect was persistent for a period of 7 days (Kleim *et al.*, 2005b). These observations indicate that Stinging Nettle has the potential to be used to promote rumen health in animals consuming high levels of readily fermentable carbohydrate by stabilising the rumen environment with respect to rumen pH. Therefore our objective was to evaluate the effects of adding stinging nettle haylage to a high-starch total mixed ration on feed intake, eating and rumination activity, rumen pH, milk yield, and milk composition of lactating dairy cows.

**Materials and methods** Six rumen fistulated lactating Holstein-Friesian cows averaging 20 litres milk/day were used in a replicated 3 x 3 Latin Square design experiment with 3 treatments and 3 week periods. Treatments were a control (C) high-starch total mixed ration (TMR) and two treatment diets containing 5% (N5) and 10% (N10) DM nettle haylage. The control diet contained on a dry matter (DM) basis: 29% maize silage, 10% grass silage, and 61% concentrate blend with minerals. Nettle haylage was included as a direct replacement for the grass silage element of the TMR (DM basis). Eating and rumination activity were measured as described previously by Aikman (2008). Rumen pH was measured using an indwelling pH electrode and rumen VFA concentrations were measured on 9 samples obtained over 8.5 hours. Measurements were made in the last week of each period. The dependent variables were analyzed using Mixed Models procedures and a model testing fixed effects of square, diet, period and diet by period interactions and random effects of cow. Orthogonal contrasts were used to test for linear and quadratic effects of increasing amounts of nettle haylage in the diet. Particle size of the rations was measured using a Penn State Separator.

**Results** There was an increase (linear,  $P < 0.01$ ) in the proportion of large particles and a reduction (linear,  $P < 0.05$ ) in medium and fine particles with increasing nettle inclusion. A trend for a linear decrease in DMI intake was observed as nettle inclusion in the diet increased (Table 1). Milk yield averaged 20.3 kg/d and was not affected by diet. Ruminating jaw movements declined linearly ( $P < 0.024$ ; data not shown) with a corresponding linear decrease in the time spent ruminating as nettle inclusion in the diet increased (Table 1). Rumen pH increased (quadratic) with nettle inclusion in the diet (Table 1). Minimum rumen pH also increased when nettle haylage was included at 10% of ration DM (quadratic). The time that rumen pH was below 5.5, 5.6 and 5.8 was least in cows with a 10% nettle inclusion in the diet and highest for cows with a 5% nettle inclusion (Table 1). There was a tendency for rumen acetate: propionate ratio to increase linearly with increasing nettle inclusion in the diet (Table 1).

**Table 1** Effects of feeding diets containing 5 (N5) or 10 (N10) % nettle haylage on feed intake, rumen pH measurements, length of time rumen fluid was below a specified pH, and rumen acetate:propionate concentration ratio during the last week of each period.

	Diet			SEM	P		
	C	N5	N10		Diet	Linear	Quadratic
DM intake, kg/d	20.0	19.7	18.5	0.93	0.220	0.106	0.593
Time ruminating, %	25.9 <sup>a</sup>	20.5 <sup>ab</sup>	18.7 <sup>b</sup>	2.56	0.175	0.080	0.525
Mean rumen pH	5.96 <sup>a</sup>	6.00 <sup>a</sup>	6.09 <sup>b</sup>	0.095	0.668	0.119	0.061
Minimum rumen pH	5.32 <sup>a</sup>	5.31 <sup>a</sup>	5.38 <sup>b</sup>	0.097	0.020	0.018	0.096
Time (h) pH<5.5	3.07 <sup>ab</sup>	3.30 <sup>a</sup>	2.50 <sup>b</sup>	0.994	0.059	0.292	0.152
Time (h) pH<5.6	4.86 <sup>ab</sup>	5.04 <sup>a</sup>	3.81 <sup>b</sup>	1.387	0.131	0.260	0.262
Time (h) pH<5.8	8.81 <sup>ab</sup>	8.78 <sup>a</sup>	7.29 <sup>b</sup>	2.068	0.195	0.276	0.159
Acetate:propionate ratio	2.66	2.74	2.85	0.313	0.283	0.122	0.883

<sup>a, b</sup> Values with different superscripts are statistically different ( $P < 0.10$ )

**Conclusions** Production levels in terms of milk output were maintained when nettles replaced grass silage in the diet of lactating dairy cows, in spite of a reduction in feed intake. Rumination activity was reduced by the addition of nettle haylage to the diet, but there were indications of shifts in rumen fermentation patterns that were potentially beneficial to lactating dairy cows on high grain content diets. Shifts in rumen pH patterns suggest potential benefits of feeding nettle haylage for reducing rumen acidosis. However, it is not certain if the effects observed were due to differences in the chemical composition of grass versus nettles or specific bioactive components of stinging nettles.

**Acknowledgements** The authors gratefully acknowledge funding from the EU Framework 6 programme (REPLACE).

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## The fatty acid composition of milk available at retail over the course of one year

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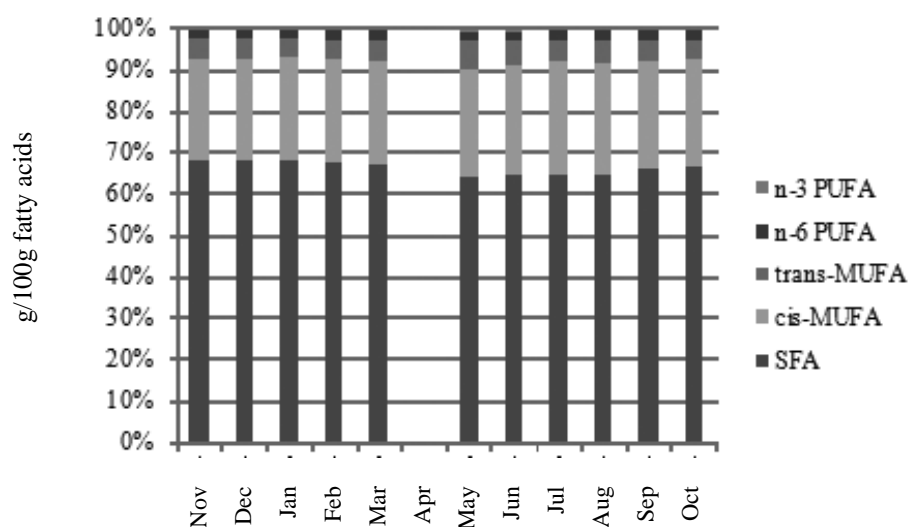
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**Introduction** Milk and dairy products are the greatest single source of saturated fatty acids (SFA) in the human diet, contributing to between 30 and 40 % of total SFA intake in the UK (Henderson *et al.*, 2003; Hulshof *et al.*, 1999). Intake of SFA in the UK exceeds current guidelines thus increasing cardiovascular disease risk. Research has focused on strategies to reduce SFA in milk fat by altering dairy cow diet (Givens and Kliem, 2008). However such studies have only been conducted on an experimental scale and it is not known if these changes would still be apparent in retail milk composited from numerous farms. There are also no contemporary data on SFA in retail milk and how this changes throughout the year. Since UK consumption of semi-skimmed milk is still rising (Givens and Kliem 2008) the objective of the current study was to assess concentration of SFA and other fatty acids in retail milk over the course of one year.

**Materials and methods** Semi-skimmed milk (550 ml) was purchased from five supermarkets within a 5 mile radius of the laboratory at the same time every month over the course of one year (one month was omitted). Milk was aliquotted and stored at -20°C until analysis. Lipid in 1 ml milk was extracted in duplicate using ethanol, diethylether and hexane, and transesterified to fatty acid methyl esters (FAME) using methanolic sodium methoxide (Kliem *et al.*, 2008). FAME were separated using a gas chromatograph temperature programme (Kliem *et al.*, 2008). Milk fatty acids were expressed as g/100 g fatty acids. Data were analysed as groups of fatty acids using analysis of variance for effects of month and supermarket.

**Results** The SFA content of retail milk peaked in January (mean  $\pm$  SEM  $67.6 \pm 0.35$  g/100 g fatty acids) and was at its lowest in May ( $62.7 \pm 0.45$  g/100 g fatty acids, Figure 1). The spring and summer months corresponded with an increase in *cis*-monounsaturated fatty acids (MUFA, peaking in July,  $27.2 \pm 0.24$  g/100 g) and *trans*-MUFA (peaking in May,  $6.6 \pm 0.26$  g/100 g). The concentration of total n-3 polyunsaturated fatty acids (PUFA) was highest ( $0.88 \pm 0.044$  g/100 g) in June. The effect of month was significant ( $P < 0.05$ ) for SFA, *cis*-MUFA, *trans*-MUFA and n-3 PUFA. There was also an effect ( $P < 0.05$ ) of supermarket on *cis*-MUFA, *trans*-MUFA, n-3 PUFA and n-6 PUFA.



Where  
PUFA = polyunsaturated fatty acids  
MUFA = monounsaturated fatty acids  
SFA = saturated fatty acids

**Figure 1** Fatty acid composition of semi-skimmed milk from five supermarkets over the course of one year (2008-2009)

**Conclusions** These results demonstrate that the seasonal effect of dairy cow diet on milk fatty acid profile is present even in bulked milk samples sourced from multiple suppliers. The reduction in SFA during the summer months is probably a response to increased dairy cow intake of fresh pasture, the higher PUFA content of which indirectly inhibits *de novo* synthesis and increases the availability of PUFA and *trans*-MUFA to the mammary gland. The observed effect of supermarket may reflect differences in supply pools. Despite the variation observed, within months all milk fat contained 63 to 68 g/100g SFA indicating considerable scope for reduction throughout the whole year.

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## Effect of low energy high fibre and grass silage feeding strategies on metabolic status of dairy cows in the peri-partum period

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**Introduction** The dry period is an important phase of the dairy cow's lactation cycle. Plane of nutrition during this time can have a major influence on peripartum metabolism (Grummer, 1995). Many studies have focused on developing feeding strategies for the transition period (3 wks pre-partum to 3 wks post-partum) that alleviate the extent of metabolic stress. It has been shown recently that feeding a low energy, high-fibre total mixed ration (TMR) for the entire dry period may be beneficial in terms of improving metabolic status and reducing incidence of peripartum health disorders (Dann *et al.*, 2006). The aim of this study was to compare the metabolic profiles of Holstein Friesian cows managed under a high fibre TMR dry cow regime, relative to a moderate quality grass silage diet.

**Materials and methods** Sixty mature spring-calving Holstein-Friesian cows were blocked according to expected calving date, genetic merit for milk yield, body condition score (BCS), bodyweight and parity. Cows were randomly assigned to one of the two dry period nutritional treatments at drying off: (1) high fibre TMR (HF-TMR) or (2) grass silage (GS). The HF-TMR diet consisted of 0.40 wheaten straw, 0.25 grass silage, 0.25 maize silage and 0.10 soyabean meal. Both diets were offered *ad libitum* for the duration of dry period, and were supplemented with an appropriate dry cow mineral mix. Net energy density was calculated as 0.71 UFL (Unité Fourragère Lait) and 0.78 UFL for the HF-TMR and GS diets, respectively. Blood samples were taken from cows by coccygeal venipuncture, for week 1 pre-partum to week 4 post-partum. Samples were collected via evacuated vials containing, lithium heparin as an anticoagulant. Plasma analyte profiles were analysed using appropriate kits each containing a number of reagents for each analyte on an ABX Mira Auto analyser. Plasma insulin-like growth factor I (IGF-I) concentrations were determined by a validated double-antibody radio-immunoassay after ethanol-acetone-acetic acid (60:30:10) extraction. Plasma insulin concentrations were determined by using a solid-phase fluoroimmunoassay. Repeated measures analysis of treatment effects on plasma metabolites, insulin and IGF-I was carried out using the MIXED procedure of SAS (SAS, 2006). A first order autoregressive covariance structure was used. Treatment, time and treatment by time interactions were tested. Cow was included as a random effect nested within treatment. Plasma analyte data were not normally distributed and were therefore log-transformed prior to statistical analysis. Plasma analyte data are presented in as means of log-transformed values for wk 1 pre-partum to wk 4 post-partum.

**Results** Plasma metabolite profiles were similar for both dry cow treatments during wk 1 pre-partum to wk 4 post-partum, except for a greater mean betahydroxybutyrate (BHB) concentration for treatment GS (Table 1). Plasma calcium concentration tended to be greater for HF-TMR compared to GS during this time. Liver enzyme GLDH (Glutamate-dehydrogenase) was greater for treatment HF-TMR relative to treatment GS. Dry period diet did not affect peripartum concentrations of insulin or IGF-I.

**Table 1** Effect of dry period diet on plasma analyte concentrations

	HF-TMR <sup>1</sup>	GS <sup>1</sup>	s.e.d.	P-value
Plasma NEFA, mmol/l	-0.34 <sup>2</sup>	-0.37	0.085	0.66
Plasma BHB, mmol/l	-0.12	-0.31	0.078	0.02
Plasma triglyceride, mmol/l	-1.83	-1.82	0.035	0.81
Cholesterol, mmol/l	0.91	0.90	0.053	0.87
Magnesium, mmol/l	-0.15	-0.18	0.028	0.28
Phosphorus, mmol/l	0.43	0.41	0.055	0.73
Calcium, mmol/l	0.84	0.82	0.012	0.11
Aspartate-aminotransferase, iu/l	3.79	3.75	0.045	0.34
GLDH, iul/l	2.63	2.33	0.144	0.03
Bilirubin, µmol/l	1.97	2.04	0.061	0.23
Insulin, µIU/ml	1.31	1.30	0.080	0.85
IGF-I, ng/ml	4.63	4.57	0.090	0.52

<sup>1</sup>HF-TMR= High-fibre Total Mixed Ration; GS = Grass silage <sup>2</sup>All data in table log-transformed.

**Conclusions** Effects of dry period diet on plasma analyte profiles during the peripartum period were modest overall. Differences in plasma BHB concentration are consistent with greater body tissue mobilisation for GS, arising from a higher level of body lipid accretion during the dry period. Evidence from this study of a beneficial effect of HF-TMR on peripartum Ca metabolism is tenuous. The reasons for elevated GLDH for HF-TMR compared to GS are somewhat unclear; however neither treatment had GLDH concentrations that would be indicative of compromised liver function.

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## Differential transport of *trans* fatty acids by bovine plasma lipoprotein fractions: 1. Soya oil and partially hydrogenated vegetable oil

E Vargas-Bello-Pérez, P C Garnsworthy

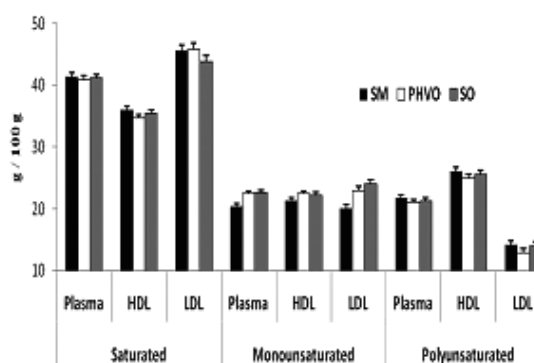
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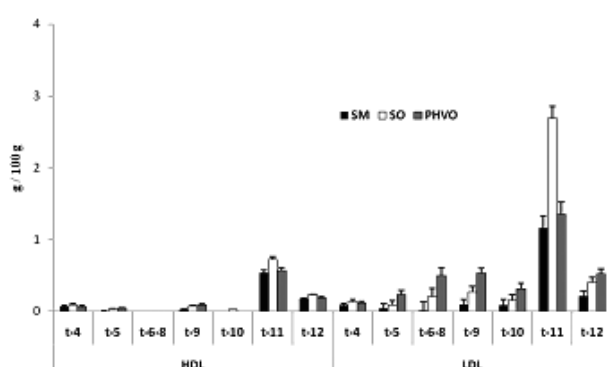
**Introduction** Dietary fatty acid (FA) source influences fat and *trans* FA (tFA) content of bovine milk. Vaccenic acid (VA; 18:1 *trans*-11) arises from rumen biohydrogenation of polyunsaturated FA (PUFA), is the predominant tFA in milk and, unlike some tFA, does not interfere with fat synthesis. Because mammary epithelial cells do not absorb HDL-lipoproteins, we hypothesized that effects of tFA on milk FA composition could be related to differential plasma transport mechanisms. The objective of this experiment was to elucidate which lipoprotein fractions are involved in plasma transport of tFA isomers by infusing oils that induce different plasma tFA profiles.

**Materials and methods** Three non-lactating Holstein cows (Live weight  $773 \pm 63$  kg), each fitted with a rumen cannula, were used in a 3 x 3 Latin square design. Cows were fed on a diet of grass hay (7 kg/d) and concentrate (based on barley, sugar beet and wheat; 2 kg/d) and treated with bolus ruminal infusions of: 1) skim milk (SM; control; 500 ml/d); 2) soya oil (SO; 250 g/d in 500 ml SM); and 3) partially-hydrogenated vegetable oil (PHVO; 250 g/d in 500 ml SM). Each three-day infusion period was followed by a four-day washout interval to minimize carryover effects. Blood samples were obtained prior to each infusion (0 h) and 1, 2, 3 and 6 h after infusion. Plasma was ultracentrifuged at  $39,000 \times g$  for 20 h at 12°C using a Beckman XL-70 ultracentrifuge to separate lipoproteins into low density (LDL) and high density (HDL) fractions. Fatty acid profiles of plasma and lipoprotein fractions were determined by gas chromatography. Data were analysed by repeated measures ANOVA to study effects of treatment, period, sampling day within period and infusion time within sampling day. Results presented are least-square means for each treatment because there was no interaction between treatment and period, day or time.

**Results** There was no difference between treatments in concentrations of saturated FA and PUFA, but monounsaturated FA were higher ( $P < 0.05$ ) in both plasma and LDL for SO and PHVO compared with SM (Figure 1). Compared with SM and PHVO, SO increased ( $P < 0.05$ ) VA concentration in both HDL and LDL. Compared with SM and SO, PHVO increased ( $P < 0.05$ ) HDL concentrations of 18:1 *trans*-9 but reduced ( $P < 0.05$ ) concentrations of 18:1 *trans*-10, *trans*-11 and *trans*-12 (Figure 2). Compared with SM and SO, PHVO, increased ( $P < 0.05$ ) LDL concentrations of 18:1 *trans*-5, *trans*-6-8, *trans*-9 and *trans*-10, and reduced ( $P < 0.05$ ) concentrations of 18:1 *trans*-11.



**Figure 1** Treatment effects on major fatty acid classes in plasma and lipoprotein fractions



**Figure 2** Concentrations of 18:1 *trans* isomers in lipoprotein fractions

**Conclusions** Effects of oil infusion of FA profiles of plasma, HDL and LDL were consistent with differences in oil composition. Soya oil is rich in PUFA, which increase VA after ruminal biohydrogenation; PHVO contains a mixture of tFA isomers, which are not changed during rumen passage. The results confirm, therefore, that tFA concentrations of plasma and lipoprotein fractions depend on dietary lipid source. This study suggests that LDL is more responsive to source of tFA, although further fractionation is required to distinguish between true LDL-cholesterol, chylomicrons and very low density lipoproteins (VLDL) that are also present in this fraction.

**Acknowledgments** E. Vargas Bello Pérez would like to thank Consejo Nacional de Ciencia y Tecnología (CONACYT-México) for the PhD studentship.

## Differential transport of *trans* fatty acids by bovine plasma lipoprotein fractions: 2. Fish oil and partially hydrogenated vegetable oil

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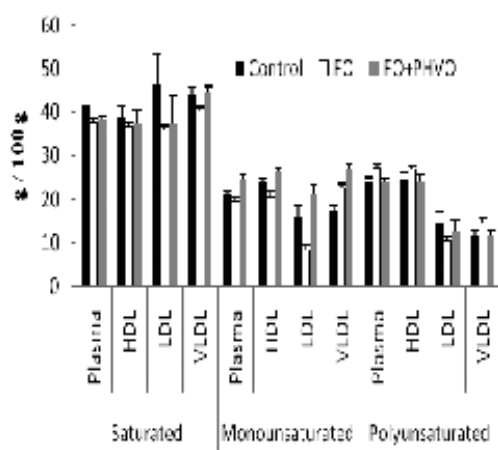
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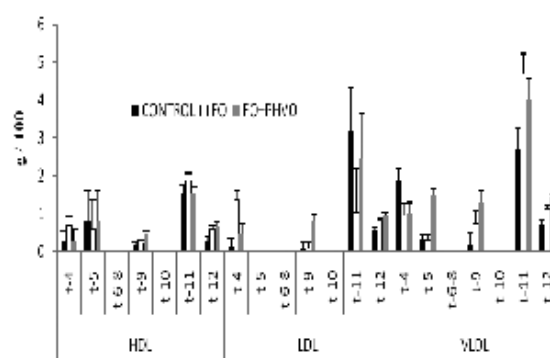
**Introduction** Fish oil (FO) either alone or in combination with vegetable oils results in increased concentrations of *trans* fatty acids (tFA) in duodenal fluid and milk fat. Our previous study demonstrated that infusion of partially hydrogenated vegetable oil (PHVO) or soya oil (SO) increased the tFA content of both HDL- and LDL- lipoprotein fractions, but responses varied according to fatty acid source. Because FO has a greater effect on rumen tFA yield than SO, we hypothesized that differences in blood FA profiles would be observed, particularly in 18:1 *trans* isomers. The objective of this study was to determine the effects of infusing oils that induce different concentrations of tFA, and to elucidate further which lipoprotein fractions are involved in tFA transport.

**Materials and methods** Two non-lactating Holstein cows (Live weight  $778 \pm 88$  kg), each fitted with a rumen cannula, were used in a 2 x 3 Cross over design with 3 d infusion periods followed by 4 d washout interval between treatments to minimize carryover effects. Cows were fed on grass hay (7 kg/d) and concentrate (based on barley, sugar beet and wheat; 2 kg/d) and treated with bolus ruminal infusions of: 1) SO (control; 250 ml/d in 500 ml/d of skim milk; SM); 2) FO (Salmon oil: 250 g/d in 500 ml/d of SM) and 3) FO+PHVO (125 + 125 g/d, in 500 ml/d of SM). Blood samples were obtained prior to each infusion (0 h) and 1 and 3 h after infusion. Plasma was ultracentrifuged at  $39,000 \times g$  for 16 h (to separate VLDL-cholesterol fraction) and a further 20 h (to separate LDL- and HDL-cholesterol fractions) at 12°C using a Beckman XL-70 ultracentrifuge. Fatty acid profiles of plasma and lipoprotein fractions were determined by gas chromatography. Data were analysed by repeated measures ANOVA to study effects of treatment, period, sampling day within period and infusion time within sampling day. Results presented are least-square means for each treatment because there was no interaction between treatment and period, day or time.

**Results** Compared with control, FO and FO+PHVO reduced ( $P < 0.05$ ) concentrations of saturated FA in plasma. Compared with control and FO+PHVO, FO reduced ( $P < 0.05$ ) concentrations of saturated FA in VLDL. Compared with control and FO, FO+PHVO resulted in higher ( $P < 0.05$ ) concentrations of monounsaturated FA in plasma and lipoprotein fractions. Compared with control and FO+PHVO, FO resulted in higher ( $P < 0.05$ ) concentrations of PUFA in plasma (Figure 1). Compared with control and FO+PHVO, FO resulted in higher ( $P < 0.05$ ) concentrations of VA in VLDL. Compared with control and FO, FO+PHVO increased ( $P < 0.05$ ) HDL concentrations of 18:1 *trans*-9 and *trans*-10, LDL concentration of 18:1 *trans*-9, and VLDL concentrations of 18:1 *trans*-5, *trans*-9 and *trans*-12 (Figure 2).



**Figure 1** Treatment effects on major fatty acid classes in plasma and lipoprotein fractions



**Figure 2** Concentrations of 18:1 *trans* isomers in lipoprotein fractions

**Conclusions** Dietary lipid source influenced FA profiles of plasma, HDL, LDL and VLDL. Fish oil is rich in PUFA, which increase VA after ruminal biohydrogenation; PHVO contains a mixture of tFA isomers, which are not changed during rumen passage. The results showed that dietary lipid source influences tFA concentrations of plasma and lipoprotein fractions. This study suggests that the VLDL-cholesterol fraction is more responsive to supply of tFA. VLDL-cholesterol fraction appears to be the major fraction involved in transportation of tFA.

**Acknowledgments** E. Vargas Bello Pérez would like to thank Consejo Nacional de Ciencia y Tecnología (CONACYT-México) for the PhD studentship.

## The association between herd size, herd expansion and breeding policy, reproduction and production performance of spring calving Irish dairy herds

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**Introduction** There are an increasing number of farmers in Ireland seeking to expand the scale of their dairy enterprises. Herd size in Ireland is small (95% of herds have less than 100 cows) but has increased from an average of 30 cows in 1994 to 55 currently. The removal of milk quotas in 2013 is expected to speed up expansion as production moves to areas of competitive advantage. Expanding a herd presents choices in terms of growing organically or sourcing non-homebred animals, as well as challenges as a result of managing more cows and the associated requirements for infrastructure and labour. The objective of this study was to characterise seasonal calving herds according to size and rate of expansion, to determine trends in sourcing new animals, breeding policy, reproduction and production performance.

**Materials and methods** Performance data from milk recording herds comprising 1,628,738 lactation records ( $n = 36,964$  herd-years) for the years 2004 to 2008 inclusive, were obtained from the Irish Cattle Breeding Federation database. Only herds with at least 20 cows, present for all five years of the study period and which had >80% of cows calving between 15<sup>th</sup> December and 30<sup>th</sup> June, inclusive, were retained (775,795 lactations from 2,555 herds). Herds were classified into three groups based on herd size and three groups based on annual rate of expansion. Linear robust regression was fitted to the annual herd size of each herd separately using PROC ROBUSTREG (SAS, 2004). The intercept (i.e. herd size in the year 2004) and a linear rate of change in herd size for each herd were retained. Herd size, predicted from the regression, was categorised into Small ( $n = 843$ ), Medium ( $n = 868$ ) and Large ( $n = 844$ ) based on predicted herd size in 2006 (i.e., the middle year of the study period). Median (5<sup>th</sup> and 95<sup>th</sup> percentile) herd size was 38 (26 and 46), 54 (47 and 62) and 79 (64 and 137) cows for Small, Medium and Large herds, respectively. If the linear regression coefficient was not different ( $P < 0.05$ ) from zero ( $n = 1585$ ) herds were coded as not expanding (Nil). Herds with a regression coefficient greater ( $P < 0.05$ ) than zero were divided into two even groups, each of 485 herds (Slow, increasing at an average rate of 3 cows/year; Rapid, increasing at an average rate of 8 cows/year). Multilevel hierarchical linear and non-linear models were fitted in ASREML (Gilmour *et al.*, 2009) with herd, and cow within herd, as random effects. Year was included in the model as a fixed effect as well as herd size and rate of expansion and their interactions.

**Results** There was no difference ( $P > 0.05$ ) in fat yield, protein yield and fat percent between herds differing in the rate of expansion. However, an association ( $P < 0.001$ ) existed between 305-day milk yield and rate of expansion (Nil 6307 litres, Slow 6242 litres, Rapid 6199 litres, SED 41.3,  $P < 0.01$ ). Expanding herds had greater ( $P < 0.001$ ) milk protein percent than herds that were not expanding. There were no associations between herd size and milk production traits except for protein percentage, which increased ( $P < 0.05$ ) with increasing herd size. Average parity number of the cows in a herd decreased as rate of expansion increased (Nil 3.0, Slow 2.9, Rapid 2.6 years, SED 0.002,  $P < 0.001$ ), but was not associated with herd-size. Relative to herds that were not expanding, the odds of a slow or rapidly expanding herd having a greater proportion of homebred animals was 0.89 (95% CI: 0.74 to 1.07, NS) and 0.44 (95% CI: 0.36 to 0.53,  $P < 0.05$ ). Relative to small herds, the odds of a medium or large herd having a higher proportion of homebred animals was 1.5 (95% CI: 1.34 to 1.90) and 2.7 (95% CI: 2.23 to 3.20). Holstein-Friesian was the predominant dairy breed of calves born to cows in herds of all herd sizes and expansion categories (73%, 76%, 77% for Small, Medium and Large herds, respectively and 74%, 74% and 77% for Nil, Slowly and Rapidly expanding herds, respectively). The proportion of beef calves born decreased ( $P < 0.001$ ) as herd size and rate of expansion increased (34, 29, 27%,  $se = 1.0$  for small, medium and large herds, respectively and 34%, 32% and 25% for Nil, Slowly and Rapidly expanding herds, respectively). The proportion of Jersey mated to Holstein-Friesian or purebred Jersey calves was very low ( $< 1\%$ ) for all herd sizes and rates of expansion, but the proportion of crossbred calves increased with increasing rate of expansion. Calving interval was longest for small and Nil expanding herds. Animals in larger and expanding herds calved for the first time at a younger age relative to smaller or non-expanding herds (804, 791, 776 days,  $se = 3.1$  for Small, Medium and Large herds, respectively and 797, 786 and 788 days,  $se = 3.6$  for Nil, Slowly and Rapidly expanding herds, respectively). There was a negative association between herd size and proportion of cows calving in the first 56 days of the calving season. A greater ( $P < 0.01$ ) percentage of calves were born to AI in medium sized herds compared to small or large herds.

**Conclusions** Rapidly expanding herds are increasing cow numbers by buying in more non-homebred cattle. The proportion of dairy sires used in their breeding program is increasing and there is more crossbreeding, albeit at a low rate. Similarly large herds are using more dairy sires and fewer beef sires. Both large and expanding herds are calving heifers at a younger age. Expansion and herd size is resulting in few production differences, although the larger herds had higher milk protein percentage.

**Acknowledgements** The Irish Cattle Breeding Federation for providing access to the herd recording database.

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## Nutrient composition and *in vitro* degradability of some tropical shrubs from Pakistan

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**Introduction** The range livestock production in Pakistan mostly depends on post harvest grazing or grazing on marginal or salty or waterlogged lands that are not suitable for traditional crops. Due to regional variations in the quality of grazing lands the forage availability for animal production is not always predictable. Unstable fodder availability with nutritional deficiencies can cause poor livestock production as animals have to rely mostly on poor quality feeds. Tree and shrub leaves can potentially alleviate some problems of feed shortages and nutritional deficiencies especially for range animals during fodder scarcity seasons. However, it would be essential to estimate the nutritive value of these leaves before their use for feeding animals. This study therefore evaluated different drought resistant and salt tolerant shrubs for their nutrient composition and *in vitro* dry matter (DM) degradability (IVD) alongside grass nuts (grass) as a good quality processed forage. The results of this study may help design future strategies for the use of these shrubs in ruminant diets to partly overcome the animal feed shortage in some tropical regions.

**Materials and methods** This 5x7 factorial study in triplicate compared the IVD of 5 plants including grass nuts as a control and 4 shrubs (Baker or *Adhatoda vasica*= AV; Sanatha or *Dodonaea viscosa*=DV; Australian acacia or *Acacia ampliceps*= AA; and saltbush or *Atriplex lentiformis*= AL) at 7 incubation hours (0, 6, 12, 24, 48, 72, 96). A sample of grass nuts that has already been tested in this laboratory was used as a standard control whereas the shrub leaves were collected, dried and transported to the UK. These samples were re-dried and ground through 1mm sieve before determining in triplicate their nutrient and total phenolic (TP) contents. Rumen fluid was obtained from 2 fistulated sheep prior to their morning feeding, strained through a cheese cloth into pre-warmed flasks under CO<sub>2</sub> and mixed with a pre-warmed buffer at 1:4 ratio to prepare the inoculum which was kept at 39°C until used. About 0.4g of each sample was weighed into a marked test tube to which 40 ml of the inoculum were added under CO<sub>2</sub>. The tubes were sealed with rubber stoppers and incubated at 39°C for the pre-determined times before placing these tubes in ice to stop fermentation. The residues were collected after centrifuging the tubes at 10,000 rpm for 10 minutes, washed with distilled water and dried at 60°C to determine IVD. The data were analyzed by using General Linear Model of SAS® to compare the differences between these plants for their nutrients and IVD for each incubation time at P<0.05. The effect of time or the plant x time interaction was not tested in this analysis. However, the Tukey's posthoc test was used to compare the treatment means for each time at P<0.05.

**Results** Table 1 shows significant differences between these plants for most nutrients and IVD at each incubation time (P<0.05). *A vasica* contained significantly more CP but less NDF and ADL than other plants including grass nuts ((P<0.05). The patterns of change in IVD of shrubs with increasing times were comparable to that of grass nuts as a control. IVD increased significantly (P<0.001) with the increased incubation time (P<0.001). However, the extent of difference between mean IVD of shrubs depended upon the shrub type and the incubation time (P<0.001). In fact, IVD of AV and AL were closer to grass nuts but greater than those of DV and AA at most incubations times. *D viscosa* contained highest TP but lowest CP and IVD than other shrubs (P<0.001).

**Table 1** Mean nutrient composition and IVD for different incubation hours of tropical shrubs and grass nuts

Items	Nutrient composition (g/kg or g/kg DM)							<i>In vitro</i> degradability at hours (g/kg)						
	DM	OM	CP	EE	NDF	ADL	TP	0	6	12	24	48	72	96
<i>A vasica</i>	918	840	285	13	231	109	22	258	306	350	393	482	512	539
<i>D viscosa</i>	922	942	83	17	311	119	73	210	239	259	287	312	339	348
<i>A ampliceps</i>	937	874	155	14	574	320	10	199	228	250	289	326	349	391
<i>A lentiformis</i>	955	780	106	9	445	134	7	229	263	291	301	398	450	534
Grass nuts	940	921	168	29	606	ND	ND	190	240	287	306	414	473	535
SEM	1.6	13.6	8	5.6	3.9	13.1	8	7	8	10	12	18	20	24
Significance	*	***	***	NS	***	***	**	***	***	***	***	***	***	***

ND=not determined; NS= non significant; \*, \*\* and \*\*\* represent significance at P<0.05, P<0.01 and P<0.001 respectively

**Conclusions** Although these shrubs showed variable nutrients and IVD in comparison with the grass nuts, they appeared to have the potential for their use in formulating ruminant diets during the feed shortage seasons of different regions of Pakistan. Further studies will look at the suitability of different amounts of these shrubs as potential supplements for forage consuming livestock particularly in tropical countries where animal production is restricted by the feed shortages.

**Acknowledgments** Thanks to Pakistan Higher Education Commission for funding and Mehedi Khan, Helio Lima Neto and M Safdar Anjum for their help during the laboratory analysis



# Effect of incubation time on chemical compositions and *in vitro* digestibility of treated extracted gambir leaf waste (*Uncaria gambir roxb*) with mix *Rhizopus sp* and *Aspergillus niger* as animal feed

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**Introduction** In Indonesia, the agricultural by-products are considered as stable source of ruminant feeds and now a days interest in their effective utilization is increasing all over the world due to economical factors and pollution. Shortage in animal feeds has been found to have a negative impact on the development of animal production. Non traditional feed sources such as extracted gambir leaf waste (*Uncaria Gambir Roxb*) are poor in nutrients such as protein content and vitamins and they are rich in fibers with low digestibility, or palatability and high lignin contents. The degree of lignifications is relatively more important in controlling hydrolysis rate in animal digestive tract (Fan *et al.*, 1981). Therefore, biological treatment is used for increasing the nutritional value of gambir leaf extracted waste, because microbial conversion of these wastes can improve their nutritional value and transforming them into animal feed with high quality (Villas-Boas *et al.*, 2002). Many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feed values by biological treatments (Abo-Eid *et al.*, 2007). The main objectives of this study were to evaluate the effect of biological treatments of extracted gambir leaf waste with two fungal (F) strains (*Rhizopus sp* and *Aspergillus niger*) for 5 to 20 days of incubation on chemical composition, and cell wall constituents as well as on the *in vitro* dry and organic matter digestibility.

**Material and method** The objective of this study was to evaluate the effect of biological treatment with three fungal strains for extracted gambir leaf waste on chemical composition, cell wall constituents and *in-vitro* digestibility. The extracted gambir leaf waste was chopped (approximate 1-3 cm) and each one was divided into 6 times of incubations treatment. The tested fungi were obtained from the extracted gambir leaf waste decay. The strains obtained were *Rhizopus sp* and *Aspergillus niger*. The strains were maintained on potato dextrose agar medium (PDA), grown at 24 - 28°C for 48 - 72 hrs, and then stored at 4°C. The medium used for the maintenance of the fungi consists of coconut water and 2.5 gram sugarcane /L. The pH value was adjusted to 5.6 before autoclaving at 121°C for 20 minutes. Precultures of the mix fungal strains were prepared by inoculating 1500 mL conical flasks containing 1000 ml coconut water (Gusmanizar, 2008) with mycelial discs of 7 days old culture. The inoculated flasks were incubated at 28°C for 7 days. The mycelia of growing fungi were diluted 10 times and used to inoculate the extracted gambir leaf waste at 10% (V/W). Two hundred and fifty grams of extracted gambir leaf waste were packed in plastic bags for each and then inoculated with the above prepared inoculums (moisture content 60 - 65%). Data were analyzed according to Statistical Analysis System user's Guide (SAS, 1998) for one way analysis of variance. Separations among means were carried out by using Duncan's (1955) multiple range test.

**Results** Table 1 illustrates the effects of time of incubation on the chemical composition and *in vitro* digestibility of treated extracted gambir leaf waste. Except the OM CF, all nutrients and *in vitro* digestibility were significantly ( $P < 0.01$ ) affected by time of incubations.

**Table 1** Time of incubation effect on chemical composition and digestibility of treated extracted gambir leaf waste (%).

Time of incubation (days)	OM	CF	CP	CP Degradation	ADF	NDF	ADF Degradation	NDF Degradation
5	95.38 <sup>a</sup>	30.53 <sup>a</sup>	13.02 <sup>b</sup>	45.72 <sup>b</sup>	29.89 <sup>b</sup>	57.20 <sup>c</sup>	12.15 <sup>a</sup>	11.59 <sup>a</sup>
10	95.29 <sup>a</sup>	31.37 <sup>a</sup>	14.52 <sup>ab</sup>	48.25 <sup>c</sup>	31.82 <sup>b</sup>	59.65 <sup>c</sup>	17.68 <sup>b</sup>	19.94 <sup>b</sup>
15	94.88 <sup>a</sup>	31.55 <sup>a</sup>	14.87 <sup>a</sup>	46.03 <sup>b</sup>	34.10 <sup>b</sup>	60.85 <sup>b</sup>	10.35 <sup>a</sup>	11.76 <sup>a</sup>
20	94.82 <sup>a</sup>	30.49 <sup>a</sup>	14.68 <sup>ab</sup>	43.00 <sup>a</sup>	36.77 <sup>a</sup>	63.44 <sup>a</sup>	13.66 <sup>a</sup>	13.21 <sup>a</sup>
SE	0.25	0.3	0.5	2.1	2.3	1.9	2.2	2.1

These results show that times of incubation influence the crude protein, ADF and NDF, and their digestion *in vitro*.

**Conclusions** Biological treatment of extracted gambir leaf waste with the mix *Rhizopus sp* and *Aspergillus niger*,) can improve chemical compositions and nutritive values at 10 days of incubation.

**Acknowledgements** The authors gratefully acknowledge funding from Dirjen Pendidikan Tinggi Indonesia

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## Development of an intra-ruminal nylon bag technique for feed evaluation which does not require the use of fistulated animals

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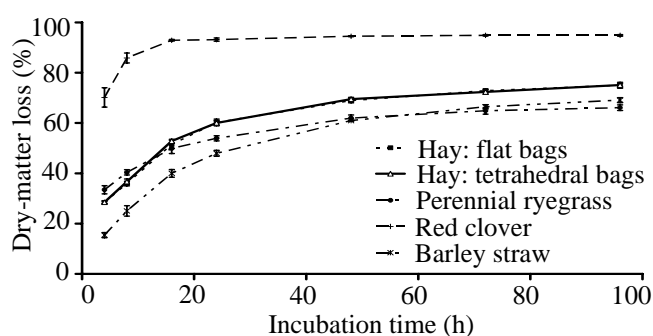
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**Introduction** For many years the nylon bag method for measuring ruminal degradation of forages and concentrates (Ørskov *et al.*, 1980) has been a widely used and reliable tool for evaluating ruminant feeds. In its current form the technique necessitates the use of surgically-prepared animals, but in many countries there is mounting pressure to discontinue their use. The purpose of this work was to develop a nylon bag method using intact sheep, by oral dosing at differing times, bags containing test feeds. The bags would be removed from the rumen following slaughter of the animals.

**Materials and methods** Three sequential experiments were carried out using individually-housed intact cast ewes fed dried grass pellets and grass hay, to evaluate orally-dosed nylon bags containing about 1g dried forage (ground to pass through a 2mm sieve). The bags were smaller than those of the conventional method, but the ratio of bag area to forage sample weight was maintained. They were constructed from the conventional fabric (Dacron, 40µm mesh size) with heat-welded seams. Two designs were tested, either flat or tetrahedral shapes. The tops of the bags were closed by machine stitching and were wrapped in tissue paper to facilitate oral dosing. Sheep received two flat and two tetrahedral bags on each of seven occasions (96h, 72h, 48h, 24h, 16h, 8h and 4h) prior to slaughter by sodium pentobarbitone injection; the fore stomachs were opened to allow removal of the bags. Recovered bags were washed in cold water and dried at 60°C before removing the stitching and weighing. In Trial 1, bags containing grass hay and two stainless steel 5g weights, were dosed to two sheep. In Trial 2, three pairs of sheep each received bags containing grass hay and having different designs of anti-regurgitation device, which were constructed from nylon cable ties and stitched to the tops of the bags. They were folded for dosing, being retained by the tissue paper wrapping, and designed to open out in the rumen. The designs of anti-regurgitation devices tested were: (i) 'z'-shaped; (ii) double 'z'-shaped; (iii) umbrella-shaped. In Trial 3, the degradation of three different forages (freeze-dried red clover, freeze-dried perennial ryegrass and barley straw) were examined using six sheep, with each sheep receiving two of the forages in separate pairs of bags, which were all fitted with 'z'-shaped devices.

**Results** In Trial 1, few intact bags were recovered from the rumen, with most of the bags having been regurgitated. In Trial 2, the recovery of intact bags from the rumen was high for all anti-regurgitation designs, being 100%, 96% and 100%, respectively, for types (i), (ii) and (iii). Missing bags were apparently lost at the time of dosing and not due to regurgitation. Reproducible dry matter losses (% units) at each time point were obtained from all sheep and no differences were found between bag shapes (mean within-sheep differences between replicates  $\pm$  SE - flat bags:  $2.04 \pm 0.718$ ; tetrahedral bags:  $1.77 \pm 0.460$ ; between-sheep SEM over all time points - flat bags: 1.000; tetrahedral bags: 0.743. In Trial 3, only one bag was not recovered from five of the sheep, but six bags were lost from the sixth animal. Using the procedure of Ørskov and McDonald (1979) and assuming the different shaped bags to be replicates, degradation curves were obtained for the three forages tested in Trial 3 (five sheep), and for the grass hay used in Trial 2. The observed and fitted degradation characteristics (Figure 1 and Table 1, respectively) were in accordance with those to be expected from the conventional nylon bag method.



**Figure 1** Observed ruminal degradation of four dried forages in nylon bags orally dosed to intact sheep (mean  $\pm$  SE).

**Conclusions** These studies show that ruminal degradation of dried forages can be characterised using a modified nylon bag method with intact sheep. Further work is required to increase the reliability of the dosing method and to test its use with additional feeds.

**Acknowledgements** Funding for this work was received from the Macaulay Development Trust and the Stapledon Memorial Trust.

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**Table 1** Ruminal degradation constants for four dried forages using nylon bags orally dosed to intact sheep

Forage	Degradation constants (means $\pm$ S.E.) <sup>1</sup>				
	n <sup>2</sup>	WL <sup>3</sup>	a (%)	b (%)	c (/h)
Grass hay	12	27.4	15.1 $\pm$ 0.65	58.4 $\pm$ 0.88	0.057 $\pm$ 0.0016
Perennial ryegrass	4	29.4	26.3 $\pm$ 2.52	39.5 $\pm$ 0.88	0.054 $\pm$ 0.0042
Red clover	3	51.0	19.1 $\pm$ 9.42	76.1 $\pm$ 9.18	0.309 $\pm$ 0.0323
Barley straw	3	12.6	4.7 $\pm$ 1.27	64.2 $\pm$ 1.77	0.048 $\pm$ 0.0032

<sup>1</sup>Obtained from the fitted equation:  $p = a + b(1 - e^{-ct})$ , where  $p$  is the percent degradation at time,  $t$  (h)

<sup>2</sup>Number of sheep observations (seven time points each).

<sup>3</sup>Washing loss (%): bags in water at 39°C; washed in cold water

## The effect of dietary proportions of kale (*brassica oleracea*) and grass silage on rumen pH and volatile fatty acid concentrations in dry dairy cows

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**Introduction** Ruminant pH is a critical factor in the normal and stable function of the rumen. A reduction in ruminal pH below normal (< 5.6) can have significant impact on microbial activity, rumen function, and animal productivity and health (Nagaraja and Titgemeyer, 2007). Forage brassicas are an alternative forage source offered to dairy cows for *in-situ* grazing. However, there is a scarcity of literature examining the effect of feeding diets composed of high proportions of kale (*brassica oleracea*) or kale alone on rumen pH and VFA production in the rumen.

**Materials and methods** In 2006/2007, an experiment was conducted at Moorepark Dairy Production Research Centre, Co. Cork, Ireland, evaluating the effect of offering differing proportions of kale and perennial ryegrass (*Lolium perenne*) silage on rumen physiology. Four multiparous pregnant dry dairy (100 ± 12 days pre-partum; 600 ± 15 kg of liveweight (LW)) cows permanently fitted with ruminal cannulae were randomly assigned to four kale: grass silage dietary proportions (0:100, K0; 60:40, K60; 85:15, K85 and 100:0, K100) on a dry matter (DM) basis in a 4x4 Latin Square design with four 20 day periods. Cows were acclimatised for fifteen days, followed by a five day period of experimental sampling and measurement. Internal pH meters were installed to continuously monitor rumen pH while rumen liquor collection tubes were used to sample rumen liquor for volatile fatty acid analysis at 0, 4, 8 and 12 h after feeding on each sampling day. Cows were offered a total of 10 kg DM of forage daily at 08.00 h. Average intake and ruminal pH variables from each cow over five days of each period and from four times within each day were recorded. Analysis with mixed model procedures of SAS (SAS Institute, 2008) was used to model the correlation structure of the repeated measurements. Treatment, block effect, day and time of day were included in the model for the data as necessary structural terms and interactions were examined. An unstructured covariance model was used for the time of day (unequal spacing) and compound symmetry for the day. There was no evidence found to support more complex covariances for the effect of day.

**Results** The chemical and mineral composition of the kale and grass silage offered in this study are presented in Keogh *et al.* (2009). Cows on treatment K85 had a lower ( $P < 0.001$ ) ruminal pH compared to cows on treatments K100, K60 or K0 (Table 1). While a gradual decrease in rumen pH was observed over an eight hour period for K85 before recovery, offering K100 did not reduce rumen pH below 6.0 or induce acidosis (Figure 1). Increasing the dietary proportion of kale did not affect ( $P > 0.05$ ) acetate: propionate ratio production between treatments (Table 1). Cows offered K60 had increased ( $P < 0.05$ ) rumen VFA concentration relative to K0 while offering K85 and K100 had no affect on rumen VFA concentration (Table 1). Offering dry cows K100 relative to K0 reduced dry matter intake by 17.5% (7.32 vs. 8.87 kg DM day<sup>-1</sup>, respectively) which may be associated with the presence S-methyl-L-cysteine sulfoxide (Barry and Manley, 1985) which can cause haemolytic anaemia and depressed DM intake.

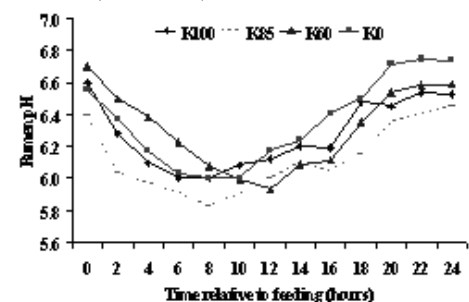
**Table 1** Effect of four pre-calving dietary proportions of kale: grass silage on dry matter intake (DMI kg<sup>-1</sup> day), Acetic: Propionic ratio (AC:PR; mmol L<sup>-1</sup>) and total volatile fatty acid (VFA) concentration (mmol L<sup>-1</sup>).

	K100	K85	K60	K0	s.e.	P
Kale DMI	-	6.35	4.90	-		
Grass silage DMI	-	1.71	3.28	-		
Total DMI	7.32 <sup>b</sup>	8.06 <sup>ab</sup>	8.18 <sup>ab</sup>	8.87 <sup>a</sup>	0.531	0.05
AC: PR	3.78	3.86	3.65	3.71	0.195	n.s.
Total VFA	59.35 <sup>ab</sup>	61.74 <sup>ab</sup>	71.67 <sup>a</sup>	53.45 <sup>b</sup>	4.887	0.05
Mean rumen pH	6.26 <sup>a</sup>	5.91 <sup>b</sup>	6.32 <sup>a</sup>	6.32 <sup>a</sup>	0.05	0.001

**Conclusions** The results suggest that increasing the dietary proportion of kale was associated with a progressive decrease in dry matter intake but with minimal effects on rumen pH and total volatile fatty acid concentration. The progressive decrease in dry matter intake may warrant further research on a larger scale, over a longer period of time while offered *in-situ* to elucidate potential effects on dry dairy cow performance.

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**Figure 1** The diurnal pattern of rumen pH in dry dairy cows offered on a DM basis four kale: grass silage dietary proportions; 100:0, K100 (●—●), 85:15, K85 (○---○), 60:40, K60 (▲—▲) and 0:100, K0 (■---■). Feeding time was at 0800 h.

## The effects of individual or blended essential oils on rumen gas production and ammonia accumulation *in vitro*

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**Introduction** The incorporation of essential oils into ruminant diets has the potential to beneficially modify rumen fermentation. In the literature, essential oils have been reported to alter several rumen parameters such as molar proportions of volatile fatty acids (VFA), methane production and amino acid degradation (reviewed by Calsamiglia *et al.* 2007). The majority of studies that have investigated the effects of essential oils have largely focused on inclusion levels of individual oils. Few studies have investigated the potential synergistic effects of blending different essential oils. The aim of this study was to analyse the effects of three essential oils, eugenol, limonene and terpinene and their respective blends on rumen fermentation *in vitro*.

**Materials and methods** A 70:30 grass hay (*Lolium perenne*) and concentrate basal diet milled through a 1 mm screen, was incubated in 125ml serum bottles. Incubation buffer (90 ml), prepared according to Theodorou *et al.* (1994), and 10 ml of strained rumen fluid were added to each bottle. The rumen fluid used was sourced from three sheep rumens obtained from a local abattoir. Essential oils were added to the bottles to achieve a dosage level of 500 mg/litre either as individual or blended (50:50) mixes of essential oils. The seven experimental treatments were as follows: control (CON, unsupplemented diet), eugenol (EUG, 100%), limonene (LIM, 100%), terpinene (TER, 100%), eugenol + limonene (EuLi, 50:50), limonene + terpinene (LiTe, 50:50), terpinene + eugenol (TeEu, 50:50). Four replicates of each treatment were incubated and gas production was measured at 3, 6, 12, 24, 48 and 72 hours using a pressure transducer (Mauricio, *et al.* 1999). Another set of extra bottles, treated as above, were incubated alongside and stopped after 12, 24, 48 and 72 hours and their contents analysed for ammonia nitrogen (NH<sub>3</sub>-N) and VFA. Treatment effects were analysed using analysis of variance with Genstat 11<sup>th</sup> Edition.

**Results** Gas production data are summarised in Table 1. After 3, 6, 12 and 24 hours of incubation all essential treatments significantly reduced gas production compared to the control ( $P<0.001$ ). Blending terpinene and eugenol (TeEu) consistently caused the greatest reduction in gas production compared to other treatments at all times ( $P<0.001$ ). Other essential oil blends (EuLi and LiTe) generally did not alter gas production compared to individual supplements of these essential oils. The effects of essential oils on NH<sub>3</sub>-N concentration are shown in Table 2. There was no difference in NH<sub>3</sub>-N after 12 hours of incubation across all treatments. In general, all essential oil treatments reduced NH<sub>3</sub>-N concentration at 24 and 48 hours compared to the control. LIM elicited the most significant reduction in NH<sub>3</sub>-N at 24 hours.

**Table 1** Effects of either individual or combinations (50:50) of essential oils on cumulative gas production (ml/g DM) *in vitro* (n=4)

Time (hr)	Experimental Treatments							sed	P-value
	CON	EUG	LIM	TER	EuLi	LiTe	TeEu		
3	31	19	21	23	18	23	16	1.4	$P<0.001$
6	60	39	40	41	35	44	33	2.8	$P<0.001$
12	98	72	66	72	64	72	57	5.1	$P<0.001$
24	138	118	83	91	90	88	82	4.7	$P<0.001$
48	178	174	116	116	117	109	104	7.2	$P<0.001$
72	193	193	136	134	132	126	114	8.0	$P<0.001$

**Table 2** Effects of either individual or combinations (50:50) of essential oils on NH<sub>3</sub>-N (mg/litre) concentration *in vitro*

Time (hr)	Experimental Treatments							sed	P-value
	CON	EUG	LIM	TER	EuLi	LiTe	TeEu		
12	242	256	258	259	250	253	262	5.7	NS
24	294	308	272	273	281	283	288	6.9	$P=0.001$
48	383	343	296	302	318	297	316	13.2	$P=0.002$
72	422	352	333	328	354	343	359	8.2	$P<0.001$

**Conclusion** This study demonstrates that essential oils have antimicrobial activities that depress both gas production and NH<sub>3</sub>-N concentration compared to unsupplemented controls. Although essential oil combinations reduced gas production they appear not to be as effective as individual unblended essential oils at reducing ammonia production *in vitro*.

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## Effect of phenotypic residual feed intake and diet type on ruminal microbial population in beef heifers

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**Introduction** In Ireland, methane (CH<sub>4</sub>) emissions from ruminants accounts for approximately 51% of greenhouse gas emissions from the agriculture sector (McGettigan *et al.*, 2008). Additionally, CH<sub>4</sub>, formed as a by-product of rumen microbial fermentation can account for up to 12% of dietary gross energy. Therefore reducing enteric CH<sub>4</sub> production may also improve feed efficiency. Despite its importance as a significant contributor to global warming, relatively little is known about the microbial population in the rumen. Selection for improved feed efficiency in beef cattle, measured as residual feed intake (RFI), has been shown to reduce total and feed intake corrected CH<sub>4</sub> emissions without compromising animal production (Hegarty *et al.*, 2007). However, little is known about the biological mechanisms controlling this effect. The ruminal microbial ecosystem is comprised of diverse symbiotic populations of anaerobic bacteria, archaea, ciliated protozoa and fungi, though there is a dearth of published information on how these microorganisms are influenced by either intrinsic or extrinsic factors. Quantification of these microbial populations and assessment of how they differ across different animals and diet types will increase our understanding of host-microbial interactions in ruminants.

**Materials and methods** Limousin x Friesian beef heifers (n = 86), initially selected on the basis of sire EBV for RFI, were ranked on the basis of phenotypic RFI, calculated over an 80-day period while consuming a 30:70 maize silage:concentrate TMR diet (on a DM basis). The seven highest (HRFI; least efficient) and seven lowest (LRFI; most efficient) ranking animals were selected for use in this study. Both groups had similar mean bodyweight and ADG at ranking but HRFI had, on average, 20% higher DMI. Following ranking on RFI all animals were allocated to a grass silage diet for six weeks (Period 1). Three months later all animals were again offered a 30:70 maize silage:concentrate TMR over a six week period (Period 2). Both silage and TMR diets were offered *ad libitum*. Ruminal fluid was sampled at the end of each period using a specialised trans-oesophageal sampling device. Total microbial DNA was isolated from the ruminal fluid using a repeated bead beating method. A qPCR SYBR Green assay was developed to quantify key microbial groups using PCR primers to target DNA sequences of total rumen bacteria, methanogens, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and protozoa. Abundance of these microbes was expressed as a proportion of total estimated rumen bacterial 16S rDNA according to the equation: relative quantification =  $2^{-(Ct_{\text{target}} - Ct_{\text{total bacteria}})}$ , where Ct represents threshold cycle. Data were analysed using mixed models ANOVA (PROC MIXED, SAS 2006). The final model included terms for RFI group, diet and their interaction, with differences between means statistically significant at  $P < 0.05$ .

**Results** No RFI phenotype x diet interactions for any of the microbial populations measured were observed. The effect of phenotypic RFI and diet on key microbial populations is shown in Table 1. There was no effect ( $P > 0.05$ ) of RFI phenotype on the quantity of any microbial species measured. However, dietary period affected ( $P < 0.05$ ) ruminal microbial populations manifested as a reduction in methanogen, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* numbers and an increase in protozoa numbers between periods 1 and 2.

**Table 1** Effect of phenotypic RFI and diet on ruminal microbial populations<sup>1,2</sup>

	RFI		SED	Diet		SED	Significance <sup>3</sup>		
	H	L		GS	TMR		RFI	Diet	RxD
Methanogens	4.04	4.84	0.985	6.15	2.74	0.985	NS	***	NS
<i>Ruminococcus flavefaciens</i> <sup>4</sup>	0.13	0.05	0.050	0.17	0.015	0.050	NS	***	NS
<i>Fibrobacter succinogenes</i> <sup>4</sup>	2.21	1.59	0.661	3.34	0.46	0.661	NS	***	NS
Protozoa	0.54	0.73	0.209	0.57	0.70	0.209	NS	*	NS

R=RFI; D=Diet. NS=Non-significant ( $P > 0.05$ ). \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . <sup>1</sup>Microbes measured as a proportion of total estimated rumen bacterial 16S rDNA, relative quantification =  $2^{-(Ct_{\text{target}} - Ct_{\text{total bacteria}})} \times 10^3$ . <sup>2</sup>Data corrected for DMI.

<sup>3</sup>Significance values for transformed data. Back transformed means presented for clarity. <sup>4</sup>Cellulolytic bacteria

**Conclusions** There was no clear evidence from the current study of differences in inherent ruminal microbial population between animals ranked as either efficient or inefficient for feed energy utilisation. However, it was clear that diet can influence the number of methanogenic and cellulolytic microbes with reductions observed in these when animals were offered the high starch, high energy diet. These results are consistent with lower ruminal CH<sub>4</sub> emissions recorded on this TMR diet (McDonnell *et al.*, 2009). Dennis *et al.*, 1983 reported increased protozoa numbers in cattle as the proportion of concentrate in the diet increased, as found in the present study. Further investigation is warranted to determine the effect of animal RFI phenotype and diet on the abundance of other important ruminal microorganisms to improve our understanding of feed efficiency and methanogenesis in cattle and how these processes might be influenced by diet type.

**Acknowledgements** The authors gratefully acknowledge funding provided under the National Development Plan, through the Research Stimulus Fund administered by the Department of Agriculture, Fisheries & Food, Ireland. RSF 05-224.

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## Effect of feeding level on dry matter degradation characteristics of canola meal and soybean meal

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**Introduction** In general, an increase in the level of feeding of a basal diet results in a decrease in digestion due to a shorter retention time of feed in the rumen. Many trials have examined the effect of feeding various hay to concentrate rations on disappearance values of concentrate feedstuffs but experiments on the effect of feeding level on digestibility of feedstuffs are limited and contradictory. Even though relationships between intake level and *in situ* degradation can be theorized, actual studies of such relationships are lacking (Vanzant *et al*, 1998). Our study was designed to examine the effect of feeding level (FL) on disappearance kinetics of dry matter (DM) of canola meal and soybean meal which are the most widely used protein sources in animal feeds.

**Materials and methods** Four Iranian *shal* wethers with a body weight of  $40 \pm 2$  kg (2 years old) were used in this study. Sheep were each fitted with a rumen fistula (4 cm internal diameter) and kept in individual stainless steel cages with external feed and water troughs. A diet was offered at four levels of feeding: 1 (maintenance), 1.5, 2 and 2.5 times of the maintenance requirements. The sheep were fed twice daily (08 and 14 h) a diet containing 600 g/kg concentrate and 400 g/kg forage. Animals were fed these diets 15 days before the experimental periods. Ruminal kinetic parameters were estimated using the exponential equation of Ørskov and McDonald (1979). Nylon bags (which were approximately  $6 \times 12$  cm,  $45 \mu\text{m}$ ) containing 5 g (2mm screen) of samples were incubated in the rumen for 0, 4, 8, 16, 24, 48 and 72 h. After incubation times the nylon bags removed and washed in tap water until the water remained clear. There were four replications per treatment (FL). The rate and extent of dry matter (DM) degradation were estimated according to the equation:  $P = a + b(1 - e^{-ct})$ . The experiment consisted of four periods. During the first period, the ruminal degradability of concentrate samples as measured at maintenance level of intake in all four animals, and during the second, third and fourth periods, respectively, the ruminal degradability calculated at 1.5, 2 and 2.5 times of maintenance. Data were analyzed using the general linear models procedure of SAS (1996). When a significant difference was found, means were separated using Duncan test. Differences were considered to be significant if  $P < 0.01$ .

**Result** table 1 shows that feeding level had a significant effect on *in situ* DM degradation characteristics. The increase of feeding level induced a decrease in the value of a, b and effective degradability (ED) ( $p < 0.01$ ). Both the fraction b and ED were significantly ( $p < 0.01$ ) higher for the FL=1 than for other treatments.

**Table 1** Influence of feeding level on ruminal kinetic of DM of canola meal and soybean meal

Intake <sup>1</sup>	a	b	c	PD(%)	ED (%)
Canola meal					
1	35.0 <sup>a</sup>	47.8 <sup>a</sup>	0.044 <sup>c</sup>	82.9 <sup>a</sup>	68.0 <sup>a</sup>
1.5	36.8 <sup>a</sup>	45.1 <sup>b</sup>	0.041 <sup>d</sup>	81.9 <sup>a</sup>	67.2 <sup>b</sup>
2	33.3 <sup>b</sup>	43.3 <sup>c</sup>	0.064 <sup>b</sup>	76.6 <sup>b</sup>	66.3 <sup>c</sup>
2.5	32.5 <sup>b</sup>	39.8 <sup>d</sup>	0.070 <sup>a</sup>	72.3 <sup>c</sup>	63.5 <sup>d</sup>
Significance	**	**	**	**	**
SEM	0.45	0.72	0.003	1.03	0.43
Soybean meal					
1	35.9 <sup>a</sup>	61.5 <sup>b</sup>	0.034 <sup>c</sup>	97.5 <sup>a</sup>	74.6 <sup>b</sup>
1.5	30.9 <sup>b</sup>	64.5 <sup>a</sup>	0.044 <sup>a</sup>	95.4 <sup>b</sup>	75.3 <sup>a</sup>
2	35.8 <sup>a</sup>	53.1 <sup>c</sup>	0.037 <sup>b</sup>	88.9 <sup>c</sup>	70.4 <sup>c</sup>
2.5	31.2 <sup>b</sup>	52.2 <sup>d</sup>	0.045 <sup>a</sup>	83.4 <sup>d</sup>	67.4 <sup>d</sup>
Significance	**	**	**	**	**
SEM	0.29	0.27	0.0004	0.24	0.22

1: Multiple of maintenance requirement

a: soluble fraction (%); b: insoluble but potentially degradable fraction (%); c: fractional rate of degradation (h<sup>-1</sup>); PD: Potential of degradation; ED: Effective degradability

**Conclusion** As expected, an increased FL resulted in a decreased potential of degradation. We concluded that the b fraction of canola meal and soybean meal decreases with increasing FL. The results of this experiment also indicate an important effect of FL on other degradability parameters “a” and “c”. Therefore, altering energy intake plays a major role in digestibility of forages, so feeding level should be considered in feed evaluation systems and feed formulation.

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## ***In situ* dry matter digestion of different fodder tree leaves in Pakistan**

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**Introduction** An insufficient supply and quality of nutrients is a major hindrance to ruminant production in Pakistan. The feed balance sheet reveals that available feed resources provide < two third of the total requirements of crude protein and total digestible nutrients respectively for ruminants. The area under fodder production is continuously reducing indicating high pressure on land for cash crop production. There was a dire need to explore new feed resources such as fodder tree leaves which may supplement the existing feed resources for ruminants and can help to bridge the gap between the demand and supply of nutrients. Tree leaves can be a rich source of supplementary protein, vitamins and minerals to enhance the microbial digestion of ruminant feeds (Singh *et al.*, 1989). The present study was designed with the objective that fodder tree leaves can help to bridge the gap between the demand and supply of nutrients for ruminants. As there was little information about the nutritive value of fodder tree leaves, the present study determined the nutritional values of selected fodder tree leaves for ruminants by using the *in situ* digestion method.

**Materials and methods** Leaves of *Morus alba*, *Acacia nilotica*, *Ziziphus jujube*, *Szegium cumunii*, *Melia azedarach*, *Leucaena leucocephala*, *Albizia procera* and *Magnifera indica* were collected from two different areas of Pakistan. Composite samples of leaves were prepared and a mature ruminal cannulated buffalo bull (350 kg) was used to study *in situ* digestion kinetics of tree leaves. The bull was fed a blend of berseem fodder and a concentrate along with tree leaves to meet its nutritional requirements. The experiment lasted for 35 days with initial 10 days for adjustment and the next 25 days for the data collection. Nylon bags measuring 10×23 cm with an average pore size of 50 µm were used to determine digestibility, rate of disappearance (Rate), lag time and extent of digestion (Extent) of DM. The bags containing leave samples were placed in the rumen in a reverse sequence for 0, 3, 6, 12, 24, 36, 48 and 72 h of incubations. All bags were removed at the same time to reduce variation associated with the washing procedure. For each time point, there were 3 bags where two bags were used for determining the digestion kinetics and one bag served as a blank. After removal from the rumen, the bags were washed in running tap water until the rinse was clear. These bags were dried in an oven at 60°C for 48 h and weighed. The residues from these bags were collected to estimate the disappearance of DM (DMD) at each time. The DMD values were then used to estimate Lag time, Rate, Digestibility (DMD at 48 h) and Extent (DMD at 72 h) of digestion. The data were analyzed by using analysis of variance in a completely randomized design and means were compared by using the least significant difference test using SPSS software package.

**Result** Dry matter digestion kinetics are presented in Table 1. The DM digestibility at 48 h was highest (90.2%) for *Morus alba* and lowest (54.8%) for *Szegium cumunii*. The lag time was shortest for *Acacia nilotica* and greatest for *Albizia procera*. The differences in lag times indicated that forages differed in their rates of hydration (Mertens, 1973). The rate of disappearance was highest for *Acacia nilotica* and lowest for *Szegium cumunii* (Table 1). Extent of digestion at 72 h of incubation was lowest for *Magnifera indica* and highest for *Morus alba*. Following the entry of plant material into the rumen, microbial colonization may have varied due to the inherent variations in the cell wall composition of these tree leaves.

**Table 1** *In situ* dry matter digestion kinetics of fodder tree leaves

Name of tree	Digestibility(g/kg)	Lag time (h)	Rate (%h)	Extent (g/kg)
<i>Morus alba</i>	902 <sup>a</sup>	0.76 <sup>c</sup>	5.43 <sup>e</sup>	983 <sup>a</sup>
<i>Acacia nilotica</i>	656 <sup>c</sup>	0.63 <sup>d</sup>	6.38 <sup>a</sup>	664 <sup>c</sup>
<i>Melia azedarach</i>	784 <sup>b</sup>	0.66 <sup>de</sup>	5.71 <sup>d</sup>	802 <sup>b</sup>
<i>Albizia procera</i>	578 <sup>d</sup>	0.94 <sup>a</sup>	4.86 <sup>f</sup>	775 <sup>b</sup>
<i>Magnifera indica</i>	558 <sup>d</sup>	0.69 <sup>e</sup>	5.97 <sup>b</sup>	572 <sup>e</sup>
<i>Szegium cumunii</i>	548 <sup>d</sup>	0.82 <sup>b</sup>	5.34 <sup>e</sup>	604 <sup>d</sup>
<i>Leucaena leucocephala</i>	564 <sup>d</sup>	0.73 <sup>c</sup>	5.77 <sup>d</sup>	582 <sup>e</sup>
<i>Ziziphus jujube</i>	652 <sup>e</sup>	0.71 <sup>c</sup>	5.82 <sup>cd</sup>	679 <sup>c</sup>
Standard error	24.8	0.02	0.09	27.2

Means within a column with the same superscripts are not statistically significant (p<0.05).

**Conclusion** The DM digestion kinetics of fodder tree leaves indicated that they can be fed to ruminants. Additionally, *Morus alba* is considered the best among the tree leaves evaluated due to its high DM digestibility and it should therefore be able to meet the maintenance requirements of forage consuming ruminants.

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## Comparison of ruminal degradability models using the number of runs of sign of residuals

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**Introduction** Various models are used to describe the degradation of feeds in the rumen. The use of a particular model in fitting a degradability data set and estimating the degradability parameters implies the model goodness-of-fit has been examined holistically, otherwise the validity of estimated parameters may be controvertible. There are different statistical tests for ranking and evaluating models and sometimes results from these different tests seem contradictory, so an overall assessment is needed in this situation. In this study, the goodness-of-fit of three models were evaluated using a single test, the number of runs of sign of residuals, when fitting whole soybean ruminal degradability data.

**Materials and methods** DM and CP degradability data of two Iranian soybean cultivars (Sahar and Williams) as raw, roasted and steep-roasted (6 feeds in total), which was recorded at fixed incubation periods (1, 2, 3, 4, 8, 16, 24, 36 and 48 h) for each feed and yielded a total of 6 disappearance curves, were used in this study. The evaluated models were: a segmented model with three spline-lines delimited by two nodes or break points, constraining splines 1 and 3 to be horizontal asymptotes, and follows zero-order degradation kinetics (model I); a simple negative exponential curve with first order kinetics and assuming a constant fractional rate of degradation (model II); and a rational function or inverse polynomial which assumes a variable fractional rate of degradation that declines with time (model III). The models were fitted to the DM and CP ruminal disappearance data by nonlinear regression using the PROC NLIN of the SAS (SAS, 1999) to estimate ruminal degradation parameters. The number of runs of sign of the residuals was calculated as Motulsky and Ransnas (1987). A run is a sequence of residuals with the same sign (positive or negative). For this test, the average residual of replicate observations was used for each incubation period.

**Results** All models could be fitted to the data using PROC NLIN of SAS, as convergence to a solution occurred in all cases and the degradability parameters could be estimated. The number of runs of sign of residuals was different (Table 1) for the three models. Model III gave a high percentage of curves with three or fewer runs (for both DM and CP components) indicating the residuals were not randomly distributed over the incubation times and this model was not as good as the other two for fitting these particular data.

**Table 1** Percentage of curves (both DM and CP) for each number of runs of sign of the residuals observed when fitting each model

Number of runs of sign	Model I		Model II		Model III	
	DM	CP	DM	CP	DM	CP
≤ 3	0.0	0.0	0.0	0.0	66.7	66.7
4	0.0	0.0	0.0	0.0	16.7	33.3
5	16.7	16.7	16.7	33.3	16.6	0.0
6	66.7	16.7	16.7	16.7	0.0	0.0
≥ 7	16.6	66.6	66.6	50.0	0.0	0.0

**Conclusion** The results of this study showed that all three models could to describe the degradability data without systematically over- or underestimating any section of the DM and CP degradability curves and the number of runs of sign of residuals test could be a useful statistical test as other statistical criteria (R-square, Bayesian information criteria and lack-of-fit test, data are not shown) for assessing and ranking models.

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## Effect of body weight at weaning on piglet feeding behaviour immediately post weaning and selection for two levels of dietary lysine

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**Introduction** Feeding behaviour and performance of individual piglets is highly variable immediately post weaning influenced by many factors such as litter size and weaning weight with some studies concluding that heavier piglets take longer to establish eating than smaller piglets (Brunninx *et al.* 2001). The variation in piglet performance post weaning is of significant importance to the pig industry. Lawlor *et al.* (2003) stated that the benefit from a choice of weaner foods could be the reduction in pen variation as the individual requirements of each piglet would be more effectively catered for. Indeed, Lawlor *et al.* (2003) observed that larger piglets selected a less nutrient dense diet when provided with a choice. Food choice experiments have shown that pigs are capable of selecting a diet which is appropriate to their nutritional needs (Morgan *et al.* 2003). This experiment aimed to test the hypothesis that body weight at weaning has an effect on the feeding behaviour of piglets immediately post weaning and the food selection for two different levels of lysine.

**Materials and methods** 136 piglets (JSR Healthbred) were weaned at  $26.9 \pm 0.92$  (s.e.m.) days of age into 34 flat deck pens (4 pigs per pen). Piglets were assigned at weaning to four weight categories: small (6 kg:  $5.9 \text{ kg} \pm 0.48$ ), medium (8 kg:  $8.1 \text{ kg} \pm 0.36$ ), large (10 kg:  $9.9 \text{ kg} \pm 0.25$ ) or mixed weight (control (1 small, 2 medium and 1 large piglets):  $8.1 \text{ kg} \pm 1.48$ ). Pen groups were balanced for sex and litter origin. Piglet feeding behaviour was constantly recorded by a multi-spaced feed recording system (Leeds University Feeding Behaviour System (LUFBS)) in each pen. Piglets were identified by LUFBS using an individual transponder ear tag. Each pen of four piglets was offered *ad-libitum* access to feed (16.45 MJ DE) in two troughs per pen. The control group was offered a control food in both troughs (1.55 g lysine/kg). The small, medium and large groups were offered a choice of two foods, one food in each of two troughs per pen. The two foods differed in their lysine level: Low Lysine (LL: 8 g lysine/kg) and High Lysine (HL: 18 g lysine/kg). Piglets were weighed at weaning and at d7 and d14. The experiment ran for 14 days. Preference for one food was defined as being significantly different from 50% of total feed intake. All data were analysed using the GLM procedures of SPSS 16.

**Results** Table 1 shows the performance, feed intake and HL intake and proportion during the experimental period. There was no difference between any of the performance measures of the four weight groups in either week. Large, medium or small piglets did not choose different levels of dietary lysine. Mixed piglets with no choice had the same performance as sized piglets with a choice. Large piglets had the same latency to start eating as all other weight groups post weaning, although smaller piglets were faster to initiate feeding than both the medium and the mixed groups ( $P < 0.05$ ). Figure 1 shows the weekly food choice selection by all piglets. There was no difference between the intake of either of the two foods and 50% of total intake concluding no preference for either of the foods overall.

**Table 1** Individual ADG, ADFI, FI and HL FI data for large (L), medium (M), small (S) and mixed (X) weight treatments.

(n=34)	L	s.e.m.	M	s.e.m.	S	s.e.m.	X	s.e.m.	P
Week 1									
ADFI (g/d)	203	21.7	217	23.6	190	20.7	169	21.7	NS
ADG (g/d)	177	26.7	160	29.0	169	25.5	134	26.7	NS
FCR	1.31	0.266	1.73	0.289	1.18	0.254	0.92	0.266	NS
FI (kg)	5.81	0.578	5.87	0.622	5.26	0.604	4.70	0.630	NS
FI HL (kg)	2.64	0.324	2.78	0.350	2.27	0.339	*	*	NS
FI HL (%)	39	(08-77) <sup>+</sup>	38	(06-78)	43	(09-81)	*	*	NS
Week 2									
ADFI (g/d)	456	28.0	444	30.3	379	26.6	375	27.9	NS
ADG (g/d)	382	23.3	38.1	25.3	315	22.2	343	23.3	NS
FCR	1.26	0.085	1.21	0.093	1.23	0.082	1.11	0.085	NS
FI (kg)	11.53	0.725	11.23	0.781	9.40	0.758	9.29	0.791	NS
FI HL (kg)	5.26	0.689	5.84	0.745	4.64	0.723	*	*	NS
FI HL (%)	34	(24-48)	46	(34-52)	46	(35-57)	*	*	NS

Proportional means are reported with a 95% confidence interval rather than s.e.m.

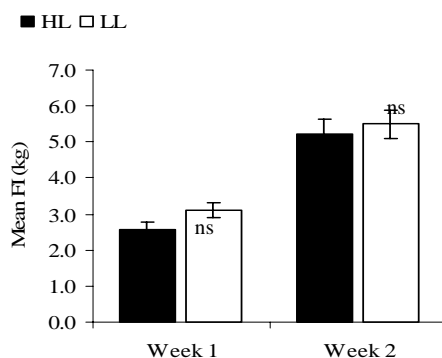
**Conclusion** Latency to first feed was influenced by body weight at weaning, with smaller piglets eating sooner than medium and mixed piglets, supporting our hypothesis that body weight would influence post weaning feeding behaviour. However, the latency of larger piglets was not different to the other weight categories. Additionally, the growth performance of each weight group did not differ and there was no difference in the feed intake or the proportion of each of the two feeds consumed in either week giving no indication of selection for a dietary requirement. There also appeared to be no disadvantage to single feed over a choice, with control pigs performing the same as choice fed pigs. Piglet variation in the immediate post weaning period warrants further research.

**Acknowledgements** The authors would like to thank the Yorkshire Agricultural Society for funding this work.

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**Figure 1** Weekly pen feed intake per food (kg).

## The effect of glycerol on nutrient digestibility in finishing pigs

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**Introduction** McCann *et al* (2009) reported that glycerol inclusion lowered the growth rate of pigs and that there was a tendency for feed conversion to become less efficient as glycerol inclusion increased. These findings suggested that the digestible energy (DE) content of glycerol is lower than that from a starch source such as wheat. There has been very little work conducted on the nutritive value of glycerol for pigs and the aim of this trial was therefore to investigate the effect of increasing glycerol inclusion on nutrient utilisation in finishing pigs.

**Materials and methods** Four experimental diets were offered to 24 Landrace x Large White boars (45kg) housed in individual metabolism crates for 14 days (7 days pre-feed and 7 days total collection of urine and faeces). The experimental diets contained 0, 40, 80 and 120g/kg crude glycerol and were formulated to be isoenergetic and isonitrogenous (15.8MJ/kg dry matter (DM) DE and 10.8g/kg DM lysine). The crude glycerol contained 850g/kg glycerol and DE content was assumed to be 15.8MJ/kg (fresh basis). The basal diet contained (g/kg) wheat 437, barley 300, soyabean meal 227, vegetable oil 13.6, limestone 9, dicalcium phosphate 3, salt 4.1, lysine 0.8 and minerals and vitamins 5. Glycerol replaced wheat in the diet formulation. Diets and faeces were analysed for DM, crude protein (CP), lipid (oil procedure B) energy and neutral detergent fibre (NDF) to determine nutrient digestibility and dietary DE content.

**Results** There was a significant quadratic effect of glycerol inclusion on DM, CP and energy digestibility and on dietary DE content (Table 1). Glycerol inclusion at 40 and 80g/kg improved DM, CP and energy digestibility and DE content relative to the control diet but there was no difference in nutrient utilisation of the diets containing 0 and 120g/kg glycerol. According to quadratic regression statistics for DE content, the optimum inclusion rate of glycerol was 74g/kg. Actual dietary DE content of the control diet was lower than the formulated DE content (15.4 vs. 15.8MJ/kg DM) and DE contents of the diets containing 40 and 80g/kg glycerol were higher than the control diet.

**Table 1** The effect of glycerol on nutrient utilisation

	0g/kg	40g/kg	80g/kg	120g/kg	SEM	P	P=LIN	P=QUAD
DM digestibility	0.852 <sup>a</sup>	0.871 <sup>b</sup>	0.868 <sup>b</sup>	0.855 <sup>a</sup>	0.0038	<0.01	NS	<0.001
CP digestibility	0.839 <sup>ab</sup>	0.859 <sup>b</sup>	0.861 <sup>b</sup>	0.826 <sup>a</sup>	0.0077	<0.05	NS	<0.01
Energy digestibility	0.850 <sup>a</sup>	0.868 <sup>b</sup>	0.867 <sup>b</sup>	0.855 <sup>ab</sup>	0.0042	<0.05	NS	<0.01
Lipid digestibility	0.719	0.725	0.724	0.713	0.0093	NS	NS	NS
DE (MJ/kg DM)	15.44 <sup>a</sup>	15.83 <sup>b</sup>	15.85 <sup>b</sup>	15.73 <sup>ab</sup>	0.076	<0.01	NS	<0.01

**Conclusions** The results indicate that glycerol inclusion at 40 or 80g/kg improves nutrient utilisation but inclusion at 120g/kg does not. This quadratic effect may be because of saturation of glycerol kinase by glycerol at the higher inclusion level, thus preventing glycerol being metabolised and representing an energy cost to excrete the excess glycerol (Doppenberg and van der Aar 2007). This theory is supported by the findings on pig growth reported by McCann *et al* (2009) who found that glycerol inclusion at 120g/kg had a negative effect on liveweight gain. The lower than expected DE content for the control diet indicates that the DE content for the basal ingredients were overestimated in the formulation matrix and the higher than expected DE content for the diets containing glycerol suggests that the assumed value for glycerol DE content was underestimated. The improvement in DE content over the control diet, at lower levels of glycerol inclusion, is in line with effects observed in broiler studies (Griffiths and McCann 2009) and it can be concluded that glycerol may be a useful source of energy for finishing pigs. The maximum inclusion rate of glycerol to optimise dietary DE content was found to be 74g/kg.

**Acknowledgments** This work was supported by the Department of Agriculture and Rural Development for Northern Ireland, John Thompson and Son Ltd. and Devenish Nutrition Ltd.

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## The effect of feeding genetically modified Bt maize (MON810) for 30 days on weanling pig growth performance organ weights and organ histopathology

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**Introduction** Genetically modified (GM) crops are now being used more frequently as animal and human food, with maize currently the second most cultivated transgenic crop (James, 2008). An ongoing debate exists as to whether transgenic plants have the same nutritional value as their conventional counterparts and as to the safety of GM organisms for human consumption (Aumaitre *et al.*, 2002). The objective of the present study was to investigate the effect of feeding the insect resistant MON810 maize for 30 days on weanling pig growth performance, organ weights and organ histopathology.

**Materials and methods** Thirty two crossbred (Large White × Landrace) weaned pigs (entire males) were used in a 31 day experiment to investigate the effects of feeding GM maize (Bt maize – Pioneer, MON810) on pig growth performance, organ weight and organ histopathology. The pigs were weaned at 28 days of age, blocked by weight and ancestry and randomly assigned to one of two treatments. The treatments were: GM maize (Pioneer PR34N44 event MON810) or the non-GM isogenic parent line (Pioneer PR34N43) at 38.8% of the diet. Following weaning the pigs were given a baseline adjustment period of 6 days, during which they were provided with *ad-libitum* access to a non-medicated non-GM starter diet. The pigs were individually penned in a total of four identical climate controlled rooms with 8 pigs per room (16 pigs/treatment). Each treatment group was equally represented in each room to avoid a room effect. Individual body weight and feed disappearance were recorded on d 0, 7, 14, 21, 28 and 31 of the study where d 0 was the first day on which experimental diets were fed. Feed was available until weighing and any waste or uneaten feed left in the feeders was dried and recorded. The pigs were slaughtered on day 31 and internal organs (heart, kidneys, spleen and liver), devoid of all blood clots and/or fat deposits, were removed from the carcass, weighed and sampled for histopathological analysis. Statistical analysis was performed using the GLM procedure of SAS and organ weights were analysed with final body weight included as a covariate in the statistical model. For all response criteria, the individual pig was the experimental unit.

**Results** Overall, pigs fed the GM maize diet tended to have higher ( $P=0.11$ ) daily gain, significantly higher feed intake ( $P<0.05$ ) and tended to be heavier ( $P=0.11$ ) than pigs fed the non-GM maize diet (**Table 1**). The weight of the heart, liver and spleen did not differ between treatments ( $P>0.05$ ). However, pigs fed GM maize had heavier kidneys than control pigs ( $P=0.05$ ; **Table 1**).

**Table 1** The effect of feeding Bt Maize (MON810) on weanling pig growth performance and organ weights

	Non-GM Isogenic Maize	GM Maize	SE	P-value
Final body weight (kg)	24.7	26.0	0.56	0.11
Daily gain (g/d)	576	620	18.2	0.11
Feed intake (g/d)	697	770	22.9	0.03
Feed conversion efficiency (g/g)	1.22	1.24	0.015	0.28
Kidneys weight(g)	145.2	161.0	4.52	0.05
Spleen weight (g)	47.5	54.3	2.71	0.14
Liver weight (g)	690.0	665.3	17.98	0.38
Heart weight (g)	133.3	142.2	3.96	0.18

**Table 2** Chemical analysis of the experimental diets

Diet	Dry matter (g/kg)	Crude Protein (g/kg)	Oil (g/kg)	Crude Fibre (g/kg)	Ash (g/kg)	DE (MJ/kg) <sup>†</sup>
Non GM maize	894	209	61	21	55	15.50
GM Maize	892	211	59	19	56	15.46

<sup>†</sup> Digestible energy (DE) was calculated using equation 22 of Noblet & Perez (1993)

**Conclusions** The difference in feed intake between treatments could not be explained by differences in chemical analysis of the diets (Table 2) or by mycotoxin or pesticide residue in the maize as these were found to be below minimum detectable levels. The difference in kidney weights found may indicate an adverse effect on kidney function, however, no indicators of kidney damage were found following histopathological examination. The difference in kidney weights found here are contrary to findings of a subsequent longer term feeding experiment conducted at our centre, which have shown no treatment effect on kidney weight.

**Acknowledgements** The authors gratefully acknowledge funding from the EU Commission 7<sup>th</sup> Framework Programme

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## An investigation into the effect of milk supplementation from birth on performance in naturally suckled piglets fed no creep feed

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**Introduction** With increasing sow prolificacy, it is now not uncommon for batches of sows to farrow litters of 13+ with the expectation that 11+ piglets are weaned. Shunt fostering and artificial rearing are traditional techniques for managing large litter sizes, however they come with inherent problems of utilisation of labour and resources particularly in batch management systems. The sow's ability to produce a sufficient quantity of milk to sustain these litters in late lactation and the competition at the udder for teat space in early lactation has led to a renewed interest in systems where all piglets have access to milk replacer ad-lib from birth.

**Materials and methods** A total of 120 litters over 5 batches were used. The 3 treatments were supplementary milk being available from birth (M0) or day 7 (M7) or no supplementary milk (NM). There was no creep feed available on any treatment prior to weaning at 24.8 days (sd1.66), and pigs were monitored to 29 days post weaning. The milk replacer was a blend of whey protein powder, vegetable oil, minerals and amino acid supplements (CP 22%, Oil 14%, Lysine 2%). It was mixed daily (150g/litre) and unused milk weighed back. The multiparous sows were allocated randomly to the treatments and were balanced by parity, breed and room. Dam breed types were PIC (Cam 23 Dx(LWxLR)) and JSR (Gene Packer 90 LWxLR). Lactation management followed the standard procedures. Fostering was undertaken within treatment and within 24 hours post farrowing. Imbalances in birth litter size were eliminated at fostering such that the overall treatments were not compromised by pregnancy variations. Housing was standard part slatted farrowing crates and fully slatted flat deck accommodation. The sows were fed a 17.5% CP ration on a standard feeding scale as determined by litter size. Postweaning 4 diets were fed in a commercial regime. Data was subjected to ANOVA using Genstat software (version 12).

**Results** The average number of pigs born alive was 12.24 (sd 3.14) and there were no significant treatment differences in the number that died between birth and fostering. Table one shows how piglet losses after week 1 were almost eliminated resulting in significantly higher numbers weaned in the milk treatments. Milk consumption was 0.5 l/d until day 14 and rose to 2 l/d/litter at weaning. Total consumption was 3.6kg of milk powder /litter. There were no significant treatment differences in post weaning performance. There was an indication that sow condition was improved in the milk treatments, however, there was no observable effect on subsequent conception rates.

**Table 1** The effect of supplementing piglets with milk during lactation on performance

	No Milk	Milk from farrowing	Milk from day 7	Sed (max)	sig
Number of litters (n)	38	40	42		
Number post fostering	11.36	11.39	11.47	0.254	0.897
Total Litter weight post fostering	16.97	16.72	16.29	0.6143	0.529
Average weight post fostering (kg)	1.50	1.47	1.43	0.055	0.447
Number died post - week 1	0.62	0.59	0.78	0.212	0.590
Number died/removed Week 1-Weaning	0.673 <sup>a</sup>	0.096 <sup>b</sup>	0.355 <sup>b</sup>	0.156	<b>0.002</b>
Number weaned	10.05 <sup>a</sup>	10.71 <sup>b</sup>	10.33 <sup>ab</sup>	0.244	<b>0.029</b>
Total litter weight at weaning	78.82	79.11	79.45	2.925	0.976
Mortality Fostering –Weaning %	10.69	5.84	9.58	2.091	<b>0.054</b>
DLWG Wean – 29 days post wean (g/d)	376	359	364	17.9	0.702
Sow weight loss inc. pregnancy (kg)	30.1 <sup>a</sup>	23.4 <sup>b</sup>	27.3 <sup>ab</sup>	2.659	<b>0.045</b>
P2 Loss (mm)	5.02	3.59	4.94	0.714	<b>0.081</b>
Parity analysis number weaned	Sow (n)	(15)	(15)	(12)	
	Gilt (n)	(23)	(25)	(30)	
				0.4202	<b>0.029</b>

Means with different superscripts (a,b) are significant  $P < 0.05$

**Conclusions** Providing milk ad-lib from farrowing increased numbers weaned by 0.65 pigs per litter. The main reduction in mortality is seen where high numbers born fade through poor nutritional support through lactation. Introduction of milk at day seven is less effective at delivering the benefits. Overall post weaning performance has not been shown to improve using supplementary milk. There is an indication that the milk treatment lightens the demand on the dam. The beneficial effects of using the milk line are greater in sows compared to gilts. Performance variance within litter has not been shown to be affected by milk treatment.

**Acknowledgements** We gratefully acknowledge BPEX as the main funders of this project and Volac International Ltd. for the supply of the milk supplement.

## Using high quality forages to improve *in vitro* rumen degradability and fermentation of low quality forages

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**Introduction** Low quality forages (LQ), like cereal straws, are the main basal feeds for ruminants in developing countries. However, animal production is low when ruminants are reared on nutrient deficient LQ as the main diet. To get more production from ruminants it is necessary to enhance the utilization of these LQ which could be achieved by using small amounts of high quality forages (HQ) as supplements with LQ. This study compared the effect of incubating *in vitro* different amounts of various HQ with LQ on the DM degradability (IVD) and fermentation (e.g. ammonia) profiles of rice straw (Straw) and grass hay (Hay) as LQ.

**Materials and methods** A 3x4x2 factorial arrangement in duplicate was used to assess the effect of 3 supplements (rye grass=RG, silage=Si and rape seed plant=RP) each at four levels of 0, 250, 500 and 750 mg/g DM on IVD and ammonia (AL) of 2 LQ (Straw and Hay) during 96h of *in vitro* incubations. Rumen fluid (RF) was obtained from 2 fistulated sheep just before feeding, strained through a cheese cloth, and then it was mixed with a pre-warmed buffer at 1:4 ratio to prepare inoculum. The incubations of forages were conducted in 50-ml polypropylene tubes containing 0.4g of ground LQ to which relevant HQ were added according to the experimental design but by enclosing these HQ individually in small polyester bags (45µ). These bags permitted the mixing of solubles but not the HQ with LQ and so helped observe the IVD of LQ without their direct mixing with HQ. About 40 ml of the inoculum was added under CO<sub>2</sub> to each tube which was sealed with a rubber stopper containing pressure release valve and incubated at 39°C for the pre-determined times. After incubation the tubes were submerged in ice to stop fermentation. The liquids and residues were separated by centrifuging the tubes at 3000 rpm for 10 minutes. The residues were washed with distilled water and dried at 60°C for 48h to determine IVD. The supernatants were acidified with 1N HCl and analysed for AL by using a colorimeter at 660 nm. The IVD and AL data were analyzed by using the General Linear Model of Minitab to study the main effects of LQ, HQ and HQ level=S and their interactions at P<0.05 for the IVD and AL.

**Results** Main effects of LQ, HQ and S were significant (P<0.003) for IVD and AL. However, due to some significant (P<0.05) interactions between these variables, the mean IVD and AL for each treatment combination are shown in Table 1. IVD was higher for Hay than Straw with all HQ. Mean IVD of LQ was higher with RG than other HQ where IVD increased with RG and Si but reduced with RP for Straw but not for Hay, hence LQ x HQ interaction (P<0.002). While IVD of both LQ did not increase with the increase in HQ after 250 mg, AL continued to increase with increase in HQ from 0 to 500mg. In fact AL was significantly (P<0.001) higher for Hay than Straw and it was highest with 500 mg RP than other HQ.

**Table 1** Effects of different level of HQ on the mean IVD and AL for LQ after 96h of incubation

LQ	HQ	IVD (g/kg)				AL (mg/L)			
		Amount of HQ (S) (mg/g) LQ				Amount of HQ (S) (mg/g) LQ			
		0	250	500	750	0	250	500	750
Straw	RG	607	666	656	652	92	165	187	188
	RP	604	583	567	563	96	175	209	205
	Si	608	633	625	562	93	135	155	168
Hay	RG	740	765	756	675	124	167	210	212
	RP	732	738	721	740	120	173	225	220
	Si	725	808	753	761	128	163	181	182
Pooled SEM		11.1				5.97			
P <for LQ, HQ, S		LQ=0.001;HQ=0.002; S=0.003; LQxHQ=0.002; LQxS=0.8; HQxS=0.09; LQxHQxS=0.02				LQ=0.001;HQ=0.001;S=0.001;LQxHQ=0.3; LQxS=0.07; HQxS=0.001; LQxHQxS=0.5			

**Conclusions** The effect of HQ on IVD and AL varied with the type and level of HQ and the LQ during *in vitro* incubation for 96h. While RP showed negative effect on IVD of LQ, other HQ showed maximum IVD at 250 mg. Possible antinutritional factors in RP might have affected the microbial activity which resulted in reduced IVD of LQ. Conversely, more crude protein might have increased AL when HQ were used at higher levels RG and Si can be used as supplements to increase the utilization of LQ but their amounts needs to be optimised in association with the target LQ

**Acknowledgements** Mr. Khan is grateful to receive UK's ORS award and funding from the Perry Foundation and Sylhet Agriculture University, Bangladesh.

## An evaluation of the effect of grass silage and concentrate feed level on ewe and subsequent progeny performance and on potential concentrate sparing effect

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**Introduction** Nutrition in late pregnancy influences lamb weight at birth and at weaning. Each 1 kg increase in lamb birth weight increases weaning weight by 3.2 kg to 3.4 kg (Keady and Hanrahan, 2009a,b). Previous studies (Keady and Hanrahan, 2009c, d) have shown that increasing silage feed value increases the weight of ewes at lambing and of their lambs at weaning. Keady and Hanrahan (2009c) reported that, when offered to pregnant ewes, high feed-value silage (FVS) supplemented with 5 kg concentrate produced lambs of similar birth and weaning weights as ewes offered medium FVS supplemented with either 15 or 25 kg concentrate. Furthermore Keady and Hanrahan (2009c) reported that when concentrate level was increased from 15 to 25 kg/ewe in late pregnancy silage feed value did not affect lamb birth or weaning weights. The aim of the present study was to quantify the production responses of prolific ewes when offered diets containing either medium or high FVS supplemented with a range of concentrates in late pregnancy. The effect of silage feed value on potential concentrate sparing effects was also calculated.

**Materials and methods** High (H) and medium (M) FVS's were ensiled precision chopped on 12 May and 14 June respectively treated with a bacterial inoculant, following a 24 h wilting period. The H silage was supplemented with either 5, 15 or 25 kg concentrate whilst the M silage was supplemented with either 15, 25, 35 or 45 kg concentrate during late pregnancy. The 7 treatments were offered to 112 ewes (Belclare x S. Blackface, Charmoise x S. Blackface) balanced for breed and age, and were allocated at random. The ewes were housed on slatted pens in groups of 5 and offered the silages *ad libitum* from 8 December until lambing in early March. Ewes rearing singles and twins were grazed at pasture and received no concentrate supplementation. Ewes rearing triplets received 0.5 kg concentrate daily for 5 weeks post lambing and their lambs had access to 300 g concentrate/head daily until weaning at 14 weeks of age. The data were analysed using Proc GLM for ewe traits, and Proc MIXED for lamb traits with ewe as a random term. Orthogonal contrasts were used to partition the effect of concentrate level into linear and quadratic components within each silage type.

**Results** The mean dry matter (DM) and metabolisable energy concentrations and DM digestibility for the H and M FVS's were 257 and 248 g/kg, 11.4 and 10.5 MJ/kg DM and 740 and 660 g/kg, respectively. The effects of silage feed value and concentrate level on animal performance are presented in Table 1. Increasing concentrate feed level with the medium FVS linearly increased ewe condition and weight post lambing and lamb birth weight. Increasing concentrate level with the high FVS tended ( $P=0.06$ ) to increase ewe condition post lambing. Ewes offered the high FVS had significantly higher condition and weight post lambing. Concentrate level offered with the high FVS did not alter ewe live weight post lambing or lamb birth weight. Whilst there was no significant interaction between silage feed value and concentrate level at the 15 and 25 kg feed levels, regression analysis, using individual animal data, suggested different relationships for lamb birth weight and concentrate level for each silage feed value as follows:

High FVS: Lamb birth weight (kg) =  $4.74 + 0.015$  (s.e. 0.0136) x

Medium FVS: Lamb birth weight (kg) =  $4.54 + 0.019$  (s.e. 0.0088) x ( $P < 0.05$ ,  $R^2 = 0.07$ )

where x = concentrate feed level in late pregnancy (kg). Consequently ewes offered the high FVS supplemented with 5, 15 and 25 kg concentrate produced lambs of similar birthweight as the medium FVS supplemented with 14, 22 and 30 kg respectively.

**Table 1** Effect of grass silage feed value on concentrate feed level on animal performance

		High feed values silage (H)			Medium feed value silage (M)				se	Contrasts		
Conc (kg)		5	15	25	15	25	35	45		H v M	Conc level linear	
Post lambing	-condition	3.43	3.65	3.80	3.10	3.30	3.52	3.80	0.139	***	P=0.06	***
	-weight	68.8	69.3	72.0	65.3	66.3	66.7	72.3	1.95	*	NS	*
(kg)												
Lambweight (kg)-birth		4.8	5.0	5.1	4.7	5.2	5.4	5.3	0.21	NS	NS	*
	-weaning	30.7	31.3	32.2	31.0	31.6	30.2	33.0	0.85	NS	NS	NS
Gain-birth-weaning (g/d)		267	272	281	271	275	258	288	7.8	NS	NS	NS

**Conclusions** Increasing silage feed value and concentrate feed level increased ewe condition at lambing. In terms of lamb birth weight the concentrate sparing effect of the high FVS supplemented either 5, 15 and 25 kg concentrate was 9, 7 and 5 respectively.

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## Using different levels of sorghum in finishing Ghezel×Arkhar-Merino crossbred lambs diets and its effects on animal performance

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**Introduction** In most of the semi-arid regions of the world such as Iran, animal feed production is difficult and farmers have to use some alternative feedstuffs such as sorghum to reduce feeding costs. In many cases a problem in utilization of these alternative feeds is the presence of anti-nutritional factors such as condensed tannins found in them (Kumar & Vaithianathan, 1990). Dietary tannin can restrict intake (Terrill *et al.*, 1989) and reduce overall weight gains by livestock (Turner *et al.*, 2005). The aim of the present study was to investigate the effects of replacing dietary barley with different levels of sorghum on lamb performances.

**Materials and methods** Sixteen Ghezel×Arkhar-merino crossbred male lambs with live weights ranging from 34 to 55 kg ( $46\pm5.8$ ) were used in the experiment. Experimental animals were kept at Research Farm of Tabriz University. Each pen was including four animals that were randomly assigned to one of the four dietary treatments (Table 1) in a completely randomized design (CRD) assignment. Animals were adapted for 3 weeks before starting main experiment. Each pen was provided with a food trough and a water container, and food was offered two times daily. During the 60-d feeding trial, body weight was recorded on individual animals at the 20 d interval and used to adjust feed intake. Live-weight gain was calculated from 12 h fasted weights taken at the start and end of each weighting. Total tannin was measured by method of Makkar (2000). Experimental treatments didn't balance for the initial weight at the beginning of the study, so we used initial weight of lambs as a covariate. Feed intake and weight gain data were analyzed using the general linear model of the Statistical Analysis Systems (SAS, 1999).

**Table 1** Experimental rations compositions

Treatment	A	B	C	D
Alfalfa hay	20	20	20	20
Barley grain	80	20	10	0
Sorghum grain	0	60	70	80
Crude protein (%)	12.27	12.33	12.34	12.35
Total tannin from sorghum (%)	0	0.588	0.686	0.784

**Result** The results of experiment indicated that there was significant difference in final weight between treatments A and B, but when we used initial weight as covariate, didn't find any significant effect of dietary treatments on dry matter intake, average daily gain and feed conversion ratio (Table 2).

**Table 2** Effect of dietary treatments on dry matter intake, weight gain and FCR

Treatment	A	B	C	D
Dry matter intake(kg/day)	$3.1\pm0.2$	$3.4\pm0.3$	$3.3\pm0.3$	$2.8\pm0.2$
Final weight(kg)	$58.1\pm1.4^a$	$62.7\pm1.1^b$	$59.7\pm1.1^{ab}$	$58.4\pm1.4^{ab}$
Total weight gain (kg)	$11.1\pm1.1$	$15.4\pm1.3$	$14.2\pm1.4$	$12.5\pm1$
Average daily gain(kg)	$0.19\pm0.02$	$0.24\pm0.02$	$0.22\pm0.02$	$0.2\pm0.02$
Feed conversion ratio	$13.3\pm1.9$	$11.8\pm1.6$	$13.4\pm1.5$	$14.1\pm2$

Mean values with different superscripts (a and b) within a row differ significantly ( $P < 0.05$ ).

**Conclusion** The results of present study showed that using of different levels of sorghum in finishing lamb's diets can not affect their performances. May be the low number of experimental units affected the accuracy of the results so it is necessary to use more animals in each treatment.

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## The effect of soaking and urea treatments on the voluntary intake of wheat straw by sheep

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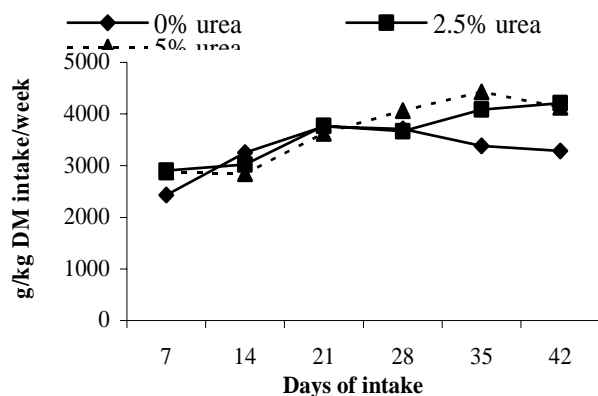
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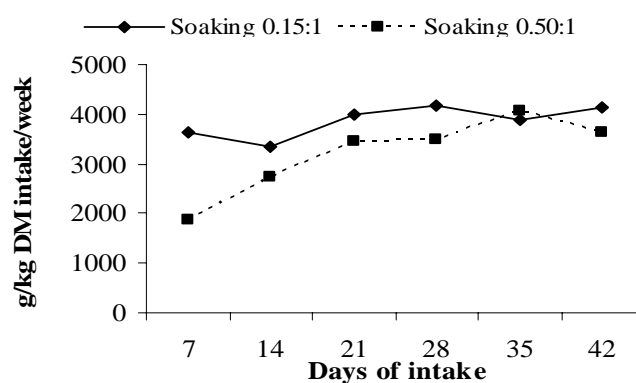
**Introduction** It is recognised that advanced maturity in forage like cereal straws was associated with high contents of detergent fibres, lignin and detergent insoluble N which can reduce the utilisation of these straws by ruminants. While urea treatments have been known to improve the chemical composition and hence utilisation of straws for ruminants, the extent of such improvements depended upon the amount of urea, method of its application and the type of a straw. This study tested the effect of treating wheat straw with different amounts of water (soaking) alone and with different urea levels on voluntary intake in wether sheep.

**Materials and methods** Nine bales of approximately 300 kg of wheat straw were chopped (10-15cm), and distributed manually into 18 polyester silo bags that were individually placed inside the galvanized mesh rings. A replicate 2 x 3 factorial design was used to apply different urea and water levels to prepare three treated straw bags as replicates per treatment as follows. Water representing 2 soaking ratios (15:1 and 0.50:1) and 3 urea solutions (0, 2.5 and 5%) were sprayed onto these straws in bags which were compressed to exclude air, sealed and left outdoors for ten weeks. An animal trial was conducted to compare the voluntary intake of these 6 straws by using 6 similar groups with 6 individual housed wethers per group over 6 weeks. The wethers were offered ad-libitum the above mentioned straws after their mixing in small bathes daily with 3% molasses for the first two weeks as an adaptation period, and then for another 6 weeks to observe the straw intake by these wethers. The wethers were also fed 200g of a concentrate plus 20g of a vitamin-mineral premix per head per day to meet the daily protein and energy requirements of a 40kg adult wether (AFRC, 1993). Straw samples and their refusals and spillages were collected daily and stored in labelled plastic bags at -20°C for each week. These samples were thawed, pooled for each treatment per week, dried, ground and analysed to determine their chemical composition and also to estimate the weekly DM intake of relevant straw per wether. Weekly live-weight (LW) of each wether was also recorded, but these are not reported in this paper. The data were statistically analysed by using the Analysis of Variance in Minitab soft ware to compare the effect of soaking, urea and soaking x urea on the weekly DM intake of these wethers at  $P < 0.05$ .

**Results** Only the main effect of urea and soaking treatments on the mean weekly DM intake per weather are shown in figure 1 and 2 respectively. The graphs give an immediate impression of these treatments.



**Figure 1** Effect of urea levels on weekly DM intake



**Figure 2** Effect of soaking on weekly DM intake

The DM intake of urea treated wheat straw was significantly increased with the increase in the urea level from 0 to 2.5 or 5% during the first 7 days and last 14 days of this trial ( $P < 0.05$ ). However, the extent of this increase in straw intake with urea treatments was less than expected and it was not proportionate to the increase in urea from 2.5 to 5%. The DM intake of straws with low soaking ratio (0.15:1) was considerably higher than the high soaking ratio straw (0.50:1) during the first 28 days ( $P < 0.05$ ) but not in the last 14 days of the trial ( $P > 0.05$ ). This change in the pattern of straw intake in response to soaking was due to the release of perhaps more soluble nutrients during the rumen fermentation of straw treated at the low soaking ratio. These soluble could have helped accelerate rumen fermentation and the production of volatile fatty acids which might have sent signals to the satiety centre to switch off animal's desire of eating according to the lipostatic theory of feed intake regulation.

**Conclusion** It appeared that the urea and soaking treatments can improve DM intake of wheat straw by sheep. However the extent of increase in DM intake depended upon the level of urea and soaking treatments which need to be optimised in the future.

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## Effect of soaking and urea treatments on *in sacco* degradability of wheat straw in sheep

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**Introduction** Several efforts have been made in the past to improve the nutritive value of straw by chemical and biological means (Sundstøl and Owen, 1984). Also numerous work was performed to evaluate the effect of these treatments on the degradability of cereal straws by using the nylon bag technique (Mehrez and Ørskov, 1977). Therefore, the objective of this study was to investigate the effect of using water for soaking of wheat straw alongside different amounts of urea on the *in sacco* organic matter degradability (OMD) of treated wheat straws following their rumen incubations in fistulated sheep at various times.

**Materials and methods** Nine bales of approximately 300 kg of wheat straw were chopped (10-15cm), and distributed manually into 18 polyester silo bags which were individually placed inside the galvanized mesh rings. A replicated 2x3 factorial design by involving 2 water: straw soaking ratios (0.15:1 and 0.50:1) and 3 urea amounts (0, 2.5 and 5%) were used to prepare 3 treated straw bags per treatment. The relevant amounts of water and urea representing these soaking ratios and urea treatments were sprayed onto these straws in bags which were compressed to exclude air, sealed and left outdoors for ten weeks. At opening the silos, sensory tests and chemical composition were determined for all treated and untreated straws. Representative samples of these straws were dried and ground through 2mm sieve for their use in *in sacco* studies using fistulated sheep consuming fixed amounts of grass hay and concentrate at 67:33 ratio. A split-plot *in sacco* study using two fistulated sheep (blocks), eight incubation times (0, 6, 18, 24, 48, 72, 96 and 120h), six straw treatments was completed to determine degradability of these straws in duplicate. At the end of each incubation time the bags were removed from the rumen and washed with cold water until the water was colourless. The bags were dried in an oven at 60°C for 24h and the un-degraded residues weighed to estimate dry matter degradability (DMD) of each straw. The un-degraded residues were weighed to estimate dry matter degradability of each straw. The un-degraded residues were ashed at 600°C to estimate organic matter degradability (OMD). Only the OMD data were statistically analysed for this paper to compare the effects of soaking, urea and soaking x urea interaction at  $P < 0.05$ .

**Results** Only the main effects of the urea and soaking treatments on the mean OMD for each incubation time are shown in figure 1 and 2 respectively. Urea treatment at either 2.5 or 5% improved the *in sacco* OMD when compared with the control (0% urea) at all incubation hours ( $P < 0.001$ ). The higher soaking ratio showed lower OMD than the low soaking ratio at most incubation hours ( $P < 0.01$ ). Sensory test did not show any visible mould growth for urea and soaked straws during the 70 days of conservation.

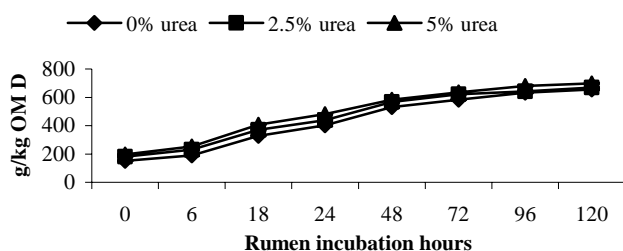


Figure 1 Effect of urea levels on OMD

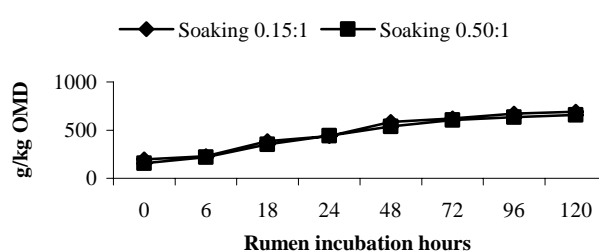


Figure 2 Effect of soaking OMD

**Conclusion:** The use of different levels of urea increased OMD at various incubation times, whereas, soaking at 0.50:1 ratio gave lower OMD than the low soaking ratio at most incubation hours. It appears that low levels of soaking may be more beneficial in improving the effect of urea to enhance the nutritive value of cereal straws for ruminant. Therefore, it would help if the correct amount of water as a readily available 'resource' was used when applying urea to treat cereal straws to enhance their nutritional quality for ruminant animals.

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## Comparison of the performance and carbon footprint of dairy-origin beef systems

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**Introduction** One of the key aims of a profitable beef rearing and finishing enterprise is to make best possible use of available resources and to adopt rearing/finishing enterprises which are efficient in terms of carcass gain per unit input costs over the lifetime of the animal. However, with increased emphasis on carbon footprint, it is important that these beef production systems are also efficient in terms of carbon emissions per kg output. On this basis a study was undertaken to evaluate the lifetime performance of a range of dairy-origin beef systems and to determine the carbon footprint of each of the systems.

**Materials and methods** Two hundred and twelve spring-born calves (Holstein, Angus, Limousin and Belgian Blue) were sourced from dairy farms from Northern Ireland at 4 weeks of age and allocated to one of four lifetime rearing/finishing regimes at weaning as follows: (1) Intensive bulls - bulls offered *ad libitum* concentrates (total concentrate input 2.2 tonnes (t) DM) (2) Forage/concentrate-based bulls - bulls offered grazed grass in their first summer followed by grass silage plus concentrates (50:50 ratio on a dry matter (DM) basis) (total concentrate input 1.5 t DM) (3) Medium concentrate input steers - steers offered grazed grass during the summer and grass silage-based diets during the winter with total concentrate input of 1.3 t DM (4) Low concentrate input steers - as for (3) but total concentrate input 0.6 t DM. Bulls were slaughtered at 550 kg live weight and steers at 650 kg live weight. Animal performance (live weight gain and dry matter intake) was monitored throughout the life of the animal. At slaughter cold carcass weight, carcass conformation and fat classification and fat depth measurements were taken. Data were analysed using REML Variance Component Analysis with year, farm of origin, breed, start age and start weight used as covariates. Carbon footprint of each of the systems was calculated using mean data for each of the systems. Methane emissions from enteric fermentation were estimated using the methane prediction equation developed by Yan *et al* (2009); methane emissions associated with manure storage and nitrous oxide emissions were estimated using IPCC (2006) Tier 1 emission factors; emissions associated with concentrate and inorganic fertiliser manufacture were taken from Lovett *et al* (2006) and Edwards-Jones *et al* (2009) respectively; a carbon sequestration rate of 1.16 tonnes CO<sub>2</sub>e/ha/year was used (Natural England 2008).

**Results** Increasing the proportion of forage in the ration of finishing bulls reduced lifetime liveweight gain by 12%, increased slaughter age by 42 days and reduced carcass weight by 11 kg ( $P<0.001$ ). Reducing total concentrate input in steers reduced lifetime liveweight gain 7% ( $P<0.01$ ), increased slaughter age by 33 days and reduced carcass weight by 12 kg ( $P<0.001$ ). The carbon footprint of forage/concentrate-based bulls was similar to intensive bulls. However, the carbon footprint of bulls was 52% of that of steers. Reducing concentrate inputs in steer-based systems reduced carbon footprint by 7%.

**Table 1** Performance and carbon footprint of four dairy-origin beef rearing and finishing systems.

	Intensive bull system	Forage/concentrate-based bull system	Medium concentrate input steer system	Low concentrate input steer system	s.e.d.	Sig
Lifetime live weight gain (kg/day)	1.22 <sup>d</sup>	1.07 <sup>c</sup>	0.82 <sup>b</sup>	0.76 <sup>a</sup>	0.021	***
Age at slaughter (months)	15.0 <sup>a</sup>	16.4 <sup>b</sup>	25.1 <sup>c</sup>	26.2 <sup>d</sup>	0.30	***
Carcass weight	309 <sup>b</sup>	298 <sup>a</sup>	340 <sup>d</sup>	328 <sup>c</sup>	3.1	***
Conformation†	2.7 <sup>b</sup>	2.7 <sup>b</sup>	2.2 <sup>a</sup>	2.1 <sup>a</sup>	0.09	***
CO <sub>2</sub> e (kg/head)	2061.4	1986.1	4023.0	3733.9		
CO <sub>2</sub> e (kg/carcass weight)	6.7	6.7	11.8	11.4		

† Conformation based on EUROP classification where E=5 and P=1

**Conclusions** Increasing the proportion of forage in the diet of dairy-origin bulls has only a marginal effect on carbon footprint. Bull-based systems of beef production have superior performance relative to steer-based systems and have a lower carbon footprint.

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## An examination of the performance, nutrient digestibility, enteric methane emissions and rumen fermentation characteristics of beef cattle fed either barley or ground ear maize based diets

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**Introduction** Ground ear maize (GEM) is a novel feedstuff produced by ensiling the chopped maize ear and offers a higher quality feedstuff compared to conventional forage maize. A high grain yield and good grain maturity is required for the successful production of GEM. Preliminary work by O'Hanlon *et al.* (2008) showed that animals offered a GEM based diet had higher dry matter intakes compared with animals offered a barley based diet. The current study aimed to build on this data and evaluate the effect of GEM on performance, nutrient digestibility and enteric methane emissions of finishing beef heifers.

**Materials and methods** A commercial crop of maize (var. Benecia) was harvested for GEM (consisting of the cobs, husks and very limited stalk) on the 29<sup>th</sup> October 2008 using an Olimac stripper maize header. The GEM was then ensiled in round wrapped plastic bales by an Orkel baler. Thirty beef heifers (Limousin x Friesian) were randomly allocated to one of two treatments (1) GEM-based diet (2) barley grain-based diet, both of which were isonitrogenous (13.0 %CP), isofibrous (26.8% NDF) and isoenergetic (18 MJ GE/kg DM). The heifers were offered *ad libitum* access to feed and individual daily intake was recorded using an electronic feeding system (Insentec, Marknesse, The Netherlands). Both diets were offered as a TMR which included soybean meal to balance for protein and grass silage in the GEM TMR and chopped straw in the barley TMR to balance as a source of fibre. Daily feed intake was recorded for each animal for a period of 52 days. Daily methane (CH<sub>4</sub>) emissions were determined using a calibrated tracer (SF<sub>6</sub>) technique as described by Johnson *et al.* (1994) and modified by Hart *et al.* (unpublished). Methane emissions were recorded over a 24 h period for 5 consecutive days starting on day 23. Faecal grab samples were taken from the animals on days 23 and 24 in order to determine nutrient digestibility using the acid insoluble ash method. All data were analysed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) in accordance with the randomised block design employed.

Results Heifers offered the GEM based diet had higher DMI ( $P<0.01$ ) and ADG ( $P<0.05$ ) and a higher digestibility of dry matter, organic matter, nitrogen, starch and gross energy compared to those offered the barley based diet (Table 1). In addition heifers offered the GEM had lower overall daily and DMI corrected CH<sub>4</sub> emissions ( $P<0.01$ ) compared to those offered the barley based diet.

**Table 1** Effect of dietary treatment on animal performance, nutrient digestibility and CH<sub>4</sub> emissions.

	GEM	Barley	SEM	P-value
DMI (kg/day)	11.8	10.5	0.2892	0.006
ADG (kg/day)	1.4	1.2	0.0697	0.051
FCR (kg/kg)	7.7	8.1	0.4478	0.562
Digestibility Coefficient				
Dry matter	0.731	0.658	0.0096	0.0001
Organic matter	0.749	0.673	0.0094	0.0001
Ash	0.387	0.354	0.0123	0.0730
Nitrogen	0.661	0.561	0.0131	0.0001
Neutral detergent fibre	0.552	0.518	0.0162	0.1438
Acid detergent fibre	0.400	0.337	0.0242	0.0738
Starch	0.990	0.964	0.0034	0.0001
Gross energy	0.720	0.597	0.0103	0.0001
Methane emissions				
CH <sub>4</sub> g/day	199	238	12.3	0.039
CH <sub>4</sub> g/kg of total DMI	18.1	22.9	1.09	0.005

**Conclusion** Heifers offered a GEM based diet had higher DMI, gained 200g more liveweight per day and emitted 21% less DMI corrected methane compared to those offered the barley diet.

**Acknowledgements** The authors gratefully acknowledge funding from the UCD Seed Funding Scheme.

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## Performance of an on-farm anaerobic digester using dairy cow slurry as the sole feedstock

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**Introduction** Anaerobic digestion (AD) is employed widely across the world to produce a source of renewable energy as biogas (mainly methane). However, there has been only limited uptake of AD in the UK, particularly at farm level. The aim of this study was to assess the performance of on-farm AD, using fresh dairy cow slurry as the sole feedstock.

**Materials and methods** An anaerobic digester at AFBI-Hillsborough was used to study the performance of AD using farm based organic materials as feedstock. The digester was a 660m<sup>3</sup> insulated above ground tank, maintained at an average of 39°C (s.d. 2.2), discharged and fed hourly and mixed *via* biogas recirculation. Slurry from dairy cows at AFBI was the sole feed at approximately 20 t d<sup>-1</sup>. Process data were automatically recorded every 30 seconds throughout the 20 week recording period (12 December 2008 to 1 May 2009). Biogas composition was recorded once daily with a hand held gas meter (Gas Data GFM400, UK). Feedstock and digestate were sampled daily, bulked weekly and analysed for DM, ash, N, NH<sub>3</sub>-N, P and K. Volatile solids (VS) contents were calculated as the difference between DM and ash contents. Hydraulic retention time was a nominal 27 days (s.d. 1.0).

**Results** The mean biogas composition during the recording period was 0.57 methane (s.d. 0.028), 0.37 carbon dioxide (s.d. 0.025) and 0.002 hydrogen sulphide (s.d. 0.0004). The average weekly feedstock temperature was 7.7°C (range 4.6–11.4°C). The proportion of gross biogas energy required to maintain digester temperature was 0.39 (range 0.30–0.51). Table 1 summarises digester performance.

**Table 1** Summary of daily digester performance using dairy cow slurry as feedstock over a 20 week period

Parameter	Unit	Value	Standard deviation
Slurry loading rate	t d <sup>-1</sup>	19.8	0.86
VS loading rate	kg VS m <sup>-3</sup> d <sup>-1</sup>	2.36	0.146
Biogas produced	Nm <sup>3</sup> d <sup>-1</sup>	310	31.4
Biogas productivity	Nm <sup>3</sup> m <sup>-3</sup> of digester d <sup>-1</sup>	0.58	0.055
Biogas yield	Nm <sup>3</sup> kg <sup>-1</sup> VS	0.25	0.022
Methane yield	Nm <sup>3</sup> kg <sup>-1</sup> VS	0.14	0.013
Gross energy in biogas	kWh d <sup>-1</sup>	1758	201
Energy to maintain AD temperature	kWh d <sup>-1</sup>	689	129

The digester produced 0.14 Nm<sup>3</sup> methane kg<sup>-1</sup> VS in feedstock (Table 1) equivalent to 89 kWh t<sup>-1</sup> of feedstock. This is an extremely useful source of renewable energy. This overall performance was very similar to that predicted by Frost *et al.* (2006), though was less than predictions of some AD suppliers e.g. approximately 0.16 less methane per unit of VS in the feedstock. Furthermore, the AFBI AD produced 0.61 less methane per unit of VS than the average of 41 Austrian digesters reported by Braun *et al.* (2009). The majority of these Austrian digesters used energy crops as the major component of their feedstock and had hydraulic retention times that were on average about five times longer than in the AFBI AD. Both of these factors would account for the higher degradation of VS in Austria (0.83) than at AFBI (0.24). In the AFBI digester approximately 0.20 of the DM and 0.24 of the VS were degraded (Table 2). The total N, P and K concentrations of the digestate were not significantly ( $P>0.05$ ) affected by digestion (Table 2). However, the crop available nitrogen (NH<sub>3</sub>-N) concentration of the digestate was significantly ( $P<0.001$ ) increased by 0.16 relative to the feedstock.

**Table 2** Summary of nutrient concentrations in dairy cow slurry feedstock and digestate over a 20 week period

g kg <sup>-1</sup> fresh	Feedstock	Digestate	s.e.d.	P
DM	81.1	64.9	0.86	<0.001
pH	7.66	8.05	0.059	<0.001
Total N	4.12	4.10	0.062	0.682
NH <sub>3</sub> -N	2.26	2.63	0.055	<0.001
Total P	0.59	0.61	0.124	0.102
Total K	4.52	4.52	0.134	0.992
VS	63.5	48.2	0.77	<0.001

**Conclusions** Dairy cow slurry is a useful source of renewable energy. The crop available nitrogen in the digestate, compared to the feedstock, was enhanced.

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## Effect of diet, bedding material and ambient temperature on the production of volatile fatty acids and ammonia in the bedding of beef steers in an arctic production system

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**Introduction** Faecal shedding in modern cattle production is often identified as an important source of microbial contamination of the environment. Moreover, microbes accumulated in the pen bedding can lead to the production of undesirable gas emissions, including volatile fatty acids (VFA) (Miller, 2001) and ammonia. Condensed tannin incubated *in vitro* with rumen liquor has been shown to lower ammonia concentration and suppress methane formation (Khiaosa-Ard *et al.*, 2009). Bacterial death may occur during freezing due to metabolic injury (Straka and Stokes, 1959). Utilising antimicrobial forages in the diet and/or the bedding of cattle raised in subzero temperatures may inhibit the bacterial growth thereby decreasing gas emissions from bedding. This work examined the impact of the use of sainfoin (*Onobrychis viciifolia*) in the diet and bedding of beef cattle on VFA concentrations in the bedding pack.

**Materials and methods** Forty beef steers were allocated by live weight into four pens with either sainfoin or straw as bedding and given water and alfalfa silage or sainfoin silage as 100% *ad libitum* diets. Rumen liquor was oral-probe aspirated and faeces grabbed by hand from individual animals. At individual pens several bedding samples were dug out at random by an ice auger, mixed in a bucket and a subsample taken per pen. All samples were collected once in the first three weeks and fortnightly afterwards for seven weeks, and stored in a -20°C freezer. Weekly temperature was averaged from daily ambient temperature (range: -4.6 to -30.0°C). Frozen rumen liquor and bedding samples were processed as described by Bhandari *et al.* (2007). Volatile fatty acids (VFA) concentrations were analysed by gas chromatograph (GC) as described by Bhandari *et al.* (2007) (GC conditions: Injector and detector temperature: 200°C; initial column temperature: 175°C/20 min; final column temperature: 200°C). Sample ammonia nitrogen concentration was determined as described by Bhandari *et al.* (2007). Ammonia and VFA concentrations were analysed by Linear Mixed Models (GenStat, 2007). The fixed model (with each value treated as a distinct factor level) was bedding type \* diet \* ambient temperature and the random model was week × pen, with two- and three-way interactions. Significance levels (P<0.05, P<0.01 and P<0.001) and standard errors of the means were tested using the Wald test.

**Results** Results are shown in Table 1. Feeding sainfoin silage and flooring pens with straw lowered the concentration of VFA in the bedding of steers. Diet had very little effect on VFA concentrations in the bedding of animals, but decreased the ammonia concentration in the bedding of steers fed on sainfoin silage. Except for isovaleric acid, ambient temperature and the bedding type × ambient temperature interaction had a greater effect on VFA concentration in the bedding samples of steers fed on sainfoin silage and with straw bedding than on those fed on alfalfa silage and with sainfoin bedding.

**Table 1** Effect of diet, bedding type and ambient temperature on volatile fatty acids and ammonia concentrations in bedding (mmol/l)

	Sainfoin bedding		Straw bedding		s.e.	Significance level			
	Alfalfa silage	Sainfoin silage	Alfalfa silage	Sainfoin silage		Bedding type	Diet	Amb. temp.	Bedding × Amb. temp.
Acetic	3.48	2.57	1.37	1.41	0.444	***	n.s.	n.s.	*
Butyric	0.18	0.09	0.01	0.01	0.031	***	n.s.	***	***
Propionic	0.32	0.17	0.08	0.10	0.053	**	n.s.	***	*
Isobutyric	0.05	0.02	0.01	0.01	0.006	***	*	**	*
Isovaleric	0.02	0.02	0.01	0.01	0.006	*	n.s.	*	n.s.
Ammonia	14.01	8.14	13.23	9.89	1.120	n.s.	***	n.s.	n.s.

Statistical significance= \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; n.s.= not significant. Amb. temp. = ambient temperature.

**Conclusions** The results showed that feeding sainfoin was effective in lowering the ammonia concentration in the bedding of steers but had little effect on VFA concentrations, partly agreeing with the findings by Khiaosa-Ard *et al.* (2009). Ambient temperature had a strong effect on VFA concentrations in samples, specially isobutyric, butyric and propionic acids. Interestingly, the concentration of VFA increased when pens were floored with sainfoin. It is not clear the reason for this, but it may be that organisms residing in the bedding were more tolerant to tannins from sainfoin (Khiaosa-Ard *et al.*, 2009) and even thrived in cold temperature possibly with sainfoin as growth medium (Straka and Stokes, 1959). Alternative plant compounds should be tested as bedding material to try to reduce the deleterious effects of modern cattle production such as undesirable gas emissions (Miller, 2001).

**Acknowledgements** The authors would like to thank Agriculture and Agri-Food Canada for the research funding.

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## Biosafety assessment of pig manure during composting with straw, sawdust and greenwaste bulking agents

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**Introduction** Utilising pig manure as a sustainable resource for solid biofuel manufacture could potentially assist the pig industry in complying with the Nitrates Directive Action plan introduced by SI No. 378 (2006) which has imposed restrictions on land spreading of pig manure. However, a pig manure-derived solid biofuel may pose a biosafety risk to end-users, as any pathogens present in the manure may be carried over to the end product. Furthermore, in order to place a processed manure product on the market, it must comply with microbiological standards as set out in EU regulation EC/1774/2002 (amended by EC/208/2006). Therefore, strategies are required to reduce the disease risk of manure if it is to be converted into a marketable solid biofuel. The aim of this study was to investigate if composting is effective in eliminating and/or reducing pathogenic and indicator micro-organisms.

**Materials and methods** Manure from the pig unit at Teagasc Moorepark was mechanically separated into solid and liquid fractions using a decanter centrifuge. The solid component was composted alone (control) or mixed with the following bulking agents in a ratio of ~2:1; sawdust, greenwaste (a mixture of tree leaves, foliage and small twigs), or straw. Composting was performed in insulated compost tumblers and each of the four treatments was replicated three times. The temperature of the compost was recorded daily. Microbiological analyses were performed on the unseparated manure, manure solids and the bulking agents and on compost samples at days 0, 7, 14, 21 and 56 as follows; samples were homogenised in buffered peptone water as 1:10 dilutions and serially diluted 10-fold in maximum recovery diluent. Appropriate dilutions were pour-plated on selective media to enumerate coliform, *E. coli*, *Enterococcus* and yeasts and moulds. To enumerate aerobic spore-forming bacteria 1:10 dilutions were heated to 80 °C for 10 min prior to plating. In addition, the presence/absence of *Salmonella* was determined in 25g samples (ISO6579:2002 Annex D). Any *Salmonella* isolates recovered were serotyped according to the Kauffman-White scheme and assayed for susceptibility to a panel of 14 antimicrobials using a broth dilution method. Data were log-transformed prior to analysis for repeated measures using the PROC MIXED procedure of SAS. Linear and quadratic polynomial contrasts were performed on all analyses to determine the effect of composting on microbial counts over time.

**Results** *Salmonella* Livingstone was detected in the sawdust prior to composting. The strain recovered was fully sensitive to all of the antibiotics tested. *Salmonella* was not detected in the two other bulking agents, the manure, manure solids or in any of the compost treatments at any timepoint. Table 1 shows the change in microbial counts over time across all treatments during the 56-day composting period. A quadratic response was observed for *E. coli* counts ( $P<0.001$ ), *Enterococcus* counts ( $P<0.001$ ) and yeast and mould counts ( $P<0.001$ ). Changes in counts of coliform and aerobic spore-forming bacteria were observed on different sampling days ( $P<0.001$ ). Tendencies towards a quadratic response over time were observed for both coliform ( $P=0.10$ ) and aerobic spore-forming bacteria ( $P=0.07$ ). Mean microbial counts were not influenced by treatment ( $P>0.05$ ).

**Table 1** Effect of sampling day on mean microbial counts (Log<sub>10</sub> CFU/g) in pig manure-derived compost

	Day						P-value		
	0	7	14	21	56	SE	Overall	Linear	Quadratic
<i>E. coli</i>	5.33	2.00	2.00	2.00	2.00	0.049	<0.001	<0.001	<0.001
Coliform	5.39	2.82	5.07	5.22	3.66	0.268	<0.001	0.83	0.10
<i>Enterococci</i>	4.89	2.03	2.04	2.05	2.00	0.044	<0.001	<0.001	<0.001
Yeasts & moulds	5.41	3.12	3.80	4.09	4.68	0.141	<0.001	0.61	<0.001
Aerobic spore-formers	5.59	5.56	5.80	5.41	5.86	0.079	<0.001	0.31	0.07

**Conclusions** By day 7, coliform, *E. coli* and *Enterococcus* counts had decreased in the compost, most likely because the temperature had increased to 59 °C. Although coliform counts subsequently increased as the temperature decreased to 37 °C, they had declined again by day 56. The resulting compost complies with EU regulations, which state that a marketable processed manure product must be free from *Salmonella*, with *E. coli* or *Enterococcus* counts not exceeding 3.0 log<sub>10</sub> cfu/g. However, regulations also state that the product 'must be subjected to a reduction in spore-forming bacteria' and although spore-former counts tended to be reduced during composting, final counts were higher than those on day 0. Although yeasts and mould counts changed over time, the relatively high counts in the final product may be potentially hazardous due to the risk posed by exposure to spores. However, identification of moulds and spore-forming bacteria is required in order to determine the potential risks posed by either. Furthermore, processing of the manure-derived compost into a solid biofuel may reduce levels of these micro-organisms. It is worth noting that bulking agents should be selected carefully, as the sawdust contained *Salmonella*, albeit a serotype not commonly associated with human infection.

**Acknowledgements** This research was funded by the HEA Technological Sector Research Strand I Programme and the Department of Agriculture and Food's Research Stimulus Fund Programme under the National Development Plan 2007-2013

**References** ISO 6579:2002, Amendment 1, Annex D. Detection of *Salmonella* spp. in animal faeces and in samples of the primary production stage.

## The effect of dietary benzoic acid concentration on nitrogen utilisation, manure ammonia and odour emissions in finisher pigs

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**Introduction** Pig production is associated with ammonia and odour emissions causing nuisance, environmental and public concerns. Recent studies have suggested that it is possible to change the production of ammonia and odour by changing the conditions in the slurry through dietary interventions (Lynch *et al.*, 2008). Slurry pH is one of the most significant factors influencing ammonia emission, ammonia volatilisation increasing with an increasing manure pH. Buhler *et al.*, (2006) demonstrated that dietary benzoic acid significantly lowered urinary pH in grower and finisher pigs. Therefore, the objective of the following study was to investigate the effects of benzoic acid concentration as a feed additive on nitrogen utilisation, manure ammonia and odour emissions in finishing pigs.

**Materials and methods** The experiment was designed as a complete randomised design comprising of four dietary treatments: (T1) 0 g/kg benzoic acid, (T2) 10 g/kg benzoic acid, (T3) 20 g/kg benzoic acid, (T4) 30 g/kg benzoic acid. Sixteen boars were selected according to a uniform weight ( $64 \pm 1.5$  kg), and allocated to one of four dietary treatments as described above ( $n=4$ ). Pigs were transferred to individual metabolism crates to facilitate studies on nitrogen utilisation, manure ammonia and odour emissions. The collection period was subdivided into three parts; 1)  $\text{NH}_3$  emission (days 1 to 2); 2) odour emission (days 3 to 5) and 3) N utilisation (days 6 to 10). Ammonia emission from the manure was measured over a 240 h period, in a laboratory scale set-up (O'Shea *et al.*, 2009). Ammonia production (mg) from manure was compared between the different dietary treatments using the quantity volatilised from 0 to 240 h/g of N intake. Air samples, used to measure odour concentration were collected directly above the storage container in 201 Nalophan sampling bags and analysed for odour concentration using an Ecoma T08 dynamic olfactometer as described by Lynch *et al.*, (2008). For the N utilisation study, urine volume was recorded daily and a 50-ml sample was collected and frozen for laboratory analysis. Total faeces weight was also recorded daily and all faeces were oven dried at  $100^\circ\text{C}$ . A sample of fresh faeces was collected twice daily for N analysis. Experimental data were analysed as a complete randomised design using the GLM procedure of the SAS Institute (1985). The statistical model included the linear and quadratic effects of benzoic acid.

**Results** There was a significant linear decrease ( $P < 0.05$ ) in urinary nitrogen excretion and total nitrogen excretion as the level of benzoic acid inclusion increased in the diet. Manure ammonia emissions were linearly reduced by 30, 41 and 72% as the dietary benzoic acid concentration increased. However, there was no effect of benzoic acid on odour concentration across all treatments.

**Discussion** In the current study, manure ammonia emissions were decreased linearly with increasing dietary benzoic acid. This may reflect the linear decrease in manure pH which was also demonstrated (Table 1). In addition, the current study reported no effect on odour emissions despite the significant reduction in manure ammonia emission as benzoic acid increased. This is in agreement with the findings of Lynch *et al.*, (2008) who reported no correlation between manure ammonia and odour emissions from finisher pigs.

**Table 1** Effect of dietary benzoic acid inclusion level on manure composition and nitrogen balance (LSM  $\pm$  s.e.m.)

Treatment	Benzoic acid concentration g/kg					Significance	
	0	10	20	30	s.e.m	Linear	Quadratic
Urinary N excretion (g/day)	25.3	21.6	23.5	19.1	1.6	*	ns
Total N excretion (g/day)	35.5	33.6	34.6	30.3	1.9	*	ns
pH 0-240 h	8.96	8.46	8.08	7.40	0.33	**	ns
Ammonia (mg/g N intake) 0-240 h	141.3	99.1	83.8	40.4	12.0	***	ns
Odour $\text{Ou}_E/\text{m}^3$ (72h)	22090	27620	35462	32627	5709	ns	ns

Linear = linear response to dietary benzoic acid, quadratic = quadratic response to benzoic acid. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , ns = non significant ( $P > 0.05$ ).

**Conclusion** Dietary inclusion of benzoic acid linearly reduced urinary nitrogen and total nitrogen excretion and furthermore linearly reduced manure ammonia emissions. Odour emissions were not influenced by benzoic acid inclusion in the current study.

**Acknowledgements** The authors gratefully acknowledge funding from the Department of Agriculture, Fisheries & Food.

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## Investigation of faecal archaeol as a biomarker for rumen methanogens

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**Introduction** There is growing interest in methane emission from ruminants, both because it represents a loss of up to 0.12 of gross energy and its role as a potent greenhouse gas. Research has been limited because chamber-based estimates of methane emission are both laborious and restricted to stall-fed animals, whilst the sulphur hexafluoride (SF<sub>6</sub>) technique involves considerable experimental errors (Pinares-Patiño and Clark, 2008). Methane is produced by Archaea and the membranes of Archaea contain distinctive dialkyl glycerol ether lipids that have been used as biomarkers in a wide range of other sample types, including rice paddy soil and marine sediments. Recent studies have demonstrated the occurrence of the archaeal biomarker 2,3-diphytanyl-O-*sn*-glycerol (archaeol) in the faeces of ruminant and pseudo-ruminant species. The present study evaluated the potential to use the concentration of archaeol in bovine faeces as a marker for methane emission from diets with widely different forage/concentrate ratios, which were expected to generate divergent amounts of methane (Johnson and Johnson, 1995).

**Materials and methods** Twelve continental cross-bred steers (541 kg (s.d.=41.8)) were blocked according to live weight and allocated at random to receive one of two dietary treatments based on grass silage and concentrates. Treatments were: (1) 5 kg/animal daily of grass silage with *ad libitum* concentrates (diet CON), and (2) 3 kg/animal daily of concentrates with *ad libitum* grass silage (diet GS). Concentrates were based on mixtures of (g/kg) barley (820 or 460), soybean meal (100 or 460), molasses (50), vegetable oil (10) and a mineral/vitamin premix (20) for the concentrates used for diets CON and GS, respectively. Individual feed intakes were recorded through Calan gates. Measurements of methane emission were taken from three animals per treatment after 5 and 16 weeks on these dietary treatments, using the SF<sub>6</sub> technique (Lovett *et al.*, 2003). At least three separate daily measurements were obtained from each steer (average 3.8/steer). Faecal samples were obtained *per rectum* over five days, stored at -20°C, then thawed, bulked and dried at 60°C (48 hours). Archaeol concentrations were measured in a faecal lipid extract. Faecal lipids were extracted and fractionated according to Bull *et al.* (2003), derivatised to trimethylsilyl ethers and analysed by gas chromatography mass spectrometry using a ThermoQuest TraceMS equipped with a non-polar fused silica capillary column (CPSil-5CB, 50 m × 0.32 mm × 0.12 µm, Varian Chrompack, Oxford, UK). Effects of dietary treatments were analyzed using analysis of variance (Genstat Release 10.1; Lawes Agricultural Trust, 2007) with 'diet' as treatment factor and 'measurement week' as block.

**Results** The grass silage and concentrates (for CON and GS diets) contained respectively: DM: 252, 866 and 860 g/kg; crude protein: 138, 159 and 297 g/kg DM; NDF: 511, 157 and 138 g/kg DM; starch: 0, 485 and 235 g/kg DM; *in vitro* digestible organic matter: 694, 806 and 820 g/kg DM. Silage pH was 3.81 and lactic acid 106 g/kg DM. Despite the lower DM intakes for the GS diet, methane emission was doubled in comparison with the CON diet. The CON diet also resulted in considerably lower faecal archaeol concentrations compared to the GS diet (Table 1). No archaeol was found in any of the feed samples analysed.

**Table 1** Effects of dietary treatment on feed intake, methane emission and faecal concentration of archaeol.

	Diet:		s.e.d.	P
	CON: <i>ad libitum</i> concentrates	GS: <i>ad libitum</i> grass silage		
Total DM intake, kg/d	11.43	9.15	0.824	0.022
Concentrate DM intake, kg/d	10.18	2.58 (fixed)		
Silage DM intake, kg/d	1.25 (fixed)	6.57		
Methane emission, g/d	174	341	60.5	0.022
Methane emission, g/kg DM intake	15.0	37.4	6.09	0.005
Faecal archaeol, mg/kg DM	5.1	30.6	5.42	0.001

**Conclusions** The absence of archaeol in the feeds confirms that faecal archaeol is produced during passage through the digestive tract. Taken together with earlier observations of archaeol in faeces from ruminant species, but not in faeces from other herbivores, these results suggest a predominant origin in synthesis by rumen micro-organisms. The concentration of archaeol in the faeces studied is interpreted to reflect the size of the methanogen population in the rumen. However, further studies will be required to describe other components of this relationship, such as selective retention of Archaea in the rumen and differences in the species composition of the methanogen community (species vary in the proportions of diether and tetraether membrane lipids).

**Acknowledgements** The authors acknowledge the financial support of the Leverhulme Trust (F/00 182/BS) and NERC.

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## Development of Tiers 2 and 3 methane emission factors for enteric fermentation and manure management of cattle and sheep using Hillsborough herd data and calorimetric methane measurements

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**Introduction** The Intergovernmental Panel on Climate Change (IPCC, 2006) recommends three methods (Tier 1, 2 and 3) to estimate methane (CH<sub>4</sub>) emissions from enteric fermentation and manure management for livestock in development of national CH<sub>4</sub> inventories. Tier 1 default emission factors (enteric and manure) provide fixed values for each species of animals in different regions of the World, irrespective of variations in animal physiological state and production level. The objectives of the present study were to develop CH<sub>4</sub> emission factors of Tiers 2 (enteric and manure) and 3 (enteric) using AFBI Hillsborough herd data and calorimeter CH<sub>4</sub> measurements and to identify sources of variations between Tier 1 and Tier 2/3 emission factors.

**Materials and methods** AFBI Hillsborough herd data used include average live weight, milk yield (7328 kg/year, dairy cows), growth rate (growing animals), birth rates (cows, heifers and ewe), age (growing animals) and dietary GE, ME and ash concentrations for each species. Calorimetric CH<sub>4</sub> and energy metabolism data included measurements undertaken at Hillsborough since 1992 with more than 900 dairy cows, 140 beef and 50 sheep. The life span for lambs was taken as 210 days. Manure was managed under the liquid/slurry system for indoor feeding animals and the pasture management system for grazing animals. Tier 2 CH<sub>4</sub> emission factors from enteric fermentation and manure management were developed using the methodologies proposed by IPCC (2006). Tier 3 enteric CH<sub>4</sub> emission factors were developed from the ratio of CH<sub>4</sub> energy output to ME intake, with ME intake estimated using the FiM models for dairy cows and AFRC systems for beef cattle, heifers and sheep.

**Results and discussion** The results are presented in Table 1. For enteric CH<sub>4</sub> emissions, Tiers 2 and 3 factors for dairy cows were 10.2 and 7.5 kg/year lower than Tier 1 factor, respectively. This indicates that the increase in Tier 1 default factor for Western Europe from 100 kg/year in the version of IPCC 1996 to the present 117 kg/year (IPCC, 2006) may over-estimate the effect of increased milk production during the period. For sheep, dairy heifer and beef cattle, in general, when compared with Tier 2 and 3 enteric CH<sub>4</sub> factors, Tier 1 enteric CH<sub>4</sub> factors produced a considerable over-prediction of CH<sub>4</sub> emissions for young animals (less than 1 year old), with exception for dairy bulls, for which Tier 1 was similar to Tier 2 (because they were managed under intensive feeding regimes). On the contrary, for animals of over 1 year age, Tier 1 enteric CH<sub>4</sub> factors were smaller than Tier 2/3 enteric CH<sub>4</sub> factors, except for dairy heifers of over 2 years for which Tier 1 was larger than Tier 2/3 (due to low growth rates). Similar results were also obtained for CH<sub>4</sub> emissions from manure management.

**Table 1** Tier 1 versus Tiers 2 and 3 methane emission factors for enteric fermentation and manure management

Species	Age	Category	Enteric (kg/y)			As % of Tier 1		Manure (kg/y)		T2/T1 (%)
			Tier 1	Tier 2	Tier 3	Tier 2	Tier 3	Tier 1	Tier 2	
Dairy cow	Milking + dry		117	106.8	109.5	91	94	21	19.4	92
	< 1 year	Dairy steer/heifer	57	34.4	37.0	60	65	6	4.0	66
	< 1 year	Dairy bull	57	57.5	50.2	101	88	6	5.3	89
Beef cattle	< 1 year	Suckler	57	32.0	29.1	56	51	6	4.6	77
	1-2 year	Dairy steer/heifer	57	65.6	66.5	115	117	6	6.8	114
	1-2 year	Suckler	57	63.6	64.0	112	112	6	6.6	110
	> 2 year	Suckler cow	57	56.9	59.1	100	104	6	6.1	101
	< 1 year	Holstein-Friesian	57	29.7	33.3	52	59	6	3.7	61
Dairy heifer	< 1 year	Cross breeding	57	27.3	30.2	48	53	6	3.3	55
	1-2 year	Holstein-Friesian	57	64.5	69.5	113	122	6	7.2	119
	1-2 year	Cross breeding	57	57.5	61.5	101	108	6	6.3	105
	> 2 year	Holstein-Friesian	57	50.3	52.9	88	93	6	5.4	91
	> 2 year	Cross breeding	57	49.7	52.4	87	92	6	5.4	90
Sheep	< 1 year	Lamb	8	6.6	4.6	83	58	0.19	0.08	41
	> 1 year	Ewe	8	8.0	10.5	100	131	0.19	0.16	85
	Replacement		8	7.5	7.5	94	94	0.19	0.13	70

**Conclusions** For both enteric fermentation and manure management, in comparison with Tier 2/3 emission factors, Tier 1 default factors over-estimated CH<sub>4</sub> emissions for dairy cows, young cattle and sheep, while under-predicted CH<sub>4</sub> emissions for beef cattle, heifers and sheep at age between 1 and 2 years old. This indicates that the development of national CH<sub>4</sub> emission inventories from the Tier 1 method can result in considerable and systematic errors.

**Acknowledgements** This study was funded by Department of Agriculture and Food (ROI) and DARDNI.

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## Effect of level of dietary soy oil supplementation and concentrate to forage ratio on feed intake, methane production and rumen fermentation variables of beef steers

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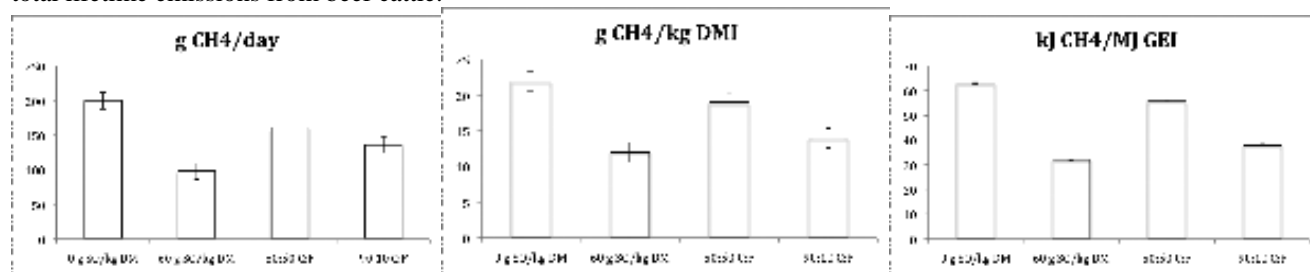
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**Introduction** Agriculture currently accounts for 12% of total global green house gas (GHG) emissions with enteric fermentation being the largest biogenic source of agricultural methane (CH<sub>4</sub>) and accounting for almost 13% of total Irish GHG emissions (McGettigan *et al.*, 2008). Ruminant methanogenesis represents a loss to the animal of up to 8.5% of gross energy intake (GEI) (Tamminga *et al.*, 2007) therefore a reduction in enteric CH<sub>4</sub> production should reduce this inefficiency as well as the environmental impact of ruminant production. Soy oil (SO) is a natural source of unsaturated long chain fatty acids and it has been shown previously that dietary SO supplementation can substantially reduce ruminal methanogenesis of beef cattle on a high concentrate diet (Jordan *et al.* 2006). However little is known on how this effect is mediated or whether it is consistent across lower concentrate to forage ratio (C:F) diets. The aim of this study, therefore, was to investigate the effect of dietary SO inclusion and C:F ratio on feed intake, CH<sub>4</sub> emissions, rumen fermentation variables and rumen microbial measurements in beef cattle.

**Materials and methods** Eight mature Limousin X steers with a mean body weight (BW) ( $\pm$  S.E.) of 521 ( $\pm$  11.7) kg and 4 ruminally cannulated Limousin X steers with a mean BW ( $\pm$  S.E.) of 484 ( $\pm$  26.4) kg were allocated at random to a 2 x 2 factorial, latin square design with four, 28 d periods. Animals were allocated within period to one of two levels of dietary C:F ratio (50:50 v 90:10; barley straw as the forage source) and one of two levels of dietary SO inclusion (0 v 60 g/kg dry matter (DM)). Diets were offered at 95% of voluntary DMI and formulated to be isonitrogenous (140 g/kg DM). Feed intake was measured daily, with CH<sub>4</sub> determined using the sulphur hexafluoride tracer (SF<sub>6</sub>) gas technique on d 21 - 25 of each period on the 8 non-cannulated steers. Rumen fluid was sampled from the ruminally cannulated steers on d 27 and 28 for analysis of ruminal VFA, ammonia and microbial profile. The mcrA gene and 16S rRNA gene were quantified using specific genomic primers and real-time quantitative polymerase chain reaction (PCR) for both liquid and solid phases of rumen digesta. Data were analysed using the MIXED procedure (PROC MIXED) of SAS.

**Results** The effect of C:F ratio and SO supplementation on CH<sub>4</sub> emissions is presented in Figure 1. There were no oil x C:F interactions detected ( $P > 0.05$ ). Inclusion of SO reduced ( $P < 0.001$ ) daily CH<sub>4</sub> by 51%, intake corrected CH<sub>4</sub> by 45% and GE intake corrected CH<sub>4</sub> by 51%. At the higher C:F ratio, dry matter intake (DMI) corrected CH<sub>4</sub> was reduced ( $P = 0.006$ ) by 27%, CH<sub>4</sub> corrected for GE intake was reduced ( $P = 0.003$ ) by 32% and there was also a trend ( $P = 0.07$ ) towards decreased overall daily CH<sub>4</sub>. Dry matter intake was greatest ( $P < 0.001$ ) at the higher C:F ratio but was reduced by 7.5% following SO inclusion ( $P = 0.02$ ). Apparent whole-tract digestibilities of DM ( $P = 0.04$ ), OM ( $P = 0.03$ ) and GE ( $P = 0.008$ ) were reduced after SO inclusion, with the higher C:F ratio increasing DM ( $P = 0.02$ ), OM ( $P = 0.03$ ) and GE ( $P = 0.04$ ) digestibility. Treatment had no effect on total ruminal concentrations of VFA. However, acetate to propionate ratio was decreased ( $P < 0.001$ ) by the higher C:F ratio and by SO inclusion. Ammonia was lower at both the higher C:F ratio ( $P < 0.001$ ) and following SO inclusion ( $P = 0.002$ ). There was a tendency towards a reduction ( $P = 0.0539$ ) in the relative abundance of the mcrA gene in digesta of animals fed SO in the ruminal liquid phase.

**Conclusion** This study demonstrated that the addition of SO, at 60 g/kg DM, to the diet of beef steers dramatically reduces daily and GEI adjusted CH<sub>4</sub> by over 50% and DMI corrected CH<sub>4</sub> by 45%. Increased C:F ratio also reduced CH<sub>4</sub> when corrected for DMI and GEI (by 26 and 32% respectively) and it tended to reduce daily CH<sub>4</sub> emissions whilst increasing DMI. This suggests that if both strategies were utilised with finishing cattle the potential exists to significantly reduce the total lifetime emissions from beef cattle.



**Figure 1** Effect of dietary SO supplementation and C:F ratio on daily ruminal CH<sub>4</sub> emissions expressed on a daily, per kg of DMI and per MJ GEI basis

**Acknowledgements** Funding for this research was provided under the Irish National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food

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## Effect of either soya or linseed oil supplementation of grazing dairy cows on milk production and methane emissions

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**Introduction** Methane (CH<sub>4</sub>) accounts for approximately 50% of total greenhouse gas (GHG) emissions (on a CO<sub>2</sub> equivalents basis) from the average Irish dairy farm and represents a loss of up to 8.5% of gross energy intake (GEI) in dairy cows (Tamminga *et al.*, 2007). Dietary polyunsaturated fatty acid (PUFA) supplementation, especially linoleic (n-6) and linolenic acids (n-3) have been shown to reduce ruminal methanogenesis (Martin *et al.*, 2008, Petrie *et al.*, 2009) in indoor housed animals. The aim of this study was to assess the impact of oils rich in either n-3 or n-6 PUFA on milk production and CH<sub>4</sub> emissions of grazing dairy cows.

**Materials and methods** Forty five Holstein Friesian cows were blocked according to parity (24 multiparous and 21 primiparous) and allocated to one of three dietary treatments balanced for days in milk (mean 143 days, S.D.  $\pm$  22) and pre-experimental milk yield (mean 24.6 litres, S.D.  $\pm$  4.8) in a randomised block design. All treatments were allocated 17 kg grazed grass DM per day per cow, following morning milking plus 4 kg (DM) of concentrates containing 160g/kg (FW) of stearic acid (C), soya oil (SO) or linseed oil (LO), daily. Concentrates were offered in equal allocations at morning and afternoon milkings. Animals were introduced to their treatment diets over a 7-day period, following which they had an adjustment period of 17 days. Individual CH<sub>4</sub> emissions were measured using the SF<sub>6</sub> technique as described by Johnson *et al.* (1994), 17 (PI) and 44 (PII) days post diet introduction. Milk production was recorded daily and milk composition was assessed on a weekly basis. Statistical analysis was performed using the mixed procedure of SAS with terms included for treatment, period and their interaction.

**Results** Data for CH<sub>4</sub> emissions are shown in Table 1. Both treatment and period affected all CH<sub>4</sub> variables measured (P<0.001). There were treatment x period interactions for daily CH<sub>4</sub> (P<0.001), CH<sub>4</sub>/kg milk (P<0.01) and CH<sub>4</sub>/kg milk solids (P<0.001). During PI both SO and LO reduced all CH<sub>4</sub> variables compared to the control, but during PII only LO reduced CH<sub>4</sub> variables compared to the control. Data for milk production across the entire experimental period are presented in Table 2. SO increased milk yield (P<0.001), milk protein % (P<0.001) and milk solids yield (P<0.05), and reduced milk fat % (P<0.01) when compared to C and increased milk yield compared to LO (P<0.001). LO increased milk protein % (P<0.001) compared to C but did not differ from C for any other milk production variable measured.

**Table 1** Effect of supplementary lipid source on methane emissions of grazing dairy cows

	Period I				Period II				Significance		
	C	SO	LO	s.e.m	C	SO	LO	s.e.m	T	P	Tx P
Daily CH <sub>4</sub> (g)	260 <sup>a</sup>	239 <sup>b</sup>	221 <sup>c</sup>	7.2	331 <sup>a</sup>	348 <sup>a</sup>	267 <sup>b</sup>	7.1	***	***	***
gCH <sub>4</sub> /kg milk	13.72 <sup>a</sup>	11.99 <sup>b</sup>	11.06 <sup>c</sup>	0.4	18.38 <sup>a</sup>	17.74 <sup>a</sup>	13.93 <sup>b</sup>	0.4	***	***	**
gCH <sub>4</sub> /kg milk solids	181.3 <sup>a</sup>	161.1 <sup>b</sup>	152.2 <sup>b</sup>	5.18	240.5 <sup>a</sup>	229.4 <sup>a</sup>	180.8 <sup>b</sup>	5.18	***	***	***

<sup>a, b, c</sup> Means with different superscripts within rows are different \*\*\*P<0.001; \*\*P<0.01

**Table 2** Effect of supplementary lipid source on milk production and milk composition of grazing dairy cows

Treatment	Control	Soya oil	Linseed oil	s.e.d	Significance
Milk yield (l/d)	20.05 <sup>a</sup>	21.52 <sup>b</sup>	20.28 <sup>a</sup>	0.120	***
Milk fat %	4.18 <sup>a</sup>	3.84 <sup>b</sup>	3.96 <sup>ab</sup>	0.068	**
Milk protein %	3.35 <sup>a</sup>	3.46 <sup>b</sup>	3.42 <sup>b</sup>	0.015	***
Milk solids yield (kg/d)	1.53 <sup>a</sup>	1.59 <sup>b</sup>	1.54 <sup>ab</sup>	0.018	*

<sup>a, b, c</sup> Means with different superscripts within rows are different \*\*\*P<0.001; \*\*P<0.01; \*P<0.05

**Conclusion** Both SO and LO have the potential to reduce enteric CH<sub>4</sub> emissions from grazing dairy cows. However, the effects of LO appear to have a greater persistency over time. Furthermore, the addition of PUFA maintained or enhanced milk production variables compared to a saturated fat supplement.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland.

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## Chemical composition of different fractions of *Solanum lycocarpum* St Hil

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**Introduction** Globally methane released from the rumen represents approximately a third of all anthropogenic actions and novel crops could be an alternative to tackle this problem (Beauchemin *et al.*, 2008). In addition the development of arid resistant plants and natural products to manipulate ruminal fermentation are being speculated as part of cutting edge technologies currently under study to optimize ruminal function. In this context the wild shrub *Solanum lycocarpum* St Hil. could be an alternative crop as well as novel rumen manipulator agent. Here its chemical composition is presented as part of a project which has been investigating the use of some fractions of this plant as a potential animal feedstuff.

**Materials and methods** Five dried meal-like fractions of *Solanum lycocarpum* (SL) (Flower=Fl, Fruit=Fr, Leaf=Lf, Stem=St and Root=Rt) from two different seasons (wet/summer –between January/March and dry/winter – between July/September of 2008) from Brazil were analysed in triplicates for their Crude Protein (CP), Ether Extract (EE), Total Sugar (TS) and Starch (ST), Total Phenols (TP), Tannins (TN) and Saponins (SP). Samples were gathered in the afternoon, sliced in small parts and spread under shadow for about 48 hours to reduce their moisture contents. Then the fractions were milled, oven dried at 60°C overnight and air transported to the UK. Proximate analysis was determined according to AOAC (1980). Phytochemicals were assayed using standard methods with little modifications. Total phenols, condensed tannins and saponins were determined by the method of Herborne (1978). The data were analyzed by using General Linear Model of Minitab to study the main effects of SL fractions and season as well as their interactions on chemical composition.

**Results** Mean chemical components for different fractions of *Solanum lycocarpum* different seasons are shown in Table 1. The main effects of SL fractions were significant for CP, TS, ST, TP and SP ( $P<0.05$ ). Flower had the highest CP level with difference between seasons ( $P<0.01$ ). Total sugar was higher for the stem and starch for the root ( $P<0.05$ ). Total phenols were significantly higher in Flower and Fruits than other fractions. Saponins were higher in Fruits and Leaves but did not vary between seasons ( $P>0.05$ ). Additionally, no significant interactions between fractions and seasons were observed ( $P>0.01$ ) with the exception of CP and SP ( $P<0.05$ ).

**Table 1** Chemical composition of *Solanum lycocarpum* fractions from two different seasons (g/100g) \*\*

Fraction	Season	CP	EE	Total Sugar	Starch	Total Phenols (GAE)	Condensed Tannins (CT)	Saponins
Flower	S	22.7	1.5	0.11	0.20	2.32	16.2	1.6
	W	15.2	1.5	0.10	0.18	2.30	16.1	1.7
Fruit	S	5.3	3.3	0.02	0.12	1.68	12.7	2.5
	W	4.2	3.0	0.01	0.11	1.68	10.0	2.3
Leaf	S	19.7	2.2	0.11	0.12	1.20	16.3	2.2
	W	18.0	2.0	0.11	0.11	1.21	14.9	1.1
Stem	S	4.5	2.5	0.13	0.14	0.54	12.4	1.2
	W	1.2	2.2	0.12	0.14	0.52	11.9	1.1
Root	S	5.2	3.0	0.08	0.22	0.34	13.2	0.8
	W	5.0	2.8	0.07	0.22	0.33	13.1	0.5
SEM (fractions)		3.2	1.2	0.12	0.15	0.29	1.3	0.9
Significance	*	**		*	*	**		*

\*\*  $P<0.01$ ; \*  $P<0.05$  (in each of the columns, shows statistical differences between fractions); SEM (Standard error of means); S= Summer & W=Winter; CP= Crude Protein; EE = Ether Extract; GAE= Gallic Acid Equivalent

**Conclusions** All fractions showed statistical differences regarding all chemical constituents with the exception of EE and CT. Secondary metabolites are present in high levels and demand further investigations to evaluate their effect on ruminal metabolism.

**Acknowledgements** Mr. Helio Lima is grateful to Brazilian Government by providing his CAPES Doctoral Scholarship

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## Biological and economic consequences heat stress due to a changing climate on UK livestock

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**Introduction** Weather and climate can directly and indirectly determine the production and welfare of livestock. An example of a direct influence is the heat balance of livestock as average temperatures increase. Excessive heat or cold increases the metabolic energy required to maintain the animal's body temperature, thus reducing the energy available for productivity and maintaining functional fitness of the animal. This requires an understanding of how environmental stressors (e.g., temperature, humidity, thermal, air speed) can directly and adversely affect animal performance, health, and well-being when coping capabilities of the animals are exceeded and can have unfavourable economic consequences. The aim of this study was to model the change in heat stress in livestock in the UK in the future and examine the biological and economic consequences of said heat stress

**Materials and methods** Predictions of climate change in the UK were used to estimate the impact of heat stress on production and fitness traits in UK livestock under the medium-high climate change scenario for 2020, 2050 and 2080 (UKCIP02; Hulme *et al.*, 2002) to 50km<sup>2</sup> resolution. The environmental conditions that induce heat stress were estimated by calculating using the temperature humidity index (THI) for each of 10 classes of livestock, including dairy, beef, pigs and poultry. However due to space constraints only selected results are presented in this abstract. For each class of animal the biological (production, fertility and mortality) response to heat stress was modelled using methodology presented in St-Pierre *et al.* (2003). The production (e.g., kg milk loss/animal/day, MILK), fertility (e.g., change in the number of days open/animal/year, DO) and mortality (change in monthly death rate from heat stress, MORT) losses were then scaled using UK livestock census data and present day economic costs estimated across the country and the 3 time periods.

**Table 1** Annual impacts of heat stress on the duration of heat stress (hours/cow/year) and selected production, fertility and mortality in UK dairy cows by region in 2050 and 2080

Region	Year	Duration	MILK	DO	MORT
East England	2050	186	1.3	0.42	1.25
	2080	601	21.5	3.19	2.87
East Midlands	2050	132	0.4	0.19	1.04
	2080	516	15.7	2.58	2.54
North East	2050	0	0.0	0.00	0.00
	2080	111	0.0	0.05	1.71
North West	2050	0	0.0	0.00	0.00
	2080	170	1.2	0.37	1.45
South East	2050	350	4.0	1.13	1.77
	2080	852	41.9	5.28	3.73
South West	2050	137	0.6	0.27	0.87
	2080	495	15.4	2.33	2.59
Wales	2050	52	0.2	0.09	0.37
	2080	290	7.3	1.23	1.78
	2050	138	0.3	0.15	1.23
West Midlands	2080	497	14.8	2.48	2.46
Yorkshire and Humberside	2050	0	0.0	0.00	0.00
	2080	245	1.6	0.54	1.57

**Results** Table 1 shows that there was geographical variation in the extent to which animals suffered heat stress in the UK (e.g., dairy cows experiencing 852 hours of heat stress/year by 2080 in South East England compared to 0 hours in Northern Ireland, Scotland and Yorkshire and Humberside). These differences meant that the production, fertility and mortality losses varied both spatially and temporal in all categories of animals. Beef cows experienced less than half the duration of heat stress than dairy cows with a subsequent negligible impact on production, fertility and mortality. The average annual duration of heat stress in 2080 for monogastric animals was 92 hours for breeding sows, 214 hours of growing pigs and 420 hours of layer hens. The impact of heat stress on production was greatest in laying hens (24 g of egg loss/bird/year) followed by growing/finisher pigs (425 g reduction in liveweight gain/animal/year). The overall economic consequences of these heat stress losses were estimated to cost the UK livestock industry £5.8 million

(GBP) by 2080 in production losses and £34 million in mortality losses. These costs exclude adaptation or extreme events (e.g., heat wave) and reflect the costs of the impacts of the gradual change of temperature (and humidity) in the UK.

**Conclusions** This study shows that the warming climate scenario in the UK is predicted to have a negative impact on the welfare of livestock. Not only will the animals be suffering heat stress but this will have a knock-on effect the economics of the system due to losses in production and functional performance of the animal. These represent private losses and we can expect industry to adapt if guided by appropriate regulatory reform and surveillance. The addition of extreme events to gradual climate change may provide a "shock" to livestock systems. The results show that the damages due to a warming climate will be amplified with the additional stress of extreme events, such as a heat wave. Also, some of the impacts of climate change on animal production and functionality (e.g., health and welfare) may interact with externalities and/or adaptations farmers make in other areas of the farming system (e.g., adoption of mitigation tools) needs to be monitored to ensure that both mitigation and adaptation measures are compatible and sustainable into the future. Continued development of information resources and tools will help farmers improve the resilience of their systems.

**Acknowledgements** The authors gratefully acknowledge funding from Defra.

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## Conformation and its effect on laterality in the thoroughbred racehorse

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**Introduction** Within the UK race horses compete on tracks in both clockwise and anticlockwise directions. Desert Orchid was the first high profile racehorse who proved he preferred to run and jump in one direction, in his case the clockwise direction. A horse that expresses a preference to use one side more than the other is known as having motor bias. Motor bias horses can be classified as preferring a clockwise (C) or an anticlockwise (AC) direction. In humans there is a 93% bias towards preference of the right side, which would make the horse prefer a C direction (Corren and Porac 1977). However, there is ambiguous research as to whether the horse expresses motor bias at population level and in what direction. Deuel and Lawrence (1987) found that horses preferred the left lead leg in gallop whilst in contrast, Rachen-Schöneich and Schöneich (2007) and Williams and Norris (2007) both found that horses prefer the right leg during lateral exercise. If a racehorse prefers to use the right lead stride pattern it should perform better on clockwise tracks as the horse is forced to use the right lead stride pattern around C bends while a horse preferring a left lead stride pattern should prefer AC racetracks. Oliver and Langrish (1991) stated that if a horse has uneven hind limbs it can affect the speed the horse reaches around bends. Therefore measuring conformation points on the horse could help to identify one of the causes of laterality in the racehorse, which could lead to more successful horses and increased safety within the sport.

**Materials and methods** Conformation data (Fore limb length, hind limb length, shoulder length, shoulder angle and shoulder to hip length) was recorded on both sides of National Hunt racehorses in Southern England, n=57 (males= 51 females n=6). There were two ways that horses qualified for this study. The first was if they were part of the British Horseracing Authority's database 'why horse's run badly' which included steward's notes on motor bias, the database is available at [www.britishhorseracing.com](http://www.britishhorseracing.com). The second point of qualification was if the horse expresses a strong desire to race in one direction, for example if the horse had only won races on clockwise tracks.

For analysis the data was divided into two sets; horses that prefer clockwise tracks and those that prefer anticlockwise tracks (AC bias n=26, C bias n=25). A one-way anova test was performed on both sets of data in PASW statistics version 18 (formerly SPSS statistics). The anova test was used to analyse the data taken from the two sides (right and left) of the horse.

**Results** On both the C and the AC horses the left side was notably ( $P<0.05$ ) longer than right when the shoulder to hip length was recorded. In the C horses the shoulder length was significantly longer on the right ( $P<0.05$ ) while the shoulder length was significantly longer on the left in the AC horses. There were considerable differences in fore limb length on both the AC and C horses ( $P<0.05$ ) with the left leg being longer in the AC horses and the right leg being longer on the C horses. However, the AC horses were the only group to show a significant difference in hind limb length ( $P<0.05$ ) with the left leg being extensively longer than the right. In the C horses there was a significance difference in shoulder angle with the left side being steeper but there were no differences seen in the AC in regards to shoulder angle.

**Conclusion** The results indicate that a longer left shoulder means the horse is likely to be classified as AC while a longer right shoulder means the horse is significantly more likely to prefer the C direction and the same can be said for fore limb length. Therefore shoulder length and fore limb length could be a strong indicators of laterality and they could be measured and used to sculpture the horse's training plan to its individual needs. The results from this study back up the suggestions of Rachen-Schöneich and Schöneich (2007) that stated that crooked conformation can affect the way of going and the stride pattern a horse prefers to use. Further work is needed to pinpoint further effects on racehorse laterality and whether additional conformation points can indicator a horse's laterality.

**Acknowledgements** The author would like to gratefully acknowledge the following National Hunt racehorse trainers for the use of their horses throughout this study; Kim Bailey, Alan King, Charlie Mann, Richard Phillips, Oliver Sherwood and Nigel Twiston-Davies.

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## A comparison of the response of equine heart rate to different equine exercise regimes

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**Introduction** The regular monitoring of heart rate (HR) in response to exercise can be a beneficial practice in terms of improving equine training programs. It is known that equine HR increases linearly with workload (Harris *et al.*, 2007) but the relationship between HR and exercise is usually determined using an equine treadmill, or a test completed on a track, access to which is often limited. Simple alternative methods which are highly reproducible and easy to complete could be of value. The aim of this study was to produce a simple method which compared the response of equine HR to different equine exercise regimes measured using an electronic monitor that is simple and easy to use. The simplicity of the method would mean that it can be completed by all equine owners/trainers and not limit equine cardiovascular research to only those who have access to an equine treadmill or outdoor track.

**Materials and methods** Five horses completed five different exercise regimes (TRT) in a replicated 5 X 5 Latin square design: TRT 1 - flatwork; TRT 2 - lungeing (work on a constant circle); TRT 3 - lungeing with a rider; TRT 4 - jumping in an indoor school and TRT 5 - riding outdoors in woodland. Each TRT lasted 30 minutes and consisted of ten minutes walk, ten minutes trot and ten minutes canter sequentially. Within each square horses completed successive exercise regimes over a period of two weeks with one or two days interval between completions of the TRT. The experiment was completed three times. Horses were of a similar size and fitness level and were offered the same basic diet of 6-8 kg concentrates daily and haylage *ad libitum*. One mare and four geldings were used. A polar HR monitoring system (Polar Vantage NV™, Polar Electro OY, Kempele, Finland), was used to measure HR from when the horse commenced at a walk and continued for the duration of each TRT. Recovery HR was recorded for ten minutes after exercise stopped as the horse was cooling down in walk. The monitoring system recorded HR every 15 seconds for the duration of each TRT and recovery period. Average HR was taken as an average of all HR recordings taken during the 30 minute TRT period. Basal HR was measured as an average of the first four HR recordings at the initiation of exercise. Statistical analysis was performed using Minitab (Version 15) using a General Linear Model testing effects of horse, square, period and TRT on average HR, basal HR, recovery HR (time taken for HR to decrease to basal HR level), time to reach maximum HR and maximum HR. Basal HR was used as a covariate for all responses except the basal HR mean values. When effects of TRT were significant a Tukey Pairwise Comparison test was used to determine differences between individual treatments.

**Results** Type of exercise has a significant effect on average and basal HR ( $P < 0.001$ ). There was no effect on the other variables. Individual horses had different average HR but the effect of horse was not significant for other variables.

**Table 1** Effects of exercise regimes on average and basal HR (beats per minute (bpm)) in horses. (n = 15 per TRT; different superscripts show differences between individual treatments).

	Treatment					SEM	P-Value
	1	2	3	4	5		
Average HR	99.1 <sup>ad</sup>	87.0 <sup>b</sup>	96.2 <sup>d</sup>	100.2 <sup>a</sup>	111.8 <sup>c</sup>	1.56	0.001
Basal HR	36.8 <sup>a</sup>	36.6 <sup>a</sup>	37.3 <sup>a</sup>	41.4 <sup>b</sup>	41.4 <sup>b</sup>	1.57	0.001

**Conclusions** An electronic HR monitor was a useful tool for monitoring HR response to exercise without specialist facilities. Of the exercise regimes compared, the highest average HR was measured during TRT 5 whilst TRT 1 was associated with the lowest. Basal HR was significantly higher during TRT 4 and 5, (table 1). Different ground surfaces, (Sloet van Oldruitenborgh-Osterbaan and Barnevald, 1995), different energy needs for different activities (Lawrence, 1997), or environmental factors (Harris *et al.*, 2007) are a few possible reasons for the differences in average and basal HR measured. Effects of excitability cannot be ruled out; expectation may explain the higher basal HR for TRT 4 and 5. Excitability can notably affect results when HR values are below 160 bpm (Harris *et al.*, 2007). Horses 1 and 5 had significantly different HR ( $P < 0.05$ ) (data not shown). Although horses used were of similar fitness levels, a detailed assessment of fitness may be beneficial in future work. With advancement in technology available for equine cardiovascular research, such as the use of global positioning systems, (Harris *et al.*, 2007), methods of assessing equine fitness and response to exercise will continue to improve and become more accessible for all members of the equine industry in the future.

**Acknowledgements** Dr. Darren Juniper, University of Reading, for advice and help on the completion of the statistical analysis

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## The influence of equine facial dermatoglyphic profiles on English and Irish Thoroughbred (*Equus caballus*) flat racehorse performance ratings

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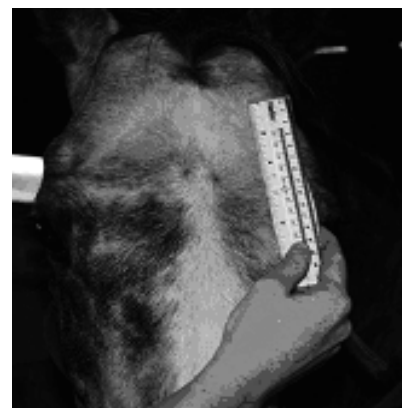
**Introduction** Relationships are established between dermatoglyph profiles and temperament, laterality and abnormal behaviour in multiple species. A further relationship between Thoroughbred dermatoglyph profiles and predisposition to exhibit non-ridden stereotypies has recently been demonstrated (Williams, 2009). The British Racing Industry exhibits high wastage of horses contributable to poor performance or injury (McGee, 2006). A non-invasive external indicator of performance could have substantial economic and welfare value. The study aimed to determine if facial dermatoglyph profiles (skin patterning: morphological or topographical parameters of trichoglyphs) would correlate to performance ratings for flat racing in Thoroughbred racehorses to establish their potential as indicators of performance.

**Materials and methods** The study population comprised English or Irish Thoroughbred horses (*Equus caballus*) (n=21) who were actively engaged in training for flat or National Hunt racing. All subjects included had attained racing post ratings (RPR) for flat racing in the UK and exhibited facial trichoglyphs. Horses presented with a mean age of  $6.81 \pm 3.28$  years, range 3-11 years, variable colour and a gender distribution of 15 (71.43%) geldings and 6 (28.57%) mares. A facial dermatoglyph profile was produced for each horse utilising previously validated methods (Murphy & Arkins, 2008). Individual trichoglyphs (Figure 1) were photographed, by the same experimenter, using a 10.2 mega-pixel Canon 400D digital camera (Adobe Photoshop: version 9.2) to enable remote analysis. Trichoglyph location in respect of vertical and horizontal position was recorded; orientation was identified as clockwise, counter-clockwise or radial. RPR data for the individuals' flat racing performance were collated from the Racing Post website; career best rating was selected to represent optimal performance. RPR interpret confounding variables (weight, distance and handicap) to produce an acknowledged comparable performance rating. The mean RPR for the sample population was calculated ( $77.52 \pm 25.12$ ) and individual RPR were ranked as <average or >average for the purpose of analysis. A series of Kruskal Wallis and Spearman Rank Correlations were performed to identify relationships between facial trichoglyph orientation, trichoglyph location, and trichoglyph orientation and location with RPR for flat racing.

**Results** Statistical analysis exposed few significant relationships. Trichoglyph position exhibited a positive correlation to less than average RPR ( $P=0.001$ ) in this population. A summary of the results obtained are presented in Table 1.

**Table 1** Summary of results

	Spearman Rank Correlation	Kruskal-Wallis
Position: <average RPR	$P=0.001$	$P>0.05$
Position >average RPR	$P>0.05$	$P>0.05$
Orientation <average RPR	$P>0.05$	$P>0.05$
Orientation > average RPR	$P>0.05$	$P>0.05$
Position & Orientation <average RPT	$P>0.05$	$P>0.05$
Position & Orientation >average RPT	$P>0.05$	$P>0.05$



**Figure 1** Facial trichoglyph

**Conclusion** Trichoglyph facial position appears to be a viable external indicator of phenotypic predisposition to poor performance levels in the English and Irish Thoroughbred engaged in flat racing. Further research within a wider population and with consideration to trichoglyph facial position, morphology, orientation and complete dermatoglyph profiles (whole body) is warranted.

**Acknowledgements** The Seale Hayne Educational Trust is acknowledged for funding this study and thanks are extended to the participating racehorse trainers and their owners for their support.

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## Factors that effect the success of embryo transfer in polo ponies

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**Introduction** Embryo transfer is becoming increasingly important as a modern reproductive technology in the equine industry. Benefits include the production of multiple offspring from one mare (donor) in the same year; the ability to allow athletic mares to continue competing without time spent breeding; the maintenance of top bloodlines (Pashen *et al*, 1993); avoidance of infertility; reduction in the risk of disease spread and, due to the above reasons, huge financial advantages (Noakes *et al*, 2001). This study aimed to assess factors suggested to be important in the success rate of this procedure.

**Materials and methods** The embryo transfer (ET) method used was modified from that described by Douglas (1982), Paschen (1985) and Sertich (1989) whereby an embryo from a genetically superior mare and stallion is transferred to a less valuable donor mare that will carry it to term. All procedures were carried out *in situ* inclusive of treatments used to aid natural oestrous cyclicity and prepare some mares' uterine environment as well as semen extraction and the artificial insemination prior to ET. The donor mares all carried a high proportion of Thoroughbred (TB) blood and were polo ponies (n= 135), aged 2.5 to 28 years, weighing 350-500kg. The stallions (n= 17) used were mostly TB, and all were registered with the Argentine Association of Breeders of Horses for Polo. They ranged in age from 3 to 28 years old and all had proven fertility. The recipient mares (n= 504) were Criollo, semi wild and handled minimally. The mares were selected on the basis of specific criteria related to age (3-9 years), size, body condition, conformation, type, temperament, and normality of the reproductive tract (as described in Paschen *et al*, 1993). If recipient mares did not retain a pregnancy and no abnormalities were detected they were reused. Synchronisation of oestrus cycles between donor and recipient mares ranged from -1, when the recipient ovulates the day before the donor, to +4 days when the recipient ovulates 4 days after the donor. This was primarily achieved through the natural cyclicity of the mares enabled by the ratio of donor to recipient mares. The main measurement of interest was pregnancy diagnosis; this was achieved by rectal palpation and ultrasonography. Success was defined as a positive pregnancy diagnosis 60 days post ovulation. Statistical analysis was carried out using SAS (SAS Institute Inc). Initially, individual variables were analysed using linear regression. In the final statistical model, data was entered into a logistic regression output incorporating all significant variables as a combination.

**Results** The results of the linear regressions are shown in table 1. The following were also significant, but were not entered into the logistic regression due to low numbers of observations. The stallion has a significant ( $p < 0.001$ ,  $df = 47$ ) effect on embryonic development and growth. There was a significant interaction between the location within the ovaries (bilateral/unilateral) and the relative timing of each ovulation (synchronised/unsynchronised) ( $p < 0.001$ ,  $df = 3$ ). The ET clinician was an important factor with variation in success rate of between 27% and 82%.

**Table 1** The effects of individual variables on the success of ET (linear regression)

Variable	Significance	Relationship	
Age of mare	$P < 0.001$	Negative	After the logistic regression, 3 variables remained significant $P < 0.001$ (highlighted in Table 1). The logistic regression suggests the following equation to predict success: $1.5001 + (x * -0.0538) + (y * -0.4038) + (z * 2.6973)$ Where x = age in years, y = ovulation to flush interval in days and z = number of embryos. A probability score is thus generated.
Ovulation to flush interval	$p < 0.001$	Negative	
Embryo number	$p < 0.001$	Positive	
Time embryo stored	NS		<b>Conclusion</b> It was established that there should be a higher success rate for younger mares, a shorter ovulation to flush interval and greater embryo number produced (ovulation). Weather (temperature and rainfall) were not significant variables which was unexpected due to the nature of the recipient management and the nutritional content of their grazing. An
Presence of uterine debris	$P = 0.011$	Negative	
Flush date	$P = 0.008$	Negative	
Hour of day	$P = 0.040$	Positive	experienced clinician and proven stallion is required to optimise this procedure. If the success of ET can be improved there are huge economical implications within all performance equine industries as it enables use of the best genetics in competitive careers as well as for breeding.
AI to flush interval	$P = 0.029$	Negative	
Environmental temperature	NS		
Flushes per mare	$P = 0.016$	Negative	
Artificial Insemination	NS		
Rainfall	NS		

experienced clinician and proven stallion is required to optimise this procedure. If the success of ET can be improved there are huge economical implications within all performance equine industries as it enables use of the best genetics in competitive careers as well as for breeding.

## Acknowledgements

Diego Rodriguez Piola (MV), the director and head veterinarian of Ellerstina's ET centre "La Grappa": (Casbas, Trenque Lauquen, Buenos Aires, Argentina); Angela Beresford (PhD) and Amber Clutton-Brock (MRCVS).

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## Characterisation of linkage disequilibrium and subsequent estimation of effective population size in Thoroughbred horses using single nucleotide polymorphism markers

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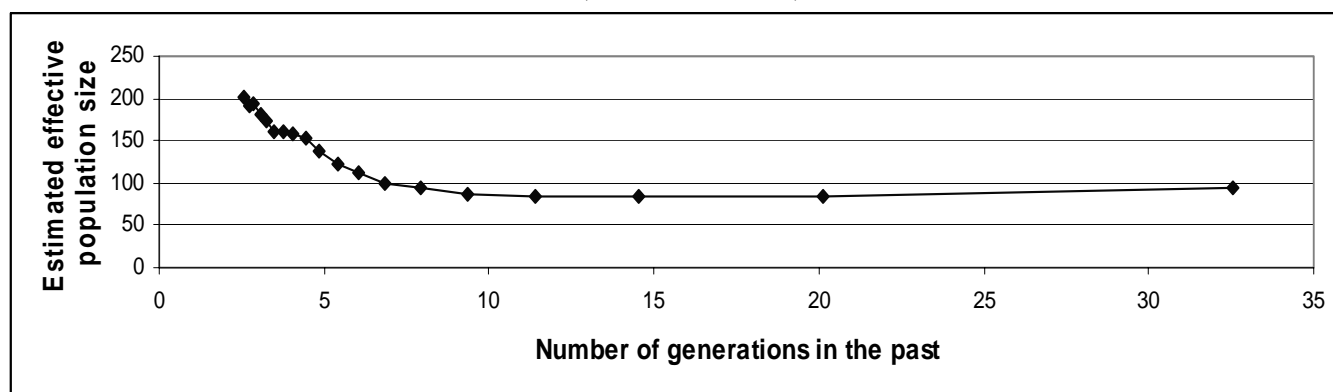
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**Introduction** The extent of linkage disequilibrium (LD) within a population is important when determining the number of markers required for genetic analyses such as association studies and can also impact on the likely accuracy of both marker-assisted and genomic selection. It has been proposed that the average LD over different genomic distances can be used to infer ancestral effective population size (de Roos *et al.* 2008; Tenesa *et al.* 2007). Whilst the extent of genome-wide LD has been investigated in a variety of livestock species, including cattle, pigs and chickens, this paper represents one of the first such studies with respect to the equine.

**Materials and methods** The data for this study consisted Illumina Equine SNP50 BeadChip genotype data for 817 UK Thoroughbred horses. 28% of the 54,602 available single nucleotide polymorphism (SNP) markers were excluded due to monomorphism, poor genotyping quality (genotyping in <95% of samples), deviation from Hardy-Weinberg equilibrium ( $p < 0.0001$ ) and low minor allele frequencies ( $< 0.05$ ). The LD measure  $r^2$  was calculated for all remaining syntenic markers pairs and expressed as a function of genomic distance for each chromosome. Marker pairs were broadly divided into three distance (Mb) categories: [0,0.1], [0.1,20] and [ $>20$ ]. Within each category, marker pairs were ordered on the basis of distance and divided into 20 equally sized bins, each containing markers of approximately equal pairwise genomic distance. The effective population size at different stages in the past was estimated from the average  $r^2$  and average marker distance of each of the bins. The equation used for this estimation was  $N_T = (1/4c(1/r^2 - 1))$ , where  $N_T$  is the effective population  $T$  generations ago, and  $c$  is the marker distance in Morgans, assuming 1Mb=1cM, and  $T = 1/2c$  (Hayes *et al.* 2003; de Roos *et al.* 2008; Tenesa *et al.* 2007).

**Results** Average  $r^2$  decreased with increasing genomic distance, as expected. The decline in LD appears to be less rapid than that seen in cattle populations, with mean  $r^2$  values at a mean distance between markers approaching 0.1Mb maintained above 0.2 for nearly all chromosomes. By way of example, in the case of Chromosome 1, at mean marker distances of 10kb, 40kb and 100kb, the mean  $r^2$  values ( $\pm$ SE) were calculated as 0.48 (0.022), 0.34 (0.018) and 0.31 (0.017), respectively. The results of most interest in terms of the prediction of past effective population size, were those produced in the distance category 0.1Mb to 20.0Mb; the number of generations ago calculated from marker distances in this group ranged from two to 100 generations in the past and the Thoroughbred population was formed around 300 years ago (representing approximately 25-30 generations). The results for Chromosome 1 can be seen in Figure 1. The pattern of  $N_T$  observed is much different to that seen in cattle (de Roos *et al.* 2008).



**Figure 1** Effective population size along the population history, estimated from the average  $r^2$  at different marker distances of markers on chromosome 1 (the number of generations in the past was truncated at 35).

**Conclusion** In the Thoroughbred population studied, LD declined with increasing genomic distance but at a less rapid rate than that seen in cattle populations. Estimations of ancestral effective population size suggest that the breed underwent a population expansion, relative to its starting population size, beginning approximately ten generations ago; a pedigree analysis would be useful to validate this hypothesis. The potential effect of population stratification on these analyses has not yet been investigated but is of interest.

**Acknowledgements** We thank the BBSRC, BEF and Biosciences KTN (formerly Genesis Faraday) for funding.

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## Genetic evaluation of sport horses for performance in Eventing competitions in Great Britain

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**Introduction** Eventing is the equestrian sport in which horse and rider compete in each of the three individual disciplines – dressage, show jumping and cross-country. In Great Britain eventing competitions are graded – prenovice (P), novice (N), intermediate (I) and advanced (A). Genetic evaluations for eventing are rarely performed. Ideally, an evaluation would evaluate the individual grades of competition separately. Disciplines and grades could then be combined to give an overall evaluation, potentially allowing for different heritabilities and competition weightings. The objective of this study was to estimate the variances and covariances (3 matrices of 78 components, i.e. 234 components in total) required by industry for the prediction of breeding values for eventing.

### Materials and methods

Eventing competition results were obtained from British Eventing. Competition results between 1999 and 2008 were analysed. Penalty points awarded for each discipline were transformed into a normal score for the competition class. Variance components for random effects (sire, horse's permanent environment and rider) were estimated, while allowing for the effect of fixed variables on the horse's performance. Horse sex, age (linear and quadratic covariates), and competition class were included as fixed effects. A sire model was implemented. This was performed using a series (n=66) of bivariate mixed effects models and Residual Maximum Likelihood (REML) in ASReml (Gilmour *et al.*, 2006), in which all combinations of discipline and grade were fitted in turn. Heritabilities of the 12 traits, genetic correlations, rider and horse permanent environment correlations were estimated.

### Results

19829 horses competed in a total of 6875 competitions. The total number of records was 345067 but eliminations during the competition resulted in less records for show jumping (n=330092) and cross-country (n=301320). 3017 sires were represented and 11841 riders. Heritabilities for all grades in all disciplines were significant, with the exception of cross-country advanced and intermediate (Table 1). Heritabilities for show jumping were highest (8.2-15.7%) followed by dressage (7.1-9.0%). Heritabilities for cross-country novice and prenovice were low (1.4%).

Within discipline, for dressage and show jumping individually, genetic correlations were high 0.63-0.99. Between disciplines, in general, genetic correlations were not significant, indicating that no discipline was a good predictor of success (due to genetics) in another discipline.

The rider explained approximately 25% of the total phenotypic variance for each of the dressage phases, and was highest in advanced. For show jumping and cross-country the rider explained approximately 10% of the phenotypic variance. The effect of the horse's permanent environment was also greatest for dressage (16 – 21% of total phenotypic variance). For show jumping and cross-country the permanent environmental variance was significant for most discipline-grades, accounting for 5 - 9% of total phenotypic variance.

Horse sex and age had significant effects on performance.

**Table 1** Heritabilities for each of the discipline-grades

Discipline-grade	heritability (SE)
Dressage A	0.090 (0.041)*
Dressage I	0.071 (0.017)*
Dressage N	0.075 (0.012)*
Dressage P	0.083 (0.010)*
Show jumping A	0.157 (0.039)*
Show jumping I	0.082 (0.014)*
Show jumping N	0.096 (0.011)*
Show jumping P	0.100 (0.009)*
Cross-country A	0.026 (0.020)
Cross-country I	0.003 (0.005)
Cross-country N	0.014 (0.005)*
Cross-country P	0.014 (0.004)*

\* indicates significant values

### Conclusions

Heritabilities for each of the eventing disciplines at every grade were significant, with the exception of the highest grades of cross-country. Heritabilities for show-jumping were 8.2-15.7%, for dressage 7.1-9.0%, and for cross-country novice and prenovice were 1.4%. Therefore there is potential to select for performance in eventing in the horse population competing in GB.

Variance-covariance matrices have been estimated, ready for use by the industry for prediction of breeding values. This will enable a multivariate analysis of all traits, yielding 12 breeding values for each horse. It is recommended that values are incorporated into an index value - overall, or possibly for each discipline - although all 12 values could potentially be presented. The advantages of this approach are that all information is included and that breeding values will be predicted for all discipline-grades irrespective of whether the horse has competed at that grade or not (based on genetic correlations between grades and performance of relatives).

**Acknowledgements** BBSRC, British Equestrian Federation and Genesis Faraday for financial support. Industry partners and sponsors BEF and British Eventing.

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## Increase in accuracy using multi-trait genomic breeding value estimation

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**Introduction** Genomic selection is becoming common practice in animal breeding. It uses genome-wide dense marker maps, to accurately predict the genetic ability of animals, without the need to record phenotypic performance from the animal itself or from close relatives. Presented applications of genomic selection have mainly been limited to implementations where genomic breeding values are estimated using single trait models. A major breakthrough in traditional breeding value estimation was the application of multi-trait breeding value estimation, for instance to combine mastitis and somatic cell count information. Therefore, our objective was to develop multi-trait genomic breeding value estimation methods.

**Materials and methods** Four different multi-trait models were considered: 1) a model with a traditional pedigree based relationship matrix (A-BLUP), 2) a model where the traditional pedigree based relationship matrix is replaced by a genomic relationship matrix based on markers (G-BLUP) (e.g. VanRaden, 2008), 3) a model that includes SNP effects drawn from a single distribution (BayesA), and 4) a model that includes SNP effects drawn from two distributions to distinguish between SNPs that are (not) associated with QTL (BayesC) (a single trait implementation is presented by Calus *et al.*, (2008)). The second model assumes equal contribution of each SNP to the total additive genetic (co)variance. Model 3 and 4 explicitly estimate the (co)variance of the SNP effects, per sampled distribution of SNP effects. The additive genetic (co)variance matrix was used as prior information for the SNP variances. The four models were applied to two simulated traits with heritabilities of 0.9 and 0.6, to reflect e.g. de-regressed proofs, having a genetic correlation of 0.2, 0.5 or 0.8 between them. In the simulated data, 2 generations of 500 animals each were available with phenotypes for both traits, and thus formed the reference population. Two additional generations of 500 animals were used as validation data, e.g. their breeding values were predicted while they had no phenotypic information of their own or from offspring in the model.

**Results** Increases in accuracy, due to applying multi-trait instead of single trait genomic breeding value estimation, depended on the genetic correlation between the traits. At a genetic correlation of 0.8, the accuracy of the breeding values of animals without phenotypes for the second trait increased by 0.03 to 0.07 (see Table 1). At a genetic correlation of 0.5, this increase ranged from 0.01 to 0.04. The highest increase was found using model BayesC, followed by BayesA, G-BLUP, and A-BLUP respectively. Regression of the simulated on the estimated breeding values showed that the estimated breeding values were generally unbiased.

**Table 1** Increase in accuracies for breeding values of the first generation of animals without phenotypes, obtained from multitrait versus single trait models.

Model	Genetic correlation	
	0.5	0.8
A-BLUP	0.009	0.034
G-BLUP	0.017	0.052
BayesA	0.024	0.056
BayesC	0.040	0.071

**Conclusions** In a scenario where all animals in the reference population have phenotypes for all traits, multi-trait genomic breeding value estimation showed an increase of up to 0.07 in accuracy for juvenile animals, compared to single trait analysis. Thus, the application of multi-trait genomic selection in this scenario proved to be more accurate than single trait genomic selection. In practice, higher accuracy increases are expected when one of the traits is measured on some of the animals only.

**Acknowledgements** The authors acknowledge funding from Hendrix Genetics, CRV, The Netherlands Organisation for Scientific Research (NWO – Casimir), and RobustMilk.

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## Response to genomic selection in the Scottish Blackface breeding programme

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**Introduction** The use of selection indices in the Scottish Blackface (SBF) breeding programme results in significant economic gains at farm and national levels (Amer *et al.*, 2007; Conington *et al.*, 2008). Recent developments in the ovine genome sequence have made dense marker panels available allowing for the prediction of genomic merit and the implementation of genomic selection (GS) in sheep. The prediction of genomic merit relies on establishing the associations between phenotypes and the dense marker panels in training populations. The aim of this study was to assess the impact on the response to selection of including genomic predictions in the SBF current breeding programme and in an alternative young ram programme considering different training population sizes.

**Materials and methods** Selection index theory was applied to predict the responses to selection for scenarios with and without the inclusion of genomic predictions. The breeding goal comprised lamb (carcass fat and lean weights and weaning weight) and maternal traits (mature size, litter weight weaned and litter size reared) and their economic values as reported by Conington *et al.* (2008). The recorded traits included the same maternal traits as in the breeding goal together with muscle and fat depths by ultrasound scanning and body weight at scanning. Phenotypic and genetic parameters were those used in the SBF national genetic evaluation (NGE). Breeding population parameters were calculated using the SBF NGE database. Two breeding program structures were considered: (i) the current breeding programme (CBP) in which young and older rams are used, and (ii) a young ram programme (YRP) which only included the new rams with no progeny information. Proportions of animals selected and generation intervals for rams and ewes are presented in Table 1. The effect of GS was considered by combining the conventional estimated breeding values (EBV) and the genomic EBV (GEBV) in the selection indices. The GEBV accuracies were calculated as a function of the heritabilities of the traits used in SBF NGE, the number of loci affecting the traits, the number of phenotypic records in the training population (TRP) and the effective population size ( $N_e$ ) (Daetwyler *et al.* 2008; Goddard, 2009). In this study the effects of  $N_e$  of 300 and 500 animals and different training population sizes were evaluated. Genetic and phenotypic correlation matrices including EBVs and GEBVs were calculated based on the approach proposed by Dekkers (2007).

**Results** Table 1 shows the economic annual responses to GS in combination with the current strategy expressed relative to the response observed in the current program (£47/year/100 ewes). The decrease of the response in the YRP compared with the CBP can be explained by the lower accuracy of EBVs (due to less phenotypic information) but this is partially traded-off by the lower generation interval. The inclusion of GEBVs increased the responses in both CBP and YRP. For training populations of 3000 animals, the response in the YRP was very similar to the one achieved in the CBP. Larger training population sizes increase the responses and the rate of improvement depends on

$N_e$  and breeding programme structure. For a given  $N_e$ , a higher increase of the annual response was observed in the YRP with the rise in training population sizes. Higher accuracies of GEBV associated with larger training population sizes had a more significant impact on the total accuracies in the young rams because of the lower EBV accuracies compared to older rams. Although similar trends were observed for both  $N_e$ , the improvement rate of the economic response to GS was higher with a  $N_e$  of 300 animals.

**Conclusions** The genetic improvement to farm profit by the inclusion of GS in the SBF CBP ranged between 1 to 14%. Proportionally greater increases were found with larger training populations. Optimising GS strategy should consider improved responses at national scale and training population costs, as well as the real undetermined SBF population size. Optimal genetic and economic responses using GS may imply the re-definition of breeding programmes.

**Acknowledgements** We are grateful to Scottish Government, Defra and QMS for funding this work. We thank Peter Amer (Abacus Biotech, New Zealand) for the index selection programme made available to us.

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**Table 1** SBF breeding programme with and without genomic markers

Parameters	Current programme		Young ram programme		
Proportion selected	Rams, 0.04; Ewes, 0.50		Rams, 0.04; Ewes, 0.50		
Generation interval	Rams, 2.30; Ewes, 3.63		Rams, 1.50; Ewes, 3.63		
Relative economic annual response to selection (%)					
Method	TRP	Ne=300	Ne=500	Ne=300	Ne=500
EBV		100		87	
EBV	1000	101	100	94	91
+	2000	105	102	101	96
GEBV	3000	108	105	107	100
	4000	111	107	112	104
	5000	114	109	117	108

## Discovery of novel single nucleotide polymorphisms in the bovine growth hormone receptor gene and their association with performance traits in Holstein-Friesian cattle in Ireland

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**Introduction** Growth hormone (GH), also known as somatotrophin, is a key molecule in many of the physiological processes that are related to cattle performance traits such as milk production (Etherton and Bauman, 1998). The actions of GH are mediated by binding to the GH receptor (GHR). Variation in the bovine *GHR* gene sequence has been shown to be associated with a number of performance traits in cattle. For example, the non-synonymous single nucleotide polymorphism (SNP) *F279Y* in exon 8 which codes for the transmembrane domain of *GHR* is widely reported to be associated with milk yield and composition (Blott *et al.* 2003). However, there is little information on the associations of bovine *GHR* SNPs with performance traits in the Irish herd. The present study aimed to discover new SNPs in the bovine *GHR* gene and quantify the associations of these, alongside previously published bovine *GHR* SNPs, with performance traits in Holstein-Friesian cattle in Ireland.

**Materials and methods** For SNP discovery, ~1 kb of sequence, surrounding bovine SNPs reported in the Btau\_4.0 build of the bovine genome ENSEMBL database, was amplified by polymerase chain reaction (PCR) of genomic DNA extracted from unique blood samples from 22 cattle of 5 different breeds (4 Simmenthal, 4 Angus x Holstein, 4 Belgian Blue x Holstein, 6 Holstein and 4 Charolais). PCR amplicons were sequenced bi-directionally using the Sanger method. To identify novel SNPs, 22 forward and reverse sequences for 19 amplified ~1 kb bovine *GHR* gene regions were aligned using ClustalW version 2.0. Chromatograms were analysed using Chromas Lite to confirm identity of the SNPs and determine whether they were homozygous or heterozygous. Genotyping for 32 putative novel bovine *GHR* SNPs and 7 previously published bovine *GHR* SNPs was carried out on genomic DNA from 848 Holstein-Friesian sires using the with the iPLEX Gold assay (Sequenom, San Diego, CA, USA). Predicted transmitting abilities (PTAs) and their associated reliabilities for traits that were evaluated by the Irish Cattle Breeding Federation in the January 2009 domestic genetic evaluations, were available for inclusion in the analysis. PTAs were de-regressed and parental contribution to the reliability of each PTA was removed. Only sires with a reliability, less parental contribution, of >60% were retained for inclusion in the association analysis. The association between each SNP and performance trait was quantified using weighted mixed models in ASREML with individual included as a random effect, and average expected relationships among individuals accounted for through the numerator relationship matrix. Year of birth (divided into 5 yearly intervals) and percent Holstein of the individual sire were included as fixed effects in the model. The dependent variable was de-regressed PTA, weighted by respective reliability less the parental contribution. Genotype was included in the analysis as a continuous variable coded as the number of copies of a given allele. A multiple regression mixed model (MRMM) was developed by backward elimination of the non-significant ( $P > 0.05$ ) segregating SNPs.

**Results** In total, 32 putative novel SNPs (30 transitions and 2 transversions, all biallelic), spanning the majority of the ~300 kb bovine *GHR* gene were discovered by resequencing ~19 kb of the gene in 22 animals of different breeds. Following genotyping of 848 Holstein-Friesians for the 32 putative novel SNPs and 7 previously published SNPs, 25 putative novel SNPs and one previously published SNP were eliminated because they were either monomorphic, had a minor allele frequency (MAF) of <2%, had abnormally high heterozygosity (>99.9%), or were in complete linkage disequilibrium (LD) with another SNP. After MRMM analysis of the 13 remaining segregating SNPs (MAF 4% - 48%), 11 SNPs (including 7 novel SNPs) remained associated with at least one of 24 performance traits including traits for milk, growth, size and survival. Of particular interest was a novel SNP (ss159831013) in the *GHR* 5' non-coding region (238 kb away from *F279Y*) which was not in linkage disequilibrium (LD) with *F279Y* and remained associated with milk yield after MRMM analysis. This suggests that the ss159831013-milk yield association is independent of the *F279Y*-milk yield association. In addition, following MRMM analysis, there were associations of 5 novel and 5 previously published bovine *GHR* SNPs with 11 growth and size traits. ss159831013 and a previously published SNP (AF126288-149) in the 5' non-coding region showed associations with progeny carcass weight and angularity that both remained following MRMM analysis. Four previously published SNPs in the coding region (1 in exon 8 and 3 in exon 10) remained associated with 9 size and growth traits (1 SNP per trait) after MRMM analysis. One of these (*H545*) is a synonymous SNP in the bovine *GHR* exon 10 and was associated with 7 size and growth traits. Association of *H545* with size and growth traits has not been reported previously.

**Conclusions** Newly discovered SNPs in the bovine *GHR* gene show associations with performance traits in Holstein-Friesian cattle in Ireland. These associations are independent of previously published bovine *GHR* SNPs and, therefore, may be of use as novel molecular breeding markers.

**Acknowledgements** The authors acknowledge the Irish Cattle Breeding Federation, the Irish Dairy Research Trust and the Research Stimulus Fund

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## The effect of the breeding value status of pedigree Suffolk and Charollais sires on lamb growth under commercial farm conditions

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**Introduction** The objective of the Pedigree Sheep Breed Improvement Programme (PSBIP), operated by the Department of Agriculture Fisheries and Food, is to increase lean tissue growth rate in breeds used as terminal sires (Murphy *et al.* 1999). Genetic evaluation (an index called LMI; base = 100, s.d. = 30) is based on live weight and ultrasonic muscle and fat depths recorded at ~120 days of age. The genetic correlation between the index and 120 day weight is ~0.7. Participation in the PSBIP by pedigree breeders has declined over recent years reflecting the absence of any clear market signal favouring rams with LMI data. The latter is assumed to reflect a perception that such rams do not enhance progeny performance compared with rams without LMI information. This study was designed to evaluate the growth benefits from rams with a positive LMI index under commercial farm conditions.

**Materials and methods** Pedigree Suffolk and Charollais rams were purchased at the principal breed society sales (and thus can be taken as good representatives of the breeds in question). The rams purchased either had high LMI values (at least 110) or had no breeding value information (non-LMI rams). The latter rams had to be from flocks that had not been in the PSBIP and the ram's sire could not be from a flock that had participated in the PSBIP. The mean LMI values were 145 (n = 27) for Charollais and 161 (n = 21) for Suffolk. The number of non-LMI rams was 28 and 24 for Charollais and Suffolk, respectively. Rams were used on one of 6 commercial flocks and both LMI and non-LMI rams from the same breed were represented in each flock each year (2005 to 2008). The breed(s) used on any particular flock was constrained by flock-owner preference. Single-sire mating groups were formed by random assignment of ewes within age (2-tooth or older) categories and joining was usually limited to 17 days. Ewe identities were provided by the National Sheep Identification System. All live lambs were tagged within 24 h of birth when dam identity, sex, birth date and live weight were recorded. These details were also recorded for dead lambs. Lambs were weighed at around 5 weeks of age and at weaning (about 14 weeks of age), when ultrasonic muscle and fat depths were recorded. Available lambs were weighed and scanned again at about 1 month post weaning. Lambs were drafted for sale as per the individual farm practice. Lamb growth data were analysed (Proc MIXED of SAS) using a model with fixed effects for year, farm, dam age, sex, birth and rearing type, sire breed and index category (LMI or non-LMI). Individual ram effects were fitted as random within breed by index sub groups.

**Results** The mean LMI for the Charollais and Suffolk rams used were 1.5 and 2.0 s.d. above the base for the respective breeds and, thus, represented the best 15% and 6% of the populations, respectively. Total progeny from LMI and non-LMI rams were 1657 and 2363 for Charollais, and 1648 and 1562 for Suffolk, respectively. The growth data for lambs are summarised in Table 1 together with estimates of the difference between LMI and non-LMI progeny. The only significant difference detected between progeny of LMI and non-LMI sires was for weight at 5 weeks for the Charollais breed (LMI significantly lighter than non-LMI). The variance components for sires yielded  $h^2$  estimates (s.e. ~0.02 in all cases), pooled across breeds, of 0.07, 0.09, 0.08 and 0.08 for birth weight, growth rate to weaning, weaning weight and live weight at 120 days, respectively. The heritability estimate for weaning weight is close to the estimate of  $0.1 \pm 0.05$  reported by Hanrahan (1999) using data obtained under research flock conditions.

**Table 1** Effect of sire lean meat index (LMI) status on progeny growth

Factor	Growth trait				
	Birth weight (kg)	Live weight (kg) at			Growth rate (g/day)
		5 weeks	14 weeks	120 days	
Birth Type					
Single	5.7	17.2	34.9	39.1	301
Twin	4.8	14.3	30.7	34.9	264
LMI versus non-LMI					
Charollais	-0.0 ± 0.04	-0.3* ± 0.11	-0.3 ± 0.25	-0.4 ±0.27	-3 ± 2.4
Suffolk	+0.1 ± 0.05	-0.0 ± 0.18	+0.3 ± 0.27	+0.3 ±0.30	+3 ± 2.7
Farm effect					
Range for twins (kg)	4.3-5.1	13-16	28-35	33-39	235-306

**Conclusions** The absence of evidence for a significant benefit to lamb growth from using above average LMI rams is consistent with the lack of positive market signals for LMI rams from commercial producers. The results highlight the importance of the current redevelopment of the genetic evaluation system for Irish sheep, and the need to avoid unrealistic projection of expected genetic gain.

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## Using standardised data as a means to overcome the limitations of fitting polynomials in a genetic analysis of lifetime ewe weights using random regression

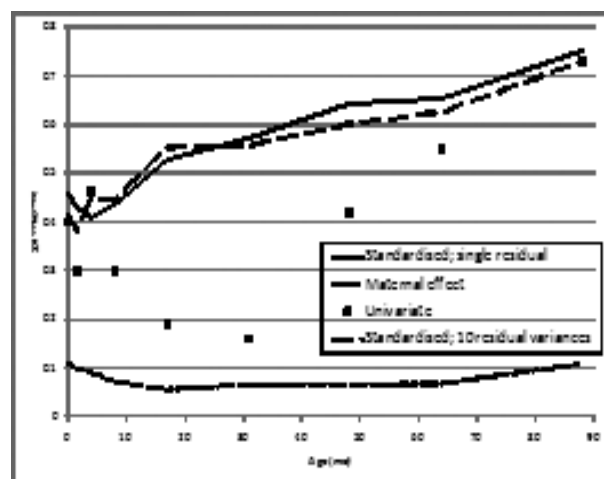
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**Introduction** It is commonly thought that by selecting for increased early lamb growth the mature weight of the breeding ewe population will increase. This notion has rarely been tested using measurements taken on the same animals since most meat animals are slaughtered at a young age and most breeding sheep are rarely weighed throughout their lives. Random regression is commonly used to study the genetics of a trait repeatedly measured on a linear scale e.g. time. In such analyses an overall curve is used to 'correct' the data for variation in the trait on the linear scale and random regression curves are fitted for each animal to investigate how additive variance changes along this scale. Polynomials are commonly used for both regressions (fixed and random) but such methods suffer from drawbacks. Firstly there may be heterogeneous variance over the linear scale and a polynomial may not best describe the overall trend in the trait. In this research the use of standardised data was investigated as a means to circumvent both problems in an analysis of lifetime liveweight in ewes and the results used to investigate the genetics of lifetime ewe weights.

**Materials and methods** The data used in this study came from a fully recorded and pedigreed flock of 600 ewes weighed three-times a year (mating, lambing and weaning) and also as lambs at birth, 6 and 16-weeks of age. Data were available for 1,390 ewes born over a 13-year period. Research aimed at finding an appropriate model to describe lifetime ewe weight (Pollott and Galea, 2010) highlighted the lack of fit for the commonly used polynomial approach and no suitable linear model was found to use in a random regression. In addition the variance of liveweight was heterogeneous over the 7-year lifespan of the ewes and using (potentially) 21 different variance components was not an option due to program limitations (Gilmour *et al.*, 2006). To overcome both problems the data was standardised for each of the 21 weights after Pollott and Greeff (2004). Standardisation was achieved by calculating the mean and SD for each of the 21 weighing times. Each individual ewe weight was then 'standardised' by subtracting the appropriate mean and dividing by the appropriate SD. The effect of this was to give each weight a similar mean and variance. The standardised data were analysed using a mixed model in ASREML (Gilmour *et al.*, 2006) fitting birth type, birth year and dam age as fixed effects, and genetic and maternal random regression terms for ewe and dam respectively. Comparative results were calculated using a univariate animal model at each weight, and by setting ASREML to calculate a different residual variance for 10 of the most variable weights. Heritability values were calculated as the ratio of the ewe variance to the phenotypic variance, where the phenotypic variance was the sum of the ewe and residual variances or the sum of the ewe, residual and dam variances as appropriate. Standard errors were calculated as described in Gilmour *et al.* (2006). The covariance functions derived from these analyses were used to investigate the change in genetic correlation between young and older ages.



**Figure 1** The heritability of liveweight calculated by 3 different methods.

**Results** The results of fitting the various models are summarised in the Figure as heritability values. The general trend in heritabilities was to increase with age but some differences were found in early life. When fitting a single residual and the maternal effect, 6-week weight heritability was lower than that at birth. The univariate analyses showed a marked fall in heritability after first mating but rose again after about 3 years of age. Both standardised random regression analyses showed a similar pattern of heritability and the change in residual variance with age made little difference to the results and were greater than the corresponding univariate estimate. The genetic correlations shown in Table 1 show a very low level of association between early lamb weights and a sample of weights from the same animals as mature ewes. Even at 4 months of age, lambs only had a genetic correlation of 0.24 with weight at 2 years; at older ages this correlation was reduced.

**Table 1** The genetic correlation between lamb weights and the mature weight of the ewe.

	Mating 2	Lambing 2	Lambing 4	Lambing 6	Mating 7
Birth	0.11	0.10	0.11	0.06	0.04
6 weeks	0.16	0.14	0.12	0.09	0.06
16 weeks	0.24	0.21	0.14	0.13	0.10

**Conclusions** Using standardised data is a suitable method for overcoming a poorly fitting overall regression model in random regression analyses. Using this method it was apparent that early lamb weight was not correlated with mature ewe weight to any great degree.

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## Phenotypic performance of progeny from UK sires selected on female fertility beef EBVs

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**Introduction** Growth and carcass trait EBVs have been produced for the UK beef industry since the 1990's, with maternal 200 day milk being the only EBV available for female fertility (FF). To provide the UK beef industry with direct and maternal EBVs for FF traits a research project was undertaken to consider trait definitions, model development and genetic parameter estimation (Roughsedge *et al.*, 2005a). Limousin heritability estimates from the study were generally low, ranging from 0.04 (calving interval (CI)) to 0.27 (age at first calving (AFC)). Being sex-limited traits means that sires have to wait until their daughters reproduce until performance information is available for a sire. Consequently, the sire is often approximately 5 years of age by the time daughter performance records for FF traits are available for use in genetic evaluation. For carcass traits, the sires own performance information is known at approximately 1-2 years of age. The generally low heritabilities and performance records of daughters only available late in life means that sires are generally older when they receive accurate FF EBVs. Despite these challenges, significant genetic improvement for FF traits is achievable (Roughsedge *et al.*, 2005b). In 2007 the EGENES/Signet genetic evaluations incorporated for the first time EBVs for AFC, CI, calving ease (direct and maternal components), life span and gestation length (direct and maternal components) (Coffey *et al.* 2007). However, feedback from industry suggests that these FF EBVs have been under utilised in the UK beef industry. The aim of this work was to demonstrate the value of the FF EBVs for breeding replacement cows.

**Materials and methods** A 2009 routine Limousin genetic evaluation was used to select sire subsets representative of the distribution tails for each trait. To ensure sufficient data only sires with relatively high accuracies were considered. There were almost 25,000 sires recorded in total, with 10,220 and 4,278 sires having accuracies greater than 50%, respectively for AFC and CI. From these sires and for each trait, the 200 best and worst ranked sires were selected. To evaluate selected sire subsets, phenotypic records from contemporary groups (CG) containing progeny of selected sires were considered. The CG definitions were the same as in the national evaluation and were based on the birth herd and date of birth for AFC and calving herd, date of first calving and a six month season for CI. The individual phenotypic records were deviated from the CG mean. These deviations from the mean were then compared for the different sire subset's progeny. These deviations were only within the CGs and were not adjusted for other effects (i.e. age effects) in the model. A 'historic' evaluation was undertaken using current models and parameters but with data truncated from 1999. This run was then used to compare the EBVs of high accuracy sires with when they were younger and less accurate.

**Results** Table 1 provides EBV summaries of selected sires and progeny phenotypic deviations from CG means. For example, the CI EBV spanned 43 days for high accuracy sires, with 23.2 days being the average difference between sires identified as being the best and worst for CI. A daughter from a best sire had a CI on average 7 days shorter than her CG, while progeny of a worst sire had a calving interval on average 9 days later than her CG. Similar trends were observed for all the FF traits (results not shown). Between 50 and 71 of selected sires were aged 0-5 years in the 1999 run. These sires were ranked the same at both time points but with greater variation (because EBVs less accurate) when they were young bulls compared to when they were high accuracy older sires. The best sires had AFC and CI EBVs of -0.08 (0.08) and -2.1 (5.0), respectively as young bulls and the worst sires had AFC and CI EBVs of 0.11 (0.10) and 4.6 (4.0), respectively as young bulls.

**Table 1** Summary of the female fertility EBVs for subsets of high accuracy sires representative of the best and worst and their progenies mean phenotypic deviations (PMPD) from contemporary group means

Trait *	Selected 200 best sires			Selected 200 worst sires		
	EBV Mean (Range)	N progeny	PMPD (std)	EBV Mean (Range)	N progeny	PMPD (std)
AFC	-0.16 (-0.33 to -0.13)	1973	-21 ( 101)	0.23 (0.19 to 0.33)	2349	17 (110)
CI	-9.8 (-19.5 to -7.0 )	2938	-7 ( 41)	13.4 (10.9 to 23.5)	3260	9 ( 48)

\* AFC = Age at first calf (score based on days (phenotype in days)), CI = Calving interval (days)

**Conclusions** This work shows that progeny phenotypes from high accuracy sires are representative of FF EBVs from the UK beef evaluations. Selection using the UK FF EBVs allows breeders to breed for cows that calve earlier and easier, have shorter calving intervals, increased longevity and improved milking abilities. However, this work has highlighted an important challenge for the UK beef industry to consider. The lower heritability and late in life performance measures of the FF traits mean that sires are older (5+years) before accurate EBVs are available. Therefore selection strategies for FF traits need to be a compromise between reduced generation intervals (i.e. selecting younger bulls) and increased EBV accuracy (i.e. selecting older more accurate bulls).

**Acknowledgements** Thank you to QMS for funding this project and Signet performance recording for the use of the data.

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## Genetic parameters for animal price and live-weight from routinely collected data at livestock marts

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**Introduction** Most international dairy breeding programmes have selected for increased milk production. However, beef output, such as calves and cull cows, remain an important financial contribution to a dairy farm. Van der Werf *et al.* (1998) reported that 10 to 20% of the gross income of a dairy farm is from the sale of calves and cull cows. Possible breeding goal traits to reflect beef revenue from dairy enterprises include calf price and cull cow value. However, there is a paucity of studies that have attempted to quantify the genetic variation present in calf and cull cow value in dairy herds, due mainly to a lack of available phenotypes. The objective of this study was to estimate phenotypic and genetic parameters for calf price, cow live-weight and cow price.

**Materials and methods** A total of 2,967,791 live-weight and/or animal value records from 2,506,110 animals sold at 71 livestock marts in Ireland between the years 2000 to 2008 inclusive, were available. Calves were defined as animals from dairy cows sold between 2 days of age and 12 weeks of age. They were categorised into 3 age groups: 2 to 24 days, 25 to 42 days and 43 to 84 days of age. Cows were defined as animals that had calved at least once or were greater than 30 months of age at sale and were categorised into two age groups of 2 to 6 years of age, and 6 to 14 years of age. Animals were removed if their sire, maternal grandsire, herd of origin, or mart of sale were unknown. For each calf only the first record in time was retained; for cows the last record in time was retained. Calves are generally not weighed at livestock marts and therefore only individual calf price information was available for inclusion in this analysis. Calves sold between €2 and €450 were retained. For inclusion in the analyses, cows were required to weigh between 300 and 1000 kg and have been sold for between €75 and €1500. Cows were classified both on their fate post-sale and, as a separate variable, on days since last calving. Two contemporary groups definitions were formed for both calves and cows: mart-by-date of sale and herd-by-year of sale. Only records from contemporary groups with at least 4 other records were retained. Following all edits 40,157 calves and 17,504 cows remained. Phenotypic and genetic (co)variance components for animal price and live-weight were estimated using animal linear mixed models in ASReml (Gilmour *et al.*, 2009). Fixed effects considered for inclusion in the models were: the two aforementioned definitions of contemporary groups, gender (for calves only), age of animal at sale (continuous variable), the proportion of the 12 most commonly found dairy and beef breeds in Ireland, calving ease (no assistance/unobserved; slight assistance; severe assistance; veterinary assistance), whether the animal was born as a singleton or twin, parity of dam (1, 2, 3, 4, 5+, missing), dam age (in months) relative to the median age within parity, heterosis, and recombination loss. When the dependent variable was cow price, the animal's lactation number was included in the model instead of dam parity and age of the animal relative to median calving age, replaced age of the dam. Fate post-sale (based on days until next calving or days to slaughter) and stage of lactation (5 classes) were also added as fixed effects. Prior to the estimation of variance components for price, residuals from a fixed effects model with price as the dependent variable and the aforementioned fixed effects, with the exception of contemporary group, were standardised to the mean residual standard deviation within the contemporary group of mart-date across the years 2004 to 2008. It is the variance components of the standardised price that are reported in the present study. Data on live-weight was not standardised. A series of bivariate analyses were also undertaken to estimate genetic correlations between traits within and across maturity categories as well as between male and female calf price.

**Results** Calves sold for an average price of €156, at on average 28 days of age. Heritability for calf price varied from 0.24±0.09 to 0.52±0.24 across the three age groups. The genetic correlation between male and female calf price was 0.60±0.04. Heritability estimates for cow price were moderately low for the two age groups ranging from 0.12±0.09 (6 to 14 years of age) to 0.36±0.05 (2 to 6 years of age). The genetic correlation for price between the age groups for cows was 0.70 (S.E. =0.18). Heritability estimates for cow live-weight was 0.25±0.05 and did not vary by age group. The correlation between calf price and cow price was weak (Table 1).

**Table 1** Number of records (N), mean price in Euro and live-weight in kgs ( $\mu$ ), genetic standard deviation ( $\sigma_g$ ), heritability ( $h^2$ ; standard error in parenthesis), and coefficient of genetic variation ( $CV_g$ ) for calves and cows. Phenotypic (above diagonal) and genetic correlations (below diagonal; standard error in parenthesis) between price and live-weight are also presented.

Trait	N	$\mu$	$\sigma_g$	$h^2$ (s.e.)	$CV_g$	Calf price	Cow price	Cow weight
Calf price	40,157	156	24.9	0.32 (0.03)	16.0		0.04	0.18
Cow price	17,504	549	25.2	0.07 (0.03)	4.6	0.22 (0.06)		0.32
Cow weight	17,504	600	27.6	0.25 (0.05)	4.6	0.11 (0.05)	0.69 (0.08)	

**Conclusion** Moderate heritability coupled with large genetic variation and the availability of routinely collected data clearly indicates that both price traits could be included in genetic evaluations of dairy cattle in Ireland as both selection criteria and as goal traits.

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## Estimation of genetic parameters for test-day somatic cell count in UK Holstein Friesian dairy herds

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**Introduction** Somatic cell count (SCC) is used widely as an indicator trait for both clinical and subclinical mastitis, and was introduced into genetic evaluations in the UK in 1998. The present evaluations of SCC are based upon a single trait repeatability lactation average model using the first five lactations. The test-day model is now widely adopted for routinely recorded traits, thus the objective of this study was to estimate genetic parameters for SCC using a test-day model.

**Materials and methods** Log<sub>e</sub> SCC (LSCC) was analysed from a dataset consisting of 1,220,344 SCC tests from 43592 Holstein/Friesian cows during their first three lactations that calved between the years 1997 and 2009, and sired by 1654 bulls. Cows required records for all three lactations and a minimum of eight herd-test days were required for lactations one and two, and a minimum of six herd-test day records for lactation three. Lactations were analysed separately and mean LSCC for lactations one, two and three were 11.66, 11.91, and 12.22, respectively. The dataset was analysed in ASReml (Gilmour *et al.*, 2006) with a sire random regression model fitting Legendre polynomials of order 2 for sire effect and permanent environmental effect of the cow. Residual variances for four classes were estimated according to lactation stage: 4-24, 25-49, 50-249, and 250-305 days in milk.

**Results** Daily heritability ( $h^2$ ) estimates for LSCC tended to increase with increased days in milk and the average daily  $h^2$  increased with increased lactation. The average daily  $h^2$  for lactations one, two, and three were 0.07, 0.10, and 0.11, respectively. Daily  $h^2$  estimates for lactations one, two, and three ranged from 0.06 to 0.10, 0.06 to 0.17, and 0.07 to 0.18, respectively. Permanent environmental variances for LSCC were at their highest at the start of lactation, generally decreased with stage of lactation, and increased with lactation number. Similarly, residual and phenotypic variances decreased with stage of lactation / days in milk and estimates were higher with increased lactation number. Phenotypic variances for LSCC ranged from 0.75 to 1.42, 0.74 to 1.71, and 0.83 to 2.01 for lactations one, two and three, respectively. Mean phenotypic variances for LSCC were 0.87, 1.01, and 1.20, respectively. As expected genetic and permanent environmental correlations were highest when days in milk were closest to each other (Table 1). Genetic and permanent environmental correlations between days in milk for LSCC tended to be higher in lactation one. Within lactations the genetic and permanent environmental correlations between days in milk had very similar values for lactations two and three.

**Table 1** Heritabilities (on diagonal), genetic correlations (below diagonal), and permanent environmental correlations (above diagonal) on selected days for LSCC in the first two lactations.

Days in milk	Lactation 1							Lactation 2						
	4	54	104	154	204	254	304	4	54	104	154	204	254	304
4	<b>0.07</b>	0.89	0.65	0.47	0.37	0.35	0.35	<b>0.06</b>	0.89	0.64	0.42	0.29	0.23	0.21
54	0.94	<b>0.08</b>	0.93	0.81	0.72	0.63	0.47	0.90	<b>0.08</b>	0.92	0.78	0.66	0.53	0.31
104	0.76	0.94	<b>0.07</b>	0.97	0.91	0.79	0.55	0.71	0.94	<b>0.09</b>	0.96	0.89	0.73	0.40
154	0.56	0.81	0.96	<b>0.06</b>	0.98	0.88	0.63	0.54	0.84	0.97	<b>0.10</b>	0.97	0.85	0.51
204	0.43	0.69	0.89	0.98	<b>0.07</b>	0.96	0.75	0.42	0.72	0.89	0.97	<b>0.10</b>	0.94	0.66
254	0.38	0.61	0.79	0.91	0.97	<b>0.09</b>	0.91	0.32	0.57	0.73	0.85	0.95	<b>0.14</b>	0.87
304	0.38	0.53	0.67	0.77	0.86	0.96	<b>0.10</b>	0.23	0.36	0.48	0.61	0.77	0.93	<b>0.17</b>

**Conclusions** Heritability estimates were generally low, but increased with stage of lactation and parity, and were in the range of those previously reported (Mrode *et al.*, 2001; Mrode and Swanson, 2003). The increase in daily  $h^2$ , particularly for lactations two and three, with increased days in milk were not solely due to increased genetic variances, but also due to reduced permanent environmental variances, and residual variances with increased stage of lactation. A model that accounts for the changes in  $h^2$  and genetic correlations with days in milk should produce more accurate evaluations.

**Acknowledgements** The authors gratefully acknowledge the input of Professor Robin Thompson and funding by the Defra LINK programme, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR.

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## Estimates of heterosis for non-production traits for an all dairy breed genetic evaluation in the United Kingdom

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**Introduction** Most countries have reported a decline in reproductive traits and other traits associated with overall fitness in recent years as a result of selecting primarily for milk yield. In an attempt to arrest the situation, cross breeding has been embarked upon by many farmers in addition to directly selecting for traits such as fertility (FERT), lifespan (LS) and somatic cell count (SCC). In general these traits are of low heritability, implying that the gains from heterosis are expected to be larger than those for production traits. While estimates of heterosis for production traits have been reported on United Kingdom (UK) national genetic evaluation data (Mrode et. al., 2008), no such estimates have been reported for non-production traits. This paper summarises the model and estimates of heterosis for FERT, LS and SCC in the UK national genetic evaluation data.

**Materials and methods** First five parity SCC data and first parity data for fertility traits: Calving interval (CI), non-return rate at day 56 (NR56), test day milk yield at day 110 (MTD), condition score (CS) and days to first service and number of services observed that resulted in a calf, were extracted from the data of milk recording organisations for cows born since 1981. The number of lactations each cow completed (up to the first five parities) and type traits were extracted over the same time period. LS evaluations were then based on a bivariate analysis of lifespan score computed from the number of lactations each cow completed and survival score computed from type information. The fertility index in the UK includes evaluations for CI and NR56 but only the results for CI have been reported in this paper. The breed composition of cows with fertility data consisted of 39% Holstein (Hol), 0.4% Friesian (Fri), 1.3% Ayrshire (Ayr), 2.5% Jersey (Jer), 0.9% Guernsey (Gue), less than 1% for Shorthorn, Brown Swiss, Montebeliarde, Normande and Meuse-Rhine, 38% crosses among the various pure breeds, with a coefficient of heterosis of at least 50% and 17% with a coefficient of heterosis of 25 to 49%. The model for fertility included the fixed effect of herd-year-season (HYS), age (linear and quadratic), days in milk (for MTD only), herd-year-visit (HYV) and stage of parity (STAG) (for CS only). Similarly the model for SCC included the fixed effects of HYS, linear effects of age within parity within breed, month of calving and random effects of animal, herd-by-sire and permanent environment. In the bivariate analysis for LS, the fixed effects were HYS for lifespan score, age (linear and quadratic), milk yield deviated from contemporaries while those for survival score were HYV, STAG, age (linear and quadratic). Random animal effect was fitted for both traits. In all trait groups, linear effects of heterosis and recombination defined for six groups of crosses were fitted in the model. The six crosses were Holstein x Friesian (Hol x Fri), Hol x Red and White breeds (Hol x R&W), with the R&W consisting of Ayr, Scandinavian Red Brown Swiss, Montebeliarde and Shorthorn, Hol x Others (Jer and Gue), Fri x R&W, Fri x others and R&W x Others. Estimates of heterosis are reported for each cross and the predicted transmitting abilities (PTAs) from the all breed analysis correlated with those from within breed analyses.

**Results** The estimates of heterosis for traits varied across the different crosses and these are presented in Table 1.

**Table 1** Estimates of 100% heterosis for some non-productive traits

Breed Cross	Calving Interval (days)	Somatic cell count (%)	Lifespan (lactations)
Hol x Fri	-1.63	-1.69	0.022
Hol x R&W	-3.13	-4.50	0.170
Hol x Others	-5.73	-3.70	0.114
Fri x R&W	-2.77	-3.13	-0.045
Fri x Others	-2.99	0.33	0.002
R&W x Others	-2.32	2.00	0.142

The estimates of heterosis For CI were largest for the Hol x Others cross with CI being reduced by about 6 days which is 0.11 standard deviation. However, the effects of heterosis were largest for SCC and LS in the Hol x R&W cross with a reduction of about 5% in SCC in 305d lactation and an increase about 0.2 lactations in a life time. Considering bulls with at least 50 daughters, the correlations of bull PTAs for SCC from the all breed analyses with those from the within breed analysis were 0.98, 0.98, 0.98 and 0.96 for Hol, Ayr, Jer and Gue respectively. Corresponding estimates for LS were 0.95, 0.96, 0.94 and 0.89 respectively. The rate of genetic change per year for SCC from the all breed run was 0.288% per year with Hol, Fri and various crosses (with at least 25% coefficient for heterosis), accounting for 50, 14 and 36% of that rate of change. Similarly, the rate of change for LS was 0.011 lactations per year with Hol, Fri and various crosses accounting for 31, 36 and 33% of the rate of change respectively.

**Conclusion** Effects of heterosis for the non-production traits when expressed in terms of the standard deviation were slightly less than observed for production. An all breed evaluation for non-production traits in the national data for UK is feasible.

**Acknowledgements** The authors gratefully acknowledge the input of Marco Winters and DairyCo for funding.

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## Development of calving ease evaluations for UK Holstein-Friesian cows

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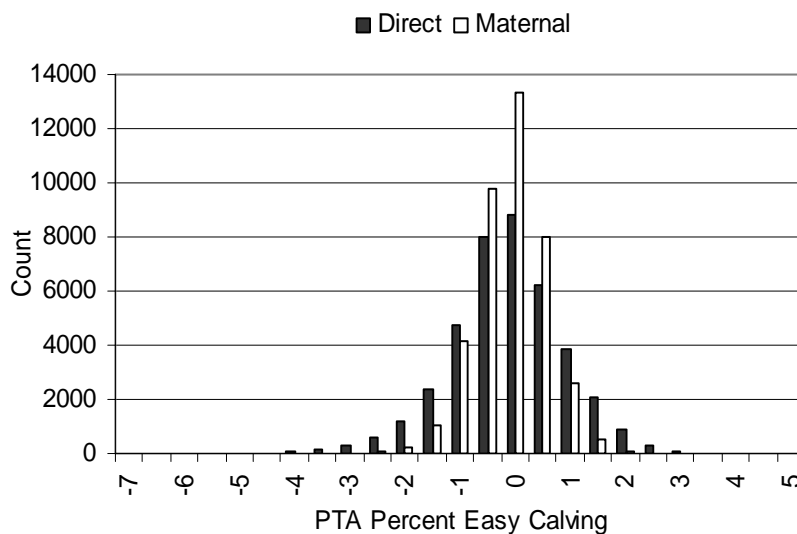
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**Introduction** The ease of calving influences the economics of a cow/calf enterprise through increased calf death loss, increased labour and veterinary costs, reduced subsequent reproductive performance of the cow, potential loss of the cow, and reduced milk production. McGuirk *et al.* (2007) estimated that a slightly difficult calving cost approximately £110 and a seriously difficult calving cost £350-£400. Many countries around the world undertake genetic evaluations for calving ease (CE). The aim of this project was to develop routine national CE evaluations for UK dairy cattle.

**Materials and methods** Data were taken from UK milk recording organisations, and covers farmer recorded CE as well as data collected as part of the progeny test scheme. Genetic parameters for CE were estimated using ASReml (version 2.00, 2006) considering a direct and indirect effect. Fixed effects in the model include herd-year, month of calving, lactation

number, calf sex and interaction between lactation number and calf sex. Age and percentage Holstein were fitted as linear regressions. A random effect of service sire was fitted to estimate direct CE predicted transmitting ability (PTA) and random effect of maternal grandsire fitted for indirect CE PTA, with a covariance between the 2 effects also fitted. A maternal CE PTA was then computed as the indirect CE PTA minus one quarter of the direct CE PTA. The direct CE PTA relates to the ease with which a bull's calves are expected to be born and the maternal calving ease PTA relates to the ease with which the bull's daughters are likely to give birth. Bull PTAs for direct and maternal CE were submitted to a test multiple across country evaluation (MACE) of calving traits performed by the



**Figure 1.** Distribution of calving ease PTAs (direct and maternal)

Interbull Centre (Uppsala, Sweden), thus allowing UK evaluation of CE to be compared to evaluations from other countries on the UK scale. A data and pedigree file were extracted in September 2009 and contained almost 400,000 calving records, representing almost 6,000 sires.

**Results** The data approximately spanned 15 years, however, the majority of the data falls after 1999. Overall, 84% of calvings were classed as “easy calving”. For first calving cows a total of 74% calvings were classed as “easy” and 85% of later calvings. The genetic analysis showed that the heritability for calving ease was low (0.066 and 0.040 for direct and indirect effects respectively with a genetic correlation of -0.685). Figure 1 shows that the calving ease PTAs were expressed as percent easy calving and centred around zero, with a positive value being favourable (i.e., less difficult calvings). There was no evidence of a genetic trend in either direct or maternal CE PTAs. The across country genetic correlations for the MACE run for direct and maternal CE were in line with other countries and suggest that UK CE PTAs would be suitable for an international MACE evaluation. The genetic correlations between countries for the direct calving trait averaged of 0.80 and ranged from 0.619 (with Hungary) to 0.944 (with Canada). The genetic correlations between countries for the maternal calving trait was a little lower with an average of 0.69 and ranged from 0.561 (with Hungary) to 0.839 (with France). These correlations with other countries are good, particularly for such low heritability traits.

**Conclusions** Having calving ease proofs available will help farmers identify bulls that are genetically good (or bad) for calving performance. This may be of particular importance when choosing bulls to use (or avoid) on heifers to avoid the costs associated with losses in production, fertility and potentially cow/calf losses.

**Acknowledgements** The authors gratefully acknowledge funding from Defra LINK, Scottish Government, DairyCo, National Milk Records, Cattle Information Services, Holstein UK, Genus ABS and Cogent Breeding

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## Implementation and first year results on the use of genomic selection in dairy cattle in Ireland

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**Introduction** Genomic selection in Holstein-Friesian dairy cattle was launched in Ireland in February 2009. The objective of this document is to outline the implementation and the uptake of genomic estimated breeding values (GEBVs) in Ireland for Spring 2009. We also outline the results of the first group of Holstein-Friesian bulls that were selected on GEBVs compared to their progeny test proofs obtained in the August 2009 evaluations.

**Materials and methods** Berry *et al.* (2009) described in detail the methodology used for the estimation of GEBVs in Ireland. Briefly, the training population was made up of just over 1,000 Holstein-Friesian bulls, genotyped using the Illumina Bovine50 Beadchip. Direct genomic values (DGVs) are estimated in Ireland using mixed models equations by replacing the traditional numerator relationship matrix with a genomic relationship matrix as outlined by VanRaden (2008). The dependent variable included in the genomic evaluation are the deregressed traditional EBVs of the animal as outlined by Berry *et al.* (2009). Genomic EBVs (GEBVs) are the combination of the DGVs and the traditional EBVs. This blending procedure is done because not all animals in the pedigree are genotyped (genotypes of no females are currently included in the genomic evaluation) and therefore not all information is included in the genomic evaluation. The uptake of the use of genomically selected (GS) bulls was assessed on 349,000 AI insemination records collected via technician handhelds from January to June 2009. Initial results on the predictive ability of genomic selection was assessed by looking at the correlation of the daughter proven EBV to the DGVs, the GEBVs, and the parent average proof for 35 young test sires used in the 2006 national progeny test program and who received a daughter proof in the August 2009 evaluation. These bulls were marketed in the Spring 2009 breeding season as GS bulls with no daughter information.

**Results** The accuracy of genomic selection using forward prediction is reported elsewhere (Berry *et al.*, 2009). After consultation with representatives from the Irish dairy industry it was decided to publish GEBVs of individual bulls without progeny on the list of active bulls for the Spring 2009 breeding season. Bulls included on the active bull list had to have sufficient progeny born to have reliability for direct calving difficulty of  $\geq 50\%$  in the country of origin. Also the reliability of the GEBV for EBI, had to be  $\geq 35\%$ . In 2008, prior to the introduction of genomic evaluations, each sire on the active bull list had to have a reliability of EBI of  $\geq 58\%$ . Compared to 2008, the average EBI of the bulls on the list was higher, but the reliability was lower. In addition, younger sires replaced sires that had occupied the list for many years, but the number of bulls with daughters in Ireland decreased. The usage for the daughter proven bulls with daughters in Ireland (DP-IRL) was the highest at 37% of inseminations with GS bulls accounting for 34%, and proven bulls but with no Irish daughters (DP-INT) at 29%. The mean number of bulls used per herd was 3 for DP-IRL bulls, 2.7 for DP-INT bulls and 4 for GS bulls. The very positive uptake of GS bulls can be attributed to the difference in genetic merit between these bulls and the daughter proven bulls. The weighted average EBI of the GS bulls was €69, more than one standard deviation (€62), ahead of the DP-IRL bulls. The weighted average across all three groups of bulls used in 2009 was €38 more than the bulls used in 2008. The correlation and mean difference between parent average, DGV, GEBV and daughter EBV are given in Table 1. In terms of the predictive ability of genomic information the correlation between parent average and daughter proofs were consistently lower than those of the GEBV and the DGV. At this stage the DGVs are the best predictors of progeny performance however one must recognize the limitations of this analysis due only 35 bulls being included in this comparison, the average reliability of the sires is 80%, and the daughter records are not completed lactation records.

**Table 1** Correlations and mean difference between daughter proofs and GEBV, DGV, and parent average for 35 GS bulls selected when in lay-off in Spring 2009 but now with greater than 70% reliability for production traits based on daughters milking in 2009.

	Correlation			Mean Difference		
	GEBV	DGV	PA	GEBV	DGV	PA
Milk (kg)	0.64	0.65	0.63	65	50	77
Fat (kg)	0.51	0.57	0.4	2	2	3
Prot (kg)	0.59	0.65	0.53	2	1.5	2.2

**Conclusion** Overall the implementation of genomic evaluations in Ireland has been very successful. The uptake of the GS bulls has been very encouraging with farmers using several bulls as recommended to reduce the risks. Initial results on how the technology is working are promising and the introduction of genomic evaluations will generate greater genetic gain in the future. Research work is underway to increase the size of the training population, by incorporating multiple-trait across country (MACE) evaluations, to improve the accuracy of GEBVs.

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## Environmental impact of three high-concentrate diets fed to bulls

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**Introduction** Bull fattening, as other farming activities, results in emissions of pollutants to air, water and soil and the use of resources (energy, land). In a trial carried out on bulls fattened with 3 diets containing 37 to 86% concentrates, enteric methane production was determined. The 86%-concentrate diet decreased enteric methane emission by more than 50% (Martin *et al.*, 2007). Enteric methane is only one of the pollutants and a comparison of feeding systems requires a more complete evaluation of the environmental impact. The data generated by this trial were analysed using life cycle assessment. Special attention was paid to emissions of greenhouse gases.

**Materials and methods** Three fattening diets have been compared in Blond d'Aquitaine bulls. Diet MSM comprised 63% maize silage, 21% maize grain and 16% soybean meal. Diet HM comprised 49% hay, 41% maize grain and 10% soybean meal. Diet M comprised 70% maize grain, 16% soybean meal and 14% straw. Enteric methane was determined in 6 animals in a 3x3 Latin square design using the SF<sub>6</sub> tracer method (Martin *et al.*, 2007). Manure methane was calculated from faecal data obtained in this trial using equations provided by IPCC (2006). Nitrous oxide and carbon dioxide emissions, eutrophication, acidification, terrestrial ecotoxicity and land use were calculated according to van der Werf (2004), updated according to IPCC (2006). Climate change was expressed as global warming potential in CO<sub>2</sub>-eq using the coefficients 1, 25 and 298 for carbon dioxide, methane and nitrous oxide, respectively. Total cumulative energy demand was calculated according to Frischknecht *et al.* (2007). The life cycle assessment was limited to the production of the four feedstuffs used in the trial, the production of inputs to produce these feedstuffs and all transport stages, using the feed intake effectively measured in the trial. Each of the feeding systems was located in France, crop production methods and transport distances were based on average data for French farming systems. Data were expressed per day and per kg liveweight gain. Liveweight gain was taken as average daily gain between 400 and 650 kg in bulls receiving the same diets in a feeding experiment with 8 bulls per diet (Mialon *et al.*, 2008).

**Results** The lower enteric methane emission with diet M was partially compensated for by a higher emission of nitrous oxide and carbon dioxide (Table 1). Acidification and terrestrial ecotoxicity were highest with diet MSM and lowest with diet HM. Eutrophication was highest with diet M and lowest with diet HM, and cumulated energy demand was highest with diet M and lowest with diet MSM. Land use was highest with diet HM. Due to a higher liveweight gain, M diet environmental criteria were improved when expressed per kg liveweight rather than when expressed per day, compared to the other two diets.

**Table 1** Emissions and environmental impacts from bulls fed three contrasting diets

	Per day			Per kg liveweight gain		
	MSM	HM	M	MSM	HM	M
Enteric methane, kg CO <sub>2</sub> eq	3.81	3.33	1.56	2.23	2.23	0.84
Manure methane, kg CO <sub>2</sub> eq	1.54	1.74	1.40	0.90	1.16	0.75
Nitrous oxide, kg CO <sub>2</sub> eq	1.37	1.15	2.03	0.80	0.77	1.09
Carbon dioxide, kg CO <sub>2</sub> eq	1.26	1.37	1.84	0.73	0.99	0.99
Global warming potential, kg CO <sub>2</sub> eq	8.02	7.65	6.89	4.70	5.12	3.70
Acidification, g SO <sub>2</sub> eq	29.6	13.1	22.6	17.3	8.8	12.1
Eutrophication, g PO <sub>4</sub> <sup>3-</sup> eq	24.1	16.3	31.9	14.0	10.9	17.1
Terrestrial ecotoxicity, g eq-1.4DB	36.9	11.5	19.7	21.6	7.7	10.6
Land use, m <sup>2</sup> .year	7.77	17.51	8.63	4.54	11.72	4.63
Total cumulative energy demand, MJ eq	22.3	28.0	36.9	13.0	18.7	19.8

**Conclusions** The relative environmental performances of three bull fattening feeding systems are not the same for climate change as for other air pollution criteria, for soil and water pollution criteria or for energy demand. Diet M has lower impact on climate change, which is a major issue for ruminant production, but higher impact on energy demand. An integrative approach is necessary to compare feeding systems, and should include a total life cycle assessment including the early life stage of the animals, and other criteria of the impacts of these systems on the environment (water use, biodiversity).

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## The effect of application timing on ammonia emissions from cattle slurry in Ireland

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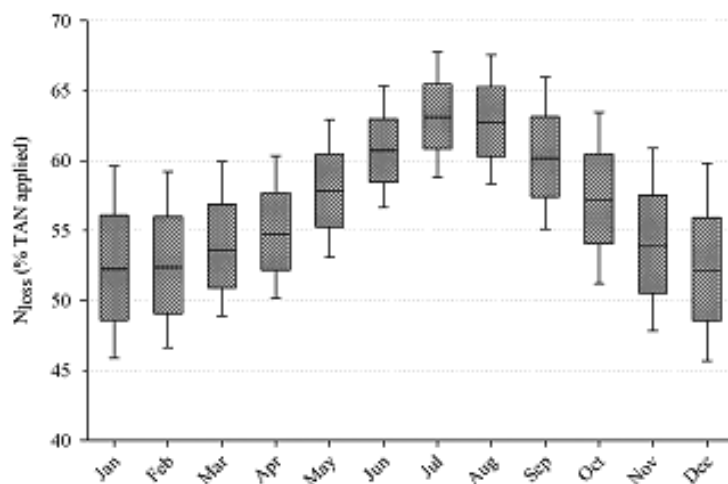
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**Introduction** Ireland is currently meeting its ammonia emission target of 116 kt per annum up to 2010. However, the requirement to further reduce ammonia emissions in the future is unknown. The volatilization of ammonia following land application of cattle slurry accounts for over 30% of the ammonia emissions from Irish agriculture. As a result, the management of slurry application has been identified as a measure that has a high potential to reduce national ammonia emissions (Hyde *et al.*, 2003). Slurry application methods such as band spreading, trailing shoe and injection are widely used in some countries as a tool for reducing ammonia emissions. However, the high cost of adopting this technology is not always compensated through increased N fertilizer value benefits. Improved management of land application, using the cheaper and more conventional splashplate method in association with optimum weather conditions, also offers benefits of reduced ammonia emission and improved N efficiency (Lalor *et al.*, 2009). The objective of this study is to estimate the potential of application timing management using the splashplate method to reduce ammonia emissions following cattle slurry application.

**Materials and methods** The ALFAM model (Søgaard *et al.*, 2002) was used to predict the total ammonia emissions following cattle slurry applications based on weather data (mean daily air temperature (T) and wind speed (W)) recorded at eight geographically dispersed Met Éireann synoptic weather stations, over a twenty year period (1988-2007). The following assumptions were applied to the ALFAM model: soil water content = dry (i.e. not waterlogged); slurry type = cattle; slurry dry matter content = 70 g/kg; total ammoniacal nitrogen (TAN) content of slurry = 1.8 g/kg; application rate = 30 m<sup>3</sup>/ha; application method = splashplate; and technique for measuring ammonia loss = Micrometeorological mass balance technique. The effect of the month, year and weather station on the predicted total ammonia emissions ( $N_{\text{loss}}$ ), expressed as a percentage of the TAN applied, was statistically analysed using PROC GLM in SAS.

**Results** Month, year and weather station all had a significant effect on  $N_{\text{loss}}$  ( $P < 0.001$  in all cases). However, only the monthly emissions over all stations and years are presented here. The month with the highest median value of predicted emissions was July (63.1%), and was significantly higher than December (52.1%), which had the lowest predicted emissions (Figure 1). This indicates that switching application timing between months would have an effect on the total emissions from land application of cattle slurry. The variation in the predicted emissions within each month is reflected in the large difference observed between the 10 and 90 percentile values for each month. These ranged from 8.7% in June to 14.1% in December, and were greater than the difference in the median value between July and December in six of the twelve months.



**Figure 1** Box plot showing median (centre line), interquartile range (boxes) and 10<sup>th</sup> and 90<sup>th</sup> percentile (whiskers) of ammonia emissions predictions ( $N_{\text{loss}}$ ) from cattle slurry application using splashplate for each month.

**Conclusions** Based on ammonia emissions predictions using the ALFAM model, managing application timing on the basis of monthly averages, and exploiting optimal weather conditions within each month, has the potential to decrease the total ammonia emissions from slurry application with splashplate. However, the development or adaptation of other ammonia emission models for Irish conditions merits further study in order to improve the prediction of ammonia losses following land application of cattle slurry.

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## The influence of strain of Holstein-Friesian cow and feeding systems on greenhouse gas emissions from pastoral dairy farms

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**Introduction** Presently, Ireland is above its national greenhouse gas (GHG) emission limit set under the Kyoto Protocol. One of Ireland's largest sources of GHG emissions is the agricultural sector (26%) (EPA, 2009). Within this sector, pastoral dairy farming is estimated to be a significant source (Lovett *et al.* 2008). Therefore, to meet the targets of the Kyoto Protocol and future reduction targets, pastoral dairy farms will be required to reduce GHG emissions. The purpose of this study was to investigate the effect different strains of Holstein Friesian cows and alternate pasture based feed systems have on GHG emissions from dairy farms.

**Materials and methods** Three strains of divergent Holstein-Friesian cows; high-production North American (HP), high-durability North American (HD) and New Zealand (NZ) were compared. The HP strain represents cows selected solely for milk production. The HD strain represents a breeding program where selection is based on improving a number of traits simultaneously; these include milk production, fertility and muscularity. The NZ strain represents the highest possible genetic merit expressed in the NZ genetic evaluation system (Breeding Worth). Each strain was allocated to one of 3 feed systems; high grass allowance (MP, control); high concentrate supplementation (HC) and a high stocking rate system (HS). The MP system had an overall stocking rate of 2.47 cows/ha and cows received 325 kg of concentrate in early lactation. The HC system had a similar overall stocking rate and N fertilizer input as the MP system, but 1,445 kg of concentrate were fed per cow. The HS system had a similar concentrate and N input as the MP system, but had an overall stocking rate of 2.74 cows/ha. The GHG emissions of the dairy production systems described were calculated using the Moorepark Dairy System Model (MDSM) of Shalloo *et al.* (2004) in combination with a new GHG emissions model (GHG model). The biological performance data required for the simulation was obtained from McCarthy *et al.* (2007). The MDSM, a whole farm simulation model of Irish grassland-based dairy systems, was used to define the parameters required by the GHG model. Parameters defined included; farm size, animal inventory, milk production, feed intakes, herbage quality (chemical composition), grazing season length, slurry, fertilizer and application of lime. The GHG model integrates the parameters defined by the MDSM with various GHG emission factors in Microsoft Excel to quantify emissions. The model calculates emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O in terms of their 100-year global warming potentials (CO<sub>2</sub> equivalents (eq)), which on a weight basis relative to CO<sub>2</sub> was set to a factor of 23 for 1 kg of CH<sub>4</sub> and 296 for 1 kg of N<sub>2</sub>O. The GHG model is also designed to simulate emissions on two levels, firstly those arising directly from farming activities (on-farm GHG emissions) and secondly those that are produced off-farm but are attributable to the production system up to the point where milk leaves the farm gate. The main outputs of the GHG model are an estimate of annual on-farm and total (on-farm plus indirect) GHG emissions. The model expresses emissions on a farm, area (CO<sub>2</sub> eq, t/ha) and product (CO<sub>2</sub> eq, kg/kg milk and per kg milk solids (MS)) basis.

**Results** The product and area GHG emissions of all dairy farms were greater when quantified at the total level than the on-farm level (Table 1). The level where GHG emissions were calculated at, affected the optimum feed system and genotype. For example, the NZ strain in terms of product emissions. The HD and HP strain produced their least product emissions in the HC feed system. The NZ strain produced their least product emissions in the HS feed system. On average the HC system produced the greatest area emissions.

**Table 1** On-farm and total GHG emissions (CO<sub>2</sub> eq) for 3 strains of Holstein-Friesian cows [high production (HP); high durability (HD) and New Zealand (NZ)] within the Moorepark (MP), high concentrate (HC) and high stocking rate (HS) feed systems

GHG Indicator	Level	MP			HS			HC		
		NZ	HD	HP	NZ	HD	HP	NZ	HD	HP
CO <sub>2</sub> eq, kg/kg milk	On-farm	0.801	0.805	0.862	0.796	0.801	0.869	0.757	0.720	0.760
	Total	1.065	1.067	1.142	1.045	1.055	1.144	1.069	1.012	1.065
CO <sub>2</sub> eq, kg/kg MS	On-farm	10.10	10.65	11.45	9.92	10.64	11.61	9.32	9.68	10.19
	Total	13.43	14.12	15.17	12.98	14.01	15.29	13.15	13.63	14.28
CO <sub>2</sub> eq, t/ha	On-farm	10.28	10.34	10.42	11.16	10.98	11.06	11.70	11.40	11.50
	Total	13.68	13.71	13.82	14.65	14.45	14.56	16.51	16.05	16.12

**Conclusion** The results show that farm product emissions do not always rank the same when estimated either as on-farm or total emissions. Thus, if effective strategies are to be developed, total emissions associated with a production system should be analyzed. The results also show how selection for increased milk production (HP strain) combined with increased concentrate supplementation within Irish grass based feed systems may result in greater total GHG emissions relative to selection on a combination of production and reproductive traits (HD and NZ strains) within feed systems with a greater reliance on grazed grass.

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## An inventory of methane emissions from ruminant animals in Northern Ireland due to enteric fermentation-a comparison using Tier 1 and Tier 3 emission factors

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**Introduction** In Northern Ireland, 21% of GHG emissions came from agriculture in 2007. The UK Climate Change Bill requires a reduction in emissions of 80% by 2050, posing a significant challenge for the industry. A calculation of the baseline GHG footprint of animal production is required to determine sustainable GHG mitigation strategies. The IPCC provide standard Emission Factors (EF) (Tier 1) for CH<sub>4</sub> produced by enteric fermentation and classify ruminant animals as dairy, non-dairy and sheep. The age and diet of the animal is taken into account by the IPCC. Previous research at AFBI Hillsborough has generated data detailing actual CH<sub>4</sub> and CO<sub>2</sub> emissions from 800 dairy cows, 146 beef cattle and 50 sheep (Tier 3 EF). The aim of this study was to compare the GHG footprint of ruminant animals in Northern Ireland in 2008 using Tier 1 (standard) and Tier 3 (actual) EF.

**Materials and methods** Tier 3 CH<sub>4</sub> emissions from dairy cows are representative of cows housed indoors and offered a range of indoor diets. Data for beef animals are representative of Friesian, Aberdeen Angus, Simmental and Charolais breeds offered diets with a forage proportion of 295-1000 g/kg dry matter (DM). Data for sheep are representative of Blackface and lowland crossbreds (Suffolk and Texel x Greyface) offered grass silage-based diets (178-1000 g/kg DM). Tier 3 EF for dairy cows, beef cattle and sheep were estimated by calculating the total ME requirement (MJ/year) and feed intake (kg DM/year) for each breed at different physiological states, followed by the conversion of ME intake to enteric CH<sub>4</sub> emissions (kg/year). Total ME requirements for dairy cows were estimated from Feed into Milk (FiM) models (Agnew *et al.*, 2004) and for beef cattle and sheep, AFRC systems (AFRC, 1993) and the Dawson and Steen (1998) model were used. Total enteric CH<sub>4</sub> emissions for each breed were calculated using the ratio of CH<sub>4</sub> energy output to ME intake, measured in calorimeter chambers at AFBI Hillsborough for each breed (Yan *et al.*, 2010).

**Results** The GHG footprint of ruminant animals in Northern Ireland using Tier 1 and Tier 3 EF is presented in Table 1.

**Table 1** GHG footprint of ruminant animals in Northern Ireland in 2008 (Tier 1 V Tier 3 calculations)

Livestock Category	Number of animals	Tier 1 Standard IPCC (2006) EF			Tier 3 Actual EF (AFBI data)		
		Tier 1 EF (kg CH <sub>4</sub> /hd/y)	Emissions (tonne CH <sub>4</sub> /y)	Footprint (tonne CO <sub>2</sub> e/y)	Tier 3 EF (kg CH <sub>4</sub> /hd/y)	Emissions (tonne CH <sub>4</sub> /y)	Footprint (tonne CO <sub>2</sub> e/y)
Dairy cows	289247	117	33842	846047	109.5	31673	791814
Dairy heifers in calf							
-2 years old	26883	57	1532	38308	52.9	1422	35553
-1 to 2 years	37389	57	2131	53279	69.5	2599	64963
Beef cows	265663	57	15143	378570	59.1	15701	392517
Beef heifers in calf							
-2 years old	24311	57	1386	34643	59.1	1437	35920
-1 to 2 years	15433	57	880	21992	64.0	988	24693
Other cattle 1-2 years	333531	57	19011	475282	64.0	21346	533650
Other cattle 6-12 months	186933	57	10655	266380	29.1	5440	135994
Ewes	935417	8	7483	187083	10.5	9822	245547
Other sheep							
-Rams for service	26868	8	215	5374	10.5	282	7053
-1 year and over	12543	8	100	2509	7.5	94	2352
-Lambs < 1 year old	998765	8	7990	199753	4.6	4594	114858
Total emissions			100369	2509220		95396	2384912

Animals in the "Breeding bulls", "Other cattle-2 years old" and "Other cattle < 6 months old" categories were not included in this inventory due to the absence of Tier 3 EF

**Conclusions** The overall GHG footprint of ruminant animals included in this study was 5% lower with Tier 3 EF than with Tier 1 EF values, but in some classifications the Tier 3 values were higher. This wide variation demonstrates a requirement for actual EF data that is representative of the age and diet of the animal. There is also a need to develop a more precise Tier 3 EF database for agriculture, particularly for the animal categories not included in this exercise, younger animals and animals at grass.

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## Field assessment of the balance between greenhouse gases and ammonia emissions from grassland under various N-management regimes

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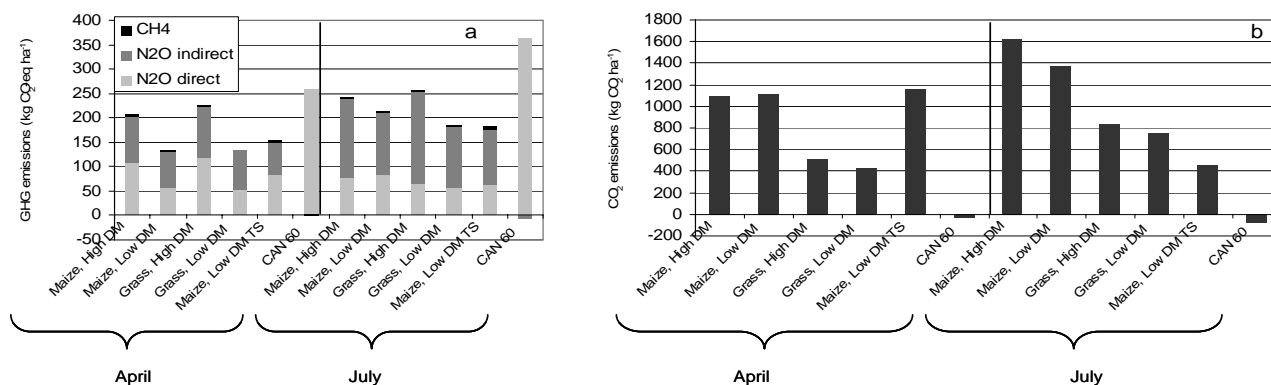
**Introduction** Agriculture in Ireland contributes 98% of ammonia (NH<sub>3</sub>) and 26% of greenhouse gas (GHG) emissions (CSO, 2008). Manure management is one of the key drivers of these emissions. This study aim at: a) measuring the effect of organic and ammonium fertilizer on GHG and NH<sub>3</sub>, b) investigate the impact of cattle slurry composition on these emissions.

**Materials and methods** A field-plot experiment (2 x 1.5 m per plot, n=3) was carried out, with grass-derived and maize-derived slurries of high and low dry matter (DM) content. Plots were spread using splash-plate application. In addition, one of the combinations of maize-derived slurry DM content was spread on three additional plots simulating trailing shoe, in order to compare both slurry application techniques. Control plots include no N fertilization and 60 kg N ha<sup>-1</sup> of calcium ammonium nitrate (CAN) fertilization. Two treatment applications were carried out in April and July 2009 under contrasting climatic conditions.

Continuous flux measurements were taken after slurry application. Ammonia was measured using a photo-acoustic analyzer (INNOVA 1412 Trace Gas Analyser, INNOVA Instruments, Copenhagen, Denmark) coupled to a dynamic chamber. Carbon dioxide and nitrous oxide were measured using static chambers and an Infra-Red Gas Analyzer (CO<sub>2</sub>, PP Systems EGM-4, PP Systems, Hitchin, Herts., UK) or a gas chromatograph (N<sub>2</sub>O, Varian) for the analysis of the gases. Ancillary data (air and soil temperature, rainfall, soil moisture, soil ammonium and nitrate concentration) were also collected.

Cumulative fluxes were calculated for the first week following fertiliser application (Figure 1). Background fluxes (control plots) were subtracted from fluxes calculated for treatments plots. These treatments were compared using ANOVA with slurry dry matter and slurry types as main factors.

**Results** The indirect effect of ammonia volatilisation on N<sub>2</sub>O emissions were calculated using IPCC default factors (IPCC, 2007). These emissions were highest for high DM grass-based slurry in July under warm dry conditions (Figure 1a). Emissions were significantly lower when slurry was spread in April and also for trailing shoe slurry in July. Reducing slurry DM content substantially lowered direct nitrous oxide emissions in spring, but not in summer. Methane emissions were generally low, but increased when slurry was trailing-shoe applied. In term of splash-plate application, maize slurry (high DM) emitted significantly (p<0.05) more methane than grass slurry. C mineralisation was substantial and increased on slurry application (Figure 1b). This priming effect on soil respiration over the measurement period varied from 400 kg CO<sub>2</sub> ha<sup>-1</sup> for grass slurry to 2000 kg CO<sub>2</sub> ha<sup>-1</sup> for maize slurry.



**Figure 1** a) Cumulative GHG fluxes of trace gases (kg CO<sub>2</sub> eq ha<sup>-1</sup>), and b) CO<sub>2</sub> emissions rates in April and June 2009, for the seven days following slurry application. Background emissions were subtracted from those calculated for treatments. Indirect N<sub>2</sub>O emissions were calculated using the IPCC default value of 1% of NH<sub>3</sub> reemitted in the atmosphere as N<sub>2</sub>O (TS: trailing-shoe application).

**Conclusion** In terms of trace gases, indirect N<sub>2</sub>O emissions sourced from ammonia losses comprised the largest proportion of landspreading emissions when slurry was spread in warm conditions. Direct nitrous oxide emissions were higher in spring and also higher for trailing shoe. There was also a substantial 'priming effect' in terms of C mineralisation when organic fertiliser was applied and these CO<sub>2</sub> emissions dwarfed other emissions over the measurement period.

**Acknowledgements** This work is funded by the Walsh Fellowship Scheme.

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## The potential effects of saponins on ruminal fermentation

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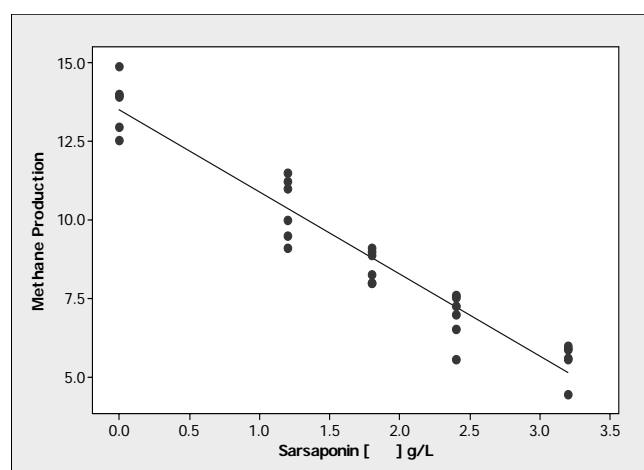
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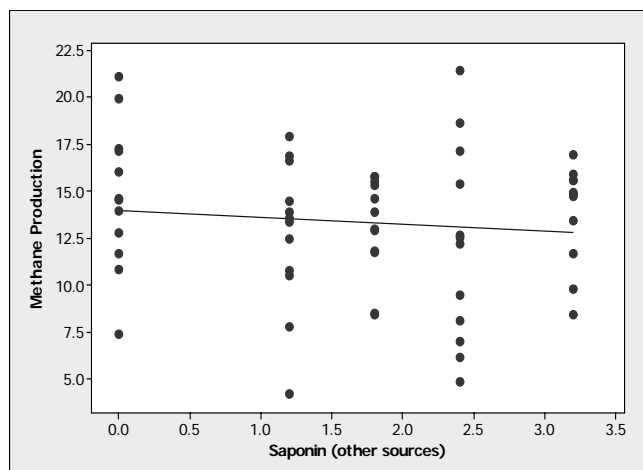
**Introduction** Mitigating greenhouse gas emissions from ruminants by feeding natural compounds has been a target for many researchers. The effect of saponins is dependant on the plant from which they are extracted and the diet fed to the animals. So, due to these constraints to the exploitation of saponins in ruminant production, a short insight of its use as a methane inhibitor will be drawn in this paper.

**Materials and methods** A literature search was performed and 59 studies were then used in a meta-analysis simple regression. The model used included the overall size effect, the effect of the size of each study, and the variation between studies. A summary was compiled and the main results of the effect of saponins for ruminants as well as sources providing beneficial results are presented (Figures 1 & 2).

**Discussion** The biological effects of saponins on microorganisms are related to the increase in microbial membrane porosity. Interaction with the outer environment tends to arise and then the effects of saponins vary considerably given that contents of rumen liquor differ widely from animal to animal. The trend observed when the compilation of 59 studies was analysed demonstrated that 16 of them significantly reduced methane production (MP) (Figure 1) (Hart *et al.*, 2008) ( $p < 0.05$ ). However we interpret these data as showing only very few examples of saponins impacting on rumen fermentation. This approach to trials included in this study can validate saponins as a potential methane inhibitor to be used in modern animal nutrition. Several authors as mentioned by Hu *et al.* (2005) have understood that the way saponins are assessed is important and can completely change the outcomes of a trial (Figure 2) ( $p > 0.05$ ).



**Figure 1** Meta-analysis of the effect of sarsaponin (S) as a methane inhibitor ( $MP = 14.10 - 2.609 \cdot S \text{ g/L}$ ) ( $p < 0.05$ )



**Figure 2** Meta-analysis of the effect of sources of saponin (OS) other than sarsaponin as methane inhibitors ( $MP = 13.98 - 0.2637 \cdot OS \text{ g/L}$ ) ( $p > 0.05$ )

**Results** The results shown above seem to have sources (*S. saponaria* fruit) and specific characteristics of saponins (chemical Sarsaponin) as the principal effect for methane suppression (Hess *et al.*, 2004). Over 50 studies in the literature evaluating the effects of saponins on rumen fermentation, including methane modification, found no evidence that these secondary metabolites could reduce the amount of this greenhouse gas. Indeed, none have used *S. saponaria* where Sarsaponin is the source of saponins. There is a need for new trials using distinct sources of saponins.

**Conclusions** Saponins can manipulate ruminal fermentation but the change in methane production is questionable and demands further investigation. These should focus on specific classes of saponins. Methanogenic archaea seems to be little affected by these chemical compounds but the real dose-response and mechanisms are still unclear.

**Acknowledgements** Mr. Helio Lima is grateful to Brazilian Government by providing his CAPES PhD Scholarship

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## Evaluation of methane emissions by spring calving Holstein Friesian dairy cows offered a grass-only diet or a total mixed ration

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**Introduction** Under the Kyoto Protocol (1997) Ireland agreed to limit its greenhouse gas (GHG) emissions to 13% above 1990 levels during the first commitment period 2008-2012. Ireland is legally bound to meet this GHG emissions reduction target. Agriculture in Ireland is the largest contributor to total GHG emissions (26.6%). Eighty per cent of the methane emissions from agriculture in 2006 came from enteric fermentation (McGettigan *et al*, 2008). Manipulating the diet of dairy cows is considered a strategy to mitigate methane production. The objective of the current experiment was to investigate the methane emissions and milk production response when spring calving Holstein Friesian dairy cows were offered a grass-only or total mixed ration (TMR) diet.

**Materials and methods** Forty eight spring calving Holstein Friesian dairy cows (18 primiparous and 30 multiparous) were randomly assigned to one of two nutritional treatments for 10 weeks: (1) Grass-only (grazing unsupplemented perennial ryegrass pasture) (2) TMR (offered *ad libitum* indoors). Animals were blocked according to parity, bodyweight (BW), body condition score, days in milk and milk yield. The TMR offered per cow per day was composed of maize silage (7.5 kg DM), concentrate blend (8.6 kg DM), grass silage (3.5 kg DM), molasses (0.7 kg DM) and straw (0.5 kg DM). The pre-grazing herbage mass was 1492 kg DM/ha (S.D=332 kg). Pre- and post-grazing sward heights were measured (platometer) before and after each grazing. The average pre- and post-grazing sward heights for the trial period were 9.4 cm (S.D=1.26 cm) and 4.1 cm (S.D=0.51 cm) respectively. Milk yield was recorded daily while milk composition was determined weekly from one evening and one morning milk sample taken on consecutive days. Bodyweight was also recorded weekly. Daily methane emissions were measured during weeks 4 and 10 of the trial for a 5-day period using the sulphur hexafluoride (SF<sub>6</sub>) tracer gas technique. Herbage DM intakes for the grass-only cows were estimated using the n-alkane technique as modified by Dillon and Stakelum (1989). DM intakes for the TMR cows were recorded using the Griffith Elder® feeding system. The data were analysed by analysis of variance with a model which included terms for treatment, lactation number and days in milk and using covariate analysis for milk yield, milk composition and bodyweight (SAS Institute, 2003).

**Results** TMR cows had significantly ( $P<0.01$ ) higher milk yield, milk solids yield, BW, total dry matter intake (TDMI) and CH<sub>4</sub> emissions (g/day) than grass-only cows in both measurement periods. However, grass-only cows had higher ( $P<0.05$ ) milk protein content and lower ( $P<0.05$ ) CH<sub>4</sub> emissions per unit milk solids and per unit BW in both measurement periods. They also had lower CH<sub>4</sub> emissions per unit intake in the second measurement period ( $P<0.001$ ).

**Table 1** Milk production and methane emissions from spring calving dairy cows offered a grass-only or TMR diet

	Week 4 – Measurement Period 1				Week 10 – Measurement Period 2			
	Grass	TMR	s.e.d	Sig.	Grass	TMR	s.e.d	Sig.
Milk yield (kg/day)	20.8	28.4	1.00	0.001	18.1	26.4	0.66	0.001
Milk solids (kg/day)	1.58	2.07	0.073	0.001	1.36	1.91	0.056	0.001
Milk fat content (g/kg)	43.2	42.5	1.38	0.63	40.7	40.1	0.130	0.68
Milk protein content (g/kg)	32.3	30.8	0.41	0.001	34.0	32.6	0.55	0.02
Bodyweight (kg)	475	534	5.0	0.001	482	547	6.7	0.001
TDMI (kg DM/day)	13.3	18.8	0.72	0.01	15.7	20.4	0.67	0.001
CH <sub>4</sub> emissions (g/day)	243	384	10.9	0.001	258	410	9.2	0.001
CH <sub>4</sub> emissions (g/kg DMI)	19.1	20.4	0.95	0.18	17.1	20.1	0.82	0.001
CH <sub>4</sub> emissions (g/kg milk solids)	157	185	6.7	0.001	196	213	7.7	0.02
CH <sub>4</sub> emissions (g/kg BW)	0.51	0.72	0.019	0.001	0.54	0.75	0.022	0.001

**Conclusions** This study indicates that grazing dairy cows emit less methane per cow and per kg of milk solids produced in comparison to TMR-fed cows. Hence, feeding grass-only diets is a strategy for decreasing enteric methane emissions from dairy production systems.

**Acknowledgements** Financial support from the Research Stimulus Fund of the Department of Agriculture, Fisheries and Food is gratefully acknowledged.

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## The effect of ploughing intensity on grassland CO<sub>2</sub> and N<sub>2</sub>O fluxes

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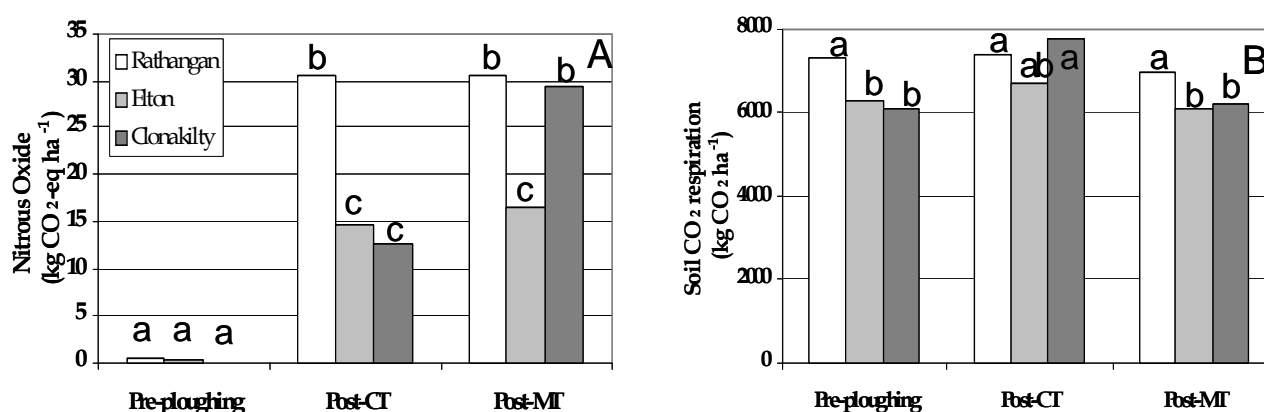
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**Introduction** The effects of land-use change on greenhouse gas fluxes are considered to be significant with the conversion of grassland to annual or perennial cropland estimated to release up to 2 tonnes CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup> (Lal 2003, Ogle *et al.* 2004). However, a large degree of uncertainty is associated with these estimates, and the transition phases between land-uses, in particular, are poorly quantified. In addition, the intensity of soil cultivation may affect emissions of both CO<sub>2</sub> and N<sub>2</sub>O via alterations to both soil carbon (C) and nitrogen (N) turnover and balances. The objective of this study was to quantify and compare the greenhouse gas (GHG) fluxes following conventional ploughing (CT) and minimum tillage (MT) on three different soil types in a small scale lysimeter study in Johnstown Castle, Wexford in order to assess the environmental impact of this step of the land use change process.

**Materials and methods** The study was carried out on isolated *Lolium perenne* lysimeters (diameter 0.75m, depth 1m) of three different soil drainage classes: Poorly-drained (Rathangan), medium-drained (Clonakilty) and well-drained (Elton). Three lysimeters of each soil type were subsequently either manually inversion-ploughed to 30cm or minimum-tilled to 10cm using a trowel. Measurements of soil CO<sub>2</sub> respiration and N<sub>2</sub>O flux were measured for 22 days pre- and post-disturbance using static chambers temporarily placed over the lysimeters. CO<sub>2</sub> respiration was measured with chambers connected to an infra-red gas analyser (EGM-4, PP Systems, Hitchin, Herts. UK), and fluxes were calculated from the rate of CO<sub>2</sub> concentration increase over a three minute period. Gas samples for N<sub>2</sub>O analysis gas were taken from each chamber over intervals of 30–45 min. These samples were subsequently analysed using a Varian 3300 GC with electron capture (ECD) detector. Analysis of variance procedures were conducted and least significant difference (LSD) calculated using SAS 3.0 (SAS Institute, Cary, NC).

**Results** The effects of tillage intensity on both N<sub>2</sub>O and CO<sub>2</sub> fluxes are shown in Figure 1. Nitrous oxide emissions increased substantially following ploughing, with emissions from the poorly-drained soil (Rathangan) 100% higher than the better-drained soils after inversion ploughing. Tillage intensity significantly affected fluxes ( $p < 0.01$ ) for the medium-drained (Clonakilty) soil, with cumulative emissions increasing from 13 kg CO<sub>2</sub>-eq ha<sup>-1</sup> to 29.8 kg CO<sub>2</sub>-eq ha<sup>-1</sup>. Increases in soil respiration post-disturbance were transient and no significant effects were observed except for the Clonakilty soil, where a small, but significant increase was observed after inversion ploughing ( $p > 0.05$ ).



**Figure 1** Cumulative fluxes of A) Nitrous oxide and B) soil CO<sub>2</sub> respiration on lysimeters (n=3) over 22 days before and 22 days after conventional inversion ploughing (CT) and minimum tillage (MT). Different letters indicate significant differences ( $p > 0.05$ ).

**Conclusions** Post-cultivation, CO<sub>2</sub> emissions were only higher for the Clonakilty soil, whereas N<sub>2</sub>O emissions remained high over a prolonged period of time for all soil types. The differences in N<sub>2</sub>O fluxes in terms of both ploughing intensity and soil type were probably driven by differences in soil moisture conditions. Initial increases in soil respiration were possibly due to degassing of CO<sub>2</sub> trapped in the soil pore spaces. However, sustained increased fluxes, that are predicted by biogeochemical models were not observed.

**Acknowledgements** The authors gratefully acknowledge funding under the DAFF Research Stimulus Fund

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## Can the nitrification inhibitor DCD decrease nitrous oxide emissions from slurry applied to grassland?

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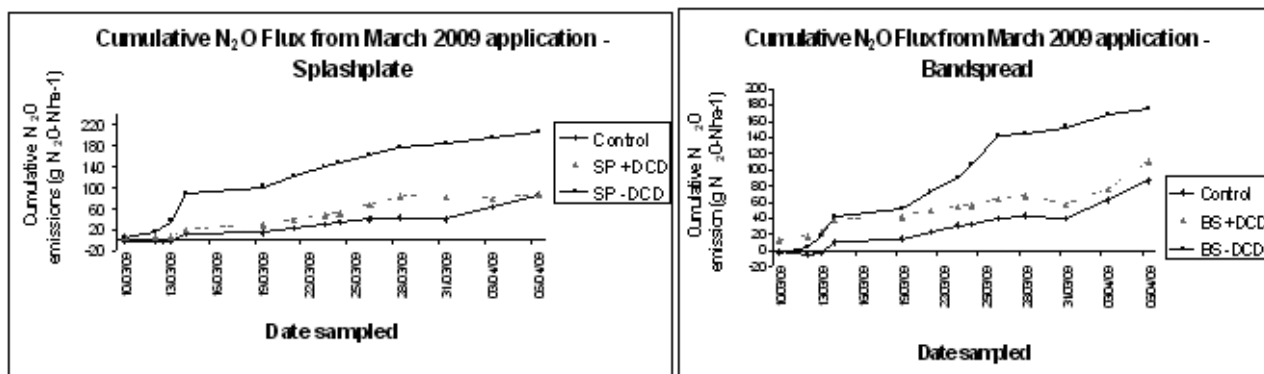
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**Introduction** In Ireland, the Kyoto Protocol sets a target to reduce greenhouse gas emissions to within 13% of 1990 level by 2012. Currently agriculture accounts for 26% of total emissions and of this figure 36% is due to trace gas emissions from the soil mainly in the form nitrous oxide ( $N_2O$ ). Landspreading of cattle slurry adds the trace gases  $N_2O$ ,  $CH_4$  and  $CO_2$  to the atmosphere in varying quantities, depending on the application method and climatic conditions at the time of application. The nitrification inhibitor Dicyandiamide (DCD) has been reported to reduce denitrification of excess nitrate within the soil by up to 73% (Di and Cameron, 2006). Dennis *et al.* (2008) reported DCD reduced  $N_2O$  emissions from poorly drained Irish soils receiving urine. Little research has been conducted on the efficacy of DCD in reducing emissions associated with landspreading of slurry. The objective of this research was to investigate the effect of DCD in reducing  $N_2O$  emissions following landspreading of slurry using band spreading and splash plate.

**Materials and methods** There were 5 treatments, with two spreading methods 1. Splashplate (SP) and 2. Bandsread (BS) both  $\pm$  DCD and a control. The experiment was conducted on an imperfectly drained fine loam in Johnstown Castle, Wexford arranged in a randomised block design with 6 replicates per treatment. Cattle slurry was applied at a rate of  $33 \text{ m}^3 \text{ ha}^{-1}$  in March, June and October 2009, which are typically important dates for slurry application before/after winter and after first cut silage. DCD was mixed with the slurry at a rate of 15% of the slurry  $NH_4\text{-N}$  content prior to application. Gases ( $N_2O$ ,  $CH_4$  and  $CO_2$ ) were collected over a 25 minute period using stainless steel chambers ( $0.4 \times 0.4\text{m}$ ) placed on water filled permanent collars. The head gas samples were analysed in the laboratory using gas chromatography. Measurements were made frequently over 1 month after slurry application. The treatments were tested for statistical differences using the GLM procedure in SAS v. 9.1, the factors being spreading method, slurry and DCD.

### Results

The daily  $N_2O$  fluxes and the cumulative flux from the March applied slurry  $\pm$  DCD are presented in figure 1. For splashplate applied cattle slurry receiving DCD reduced cumulative emissions from 110 to 42  $\text{g N ha}^{-1}$ . For bandsread slurry  $N_2O$  emissions were decreased from 112 to 60  $\text{g N ha}^{-1}$ . Overall the cumulative  $N_2O$  emissions were low after the spring applied slurry. The mean temperature at the site was  $7.5^\circ\text{C}$  during the sampling period and there was a total rainfall of  $55.2 \text{ mm}$  (avg  $1.6\text{mm d}^{-1}$ ).



**Figure 1** Cumulative daily  $N_2O$  emissions for splash plate and bandsread slurry  $\pm$  nitrification inhibitor DCD.

The  $N_2O$  emissions measured over the month after slurry application equated to  $\sim 0.2\%$  of inorganic slurry N (applied 09/03/09) which is considerably lower than the default emission factor 1% used by the IPCC. The  $N_2O$  emissions were very similar for the two spreading methods and the inclusion of DCD has significantly reduced cumulative  $N_2O$  emissions.

**Conclusions** Emission of  $N_2O$  from cattle slurry applied in March was low accounting for about 0.2% of N applied. The DCD treatments emitted significantly less  $N_2O$  than both the non-DCD treatment and the control. The incorporation of DCD with the landsread slurry reduced  $N_2O$  emissions by 46 to 62%.

**Acknowledgements** This research is financially supported under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture and Food, Ireland.

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## The effect of application method and timing of application on slurry $^{15}\text{NH}_4\text{-N}$ recovery in herbage and soil

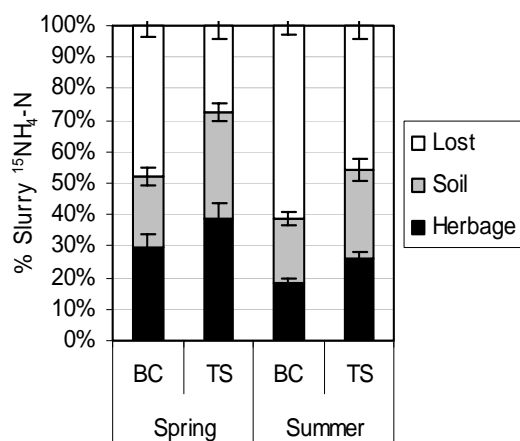
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**Introduction** In Ireland, the implementation of the EU Nitrates Directive imposes strict limits to nutrient inputs onto livestock farms, placing renewed emphasis on improving manure nutrient recovery. Low-emission slurry application techniques such as shallow injection and trailing shoe have been shown to reduce ammonia emissions compared to broadcast by 73 and 57%, respectively (Misselbrook *et al.*, 2002). For Irish grasslands, the trailing shoe appears to be the most suitable alternative to broadcast, as the high stone content of soils and undulating topography make injection unsuitable. However, it is not clear if the reduced ammonia emissions of the trailing shoe compared to broadcast result in an increase in slurry N uptake by the grass. Therefore, the objective of the experiment presented was to determine slurry  $\text{NH}_4\text{-N}$  recovery in herbage and soil in the year of application as affected by application method and timing (spring versus summer application) using a  $^{15}\text{N}$  tracer technique.

**Materials and methods** The experiment was conducted on permanent pasture at Johnstown Castle, Wexford, Ireland, as part of an agronomic experiment with similar treatments (Lalor & Schulte, 2009). Three treatments were investigated: 1) no slurry applied (N), 2) broadcast application (BC) and 3) trailing shoe application (TS). The  $\text{NH}_4\text{-N}$  fraction of slurry was spiked with highly enriched (99 atom%  $^{15}\text{N}$ ) ammonium sulphate to give a 2 atom% enrichment (2% of all N atoms were in the form of  $^{15}\text{N}$ ) and applied to 80 cm by 80 cm micro-plots by watering can at a rate equivalent to 33 tonnes per hectare (~100 kg Total N / ha and 50 kg  $\text{NH}_4\text{-N}$  / ha). The slurry was applied in April (spring application) or June (summer application) 2007 and 2008 with 6 replications. The spring applied micro-plots were harvested and sampled in June, and residual cuts (no additional fertiliser applied) were taken in July and September. Summer applied micro-plots were harvested in July with a residual cut in September. Herbage dry matter (DM) yield >5cm and the total N and  $^{15}\text{N}$  concentration in the harvested grass and the soil (top 15 cm) in the 50 by 50 cm square within the micro-plots were determined. The recovery of slurry  $\text{NH}_4\text{-}^{15}\text{N}$  in herbage and soil was calculated, and based on that the % of slurry  $\text{NH}_4\text{-}^{15}\text{N}$  lost from the system could be determined. Statistical analysis was carried out using PROC MIXED in SAS. The fixed factors were application method, timing and experimental year, including all two- and three-way interactions, and block was included as random factor.



**Figure 1** Effect of application method and season on slurry  $\text{NH}_4\text{-}^{15}\text{N}$  recovery in herbage (cum) and soil, and on calculated loss (all % of total applied), averaged over two years. Error bars =  $2 \times \text{SE}$  ( $n=12$ ).

**Results** Of all the slurry ammonium N applied, at the end of the season on average, 26% was recovered in the soil and 28% in herbage (Fig. 1). The remaining 46% of  $^{15}\text{NH}_4\text{-N}$  was lost from the plant-soil system. The main loss pathway for slurry ammonium N is volatilisation, followed by losses through leaching, denitrification and runoff. Additionally, some  $^{15}\text{N}$  was probably incorporated into the stubble and root mass, or moved into soil layers below 15 cm, which was not accounted for in this experiment.

Ammonium loss was significantly lower ( $p<0.0001$ ) for TS compared to BC. This resulted in a higher recovery of ammonium in grass and soil (on average by 9 and 10 % points, respectively). The difference between TS and BC was largest in the first cut after application, but remained visible for the residual harvests. The increase in slurry N recovery resulted in a higher yield for TS compared to BC in the first cut after application (on average 4.9 and 4.5 t DM ha<sup>-1</sup>, respectively) (results not shown).

The ammonium N loss was significantly higher ( $p<0.001$ ) during summer than spring (Fig. 1). This was related to weather conditions after application, as average temperature and radiation tended to be higher, which may have resulted in higher volatilisation (Dowling *et al.* 2008).

**Conclusions**  $^{15}\text{N}$  labelling of slurry proved to be a good tool for plot scale quantifications of N recoveries and losses after application of slurry. The slurry  $\text{NH}_4\text{-}^{15}\text{N}$  recovery was significantly higher for trailing shoe compared to broadcast applied slurry. Additionally,  $\text{NH}_4\text{-}^{15}\text{N}$  recovery from spring applied slurry was higher than for summer applied slurry. N efficiency in livestock grassland systems can be increased by changing spreading timing to spring and/or spreading method to trailing shoe.

**Acknowledgements** This research is financially supported under the National Development Plan, through the Research Stimulus Fund 2005, administered by the Department of Agriculture and Food under the National Development Plan.

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## The effects of 'Biosolids' on soil biota – a laboratory study

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**Introduction** Current EU directives (ED 86/278/EEC) promote the recycling of municipal sludge in agriculture and to set standards to protect the environment and food quality. Treated sludge is termed 'biosolids' of which an estimated 150,000 tonnes will be produced annually in Ireland by 2020. The only means of disposing of this material, in compliance with current regulations, is by land spreading. Biosolids may improve soil fertility and structure and benefit crop yields, however, their impact on agricultural ecosystems are unknown. The objective of this study was to investigate the effects of biosolids from various sources in Ireland and applied at different rates on sensitive indicator invertebrate species under laboratory conditions.

**Materials and methods** The species investigated were the earthworm *Eisenia fetida* and the springtail *Folsomia candida*. Worms and springtails of similar age and development stage were obtained following the collection of cocoons and eggs, respectively, from populations reared in a controlled environment cabinet at 20 °C and 12 h : 12 h D : N. Artificial soil, composed of kaolinite 20%, moss 10% and quartz sand 70%, was used for worm trials in boxes 10 x 10 x 10 cm and containers of 5.5 cm diam. and 6.5 cm in height were used for springtails. Worms were reared on reconstituted cow manure and springtails reared on yeast. Biosolids ( $\approx 90 - 96\%$  DM) were obtained from the five plants throughout the country. The rate of applied biosolids were equivalent to 2, 5, 10 and 20 t/ha (recommended rate is 2 t/ha) were compared with untreated controls. Replication was 6-fold. Ten worms and ten springtails were used per treatment. Mortality and biomass of the adult worms were recorded after 28 days and the number of juvenile earthworms after 56 days. Mortality/reproduction of springtails were recorded after 28 days. The data were analysed using the general linear model procedures (SAS 9.1, 2003).

**Results** Applying Biosolids at the 2 t/ha rate had no affect on the mortality of adult worms. While the 5 t/ha rate resulted in the death of a small number of worms the difference with that for the 2 t/ha was not significant. Increasing the rate of application of any of the five Biosolids from 5 t/ha to 10 t/ha caused a significant ( $p=0.01$ ) reduction in worm number. There were significantly fewer worms recovered from the 20 t/ha rate than that for 10 t/ha. Juvenile worms were significantly fewer than controls for a one Biosolid at the 2 t/ha rate and for three Biosolids at 5 t/ha. No juvenile worms were recorded at the 10 t/ha and 20 t/ha rates, respectively for two and four of the Biosolids investigated. Comparisons of Biosolids from different sources based on the number of juvenile worms produced showed differences were significant.

In the case of adult springtails, significantly fewer were recorded for the 2 t/ha rate when compared with that for untreated controls. The difference in adult numbers between the 2 t/ha and 5 t/ha rates was not significant. Neither was the difference between the 5 t/ha and 10 t/ha rates. However, the 20 t/ha rate had significantly fewer adults than that for the 10 t/ha. Each of the four rates of Biosolids had significantly fewer juveniles when compared with controls. When compared with the 2 t/ha rate the 5, 10 and 20 t/ha rates had significantly fewer juveniles. Juvenile springtail numbers recorded for the 5 t/ha rate did not differ significantly with that for 10 t/ha but were significantly greater than that for the 20 t/ha rate. The number of juveniles recorded for the 10 and 20 t/ha rates were low and were not significantly different. Comparing the number of juveniles recorded for the different Biosolids showed significant differences *i.e.* a source effect.

**Conclusions** Applying Biosolids at 2 or 5 t/ha had no effect on the mortality of adult worms. Significant worm mortality occurred at the 10 and 20 t/ha rates. The production of juvenile worms was reduced at the 2 t/ha rate while above this rate relatively few juveniles were produced. The 2 t/ha rate caused mortality of adult springtails and reduced production of juveniles. Biosolids from different locations differed in their effects on worms and springtails. Overall, the laboratory study showed that while the application of Biosolids at the normal rate of 2 t/ha does not affect adult worms it reduces the production of juveniles and negatively impacts on both adult and juvenile springtails.

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## The impact of soil type and soil biology on the survival of *Escherichia coli* O157

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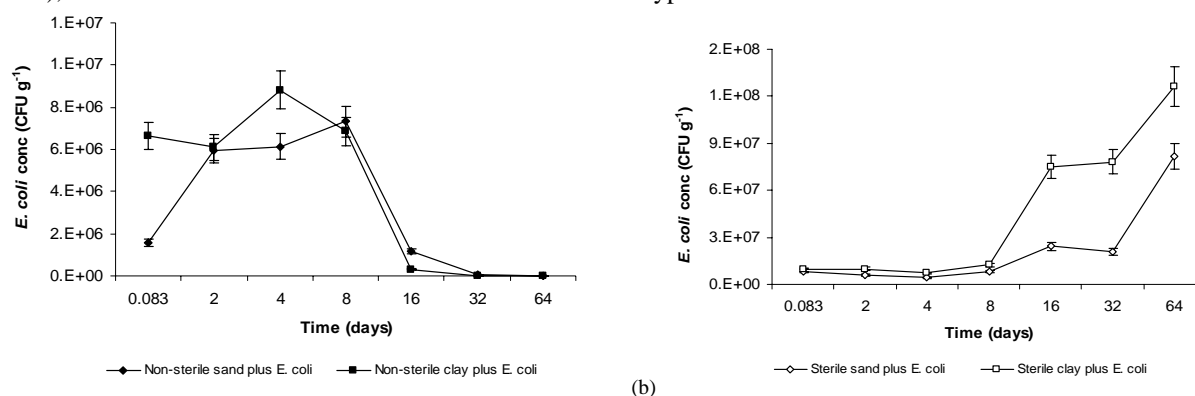
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**Introduction** *E. coli* O157 is a pathogenic micro-organism which is ubiquitous in the agricultural environment as a result of livestock defecation and slurry spreading. This pathogen can cause severe gastroenteritis and haemolytic uraemic syndrome in humans. Thus it is necessary to determine the factors that influence *E. coli* survival in soil to ensure that agricultural practices do not pose a risk to public health. The aim of this work was to determine the impact of soil biota and soil type on the survival of a non-pathogenic strain of *E. coli* O157, which shows similar survival characteristics to pathogenic *E. coli*.

**Materials and methods** Sandy loam and clay loam soils were collected from Silsoe farm, Cranfield, UK. Soil was sieved to 4mm and moisture content was adjusted to 44 % and 25 % of field capacity for clay and sand soil respectively. 5g sub-samples were transferred to microcosms (sterile glass vials, 30ml). Half of these microcosms were sterilised by autoclaving. Microcosms were stored at 4°C for the first 6 days of the experimental period, and at room temperature (18°C) thereafter. Test microcosms were inoculated with 500µl of culture, containing a known *E. coli* concentration of  $2 \times 10^7$  colony forming units (CFU) per gram of soil. The inoculum culture was prepared by adding 100µl of an overnight culture of *E. coli* O157 to 100ml fresh LB broth and incubating at 37°C for 24 hours on an orbital shaker at 150 rev min<sup>-1</sup>. This culture was subsequently centrifuged at 10 000 x g and washed three times in sterile ¼ strength Ringers solution. The culture was then serially diluted to a factor of ten, and each dilution was plated onto Sorbitol MacConkey agar amended with Cefixime-Tellurite supplement (CT-SMAC), which is selective for the O157 strain. Plates were incubated at 37°C for 24 hours. Based on these results, it was possible to determine the appropriate dilution to use for microcosm inoculation. Control microcosms were inoculated with 500µl of sterile ¼ strength Ringers solution. Microcosms were mixed gently by hand following inoculation and weighed to establish cumulative initial moisture content. They were sampled destructively immediately after inoculation, and on days 2, 4, 8, 16, 32 and 64 of the experimental period. They were monitored for evaporation by weighing, and sterile water was added when necessary. Sampling consisted of adding 10ml sterile ¼ strength Ringers solution to each microcosm and vortexing, followed by 15 minutes on a reciprocating shaker at 150 rev min<sup>-1</sup>. Microcosms were then vortexed again, and allowed to stand for 5 minutes for the heavier soil fraction to settle out of suspension. 5-fold dilutions were established for each microcosm in sterile universal bottles. 100µl of each dilution was plated onto CT-SMAC, and incubated at 37°C for 24 hours after which characteristic beige colonies of non-toxicogenic *E. coli* O157 were counted and recorded (adapted from Avery *et al.*, 2005).

**Results** *E. coli* concentrations were stable at low temperature (4°C), and there was no significant effect of time, treatment or soil type on *E. coli* survival. Conversely, the three-way interaction between time, treatment and soil type was strongly significant at room temperature (18°C,  $p < 0.001$ ). Data also suggest that this interaction differed between sand and clay microcosms. There was a significant effect of the interaction between temperature and treatment on *E. coli* survival ( $p < 0.001$ ), but there was no difference in this effect between soil types.



**Figure 1** *E. coli* concentration in (a) sterile and (b) non-sterile sand and clay microcosms over time. Values show mean CFU g<sup>-1</sup> ± SEM (n=3).

**Conclusions** These results show that the soil biota has a definite impact on *E. coli* O157 survival, which becomes more pronounced at ambient temperature. The influence of soil type on survival is less clear, due to additional confounding factors such as organic matter, soil spatial structure and microbial diversity.

**Acknowledgements** This work is funded by a Walsh Fellowship Grant from Teagasc.

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## Dry matter production of perennial ryegrass swards following varying levels of poaching damage on a free-draining earth soil

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**Introduction** Poaching damage is a limiting factor for pasture production and utilisation. Understanding the negative effects of poaching remains crucial since grazing is becoming more important for European farmers. Poaching has been shown to reduce pasture production by 13 to 45 % (Meneer *et al.*, 2001). The objective of this study was to quantify the effects of varying levels of poaching damage on the dry matter (DM) production and tiller density of a perennial ryegrass sward on a free-draining acid brown earth soil.

**Materials and methods** Twenty four 5 m x 11 m (55 m<sup>2</sup>) plots were established in a two-year old sward dominated by perennial ryegrass (*Lolium perenne* L.) at Teagasc Moorepark Dairy Production Centre in the south of Ireland. Four treading damage treatments were applied: i) Control (C), ii) Barely damaged (BR), iii) Intermediately damaged (ID) and iv) Badly damaged (BD). Forty five non-lactating dairy cows (average weight 550 kg) were used to achieve the desired levels of damage. Residency times were 0, 20, 40 and 120 minutes for C, BR, ID and BD, respectively. Half of each plot was rolled four weeks after the poaching treatments had been applied (12 March) thereby creating 48 plots (2.5 m x 5.5 m). Once preceding and five times subsequent to treatments being applied, herbage mass on each treatment plot was estimated by harvesting a strip of 10 m x 1.2 m using a motor Agria mower (Etesia UK Ltd., Warwick, UK). The fresh weight of the harvested material was recorded and a sub-sample (100 g) was dried at 40° C for 48 h, and DM% obtained. Herbage mass (kg DM/ha) was then calculated. Tiller density was assessed once before and on two occasions after the poaching event. Three turves (10 cm x 10 cm) were selected randomly from each plot, cut to a depth of >30 mm and dissected. The species of each tiller were identified and counted. All plots were fertilised with 30 kg nitrogen (N)/ha in the form of calcium ammonium nitrate (CAN) after they were grazed. Hoof depth was measured with a ruler on 20 random hoof marks in each plot. Surface roughness was measured using a 7.57 m chain placed on the soil surface following the contours of the poached soil. The chain length reduction was an estimate of the roughness of the surface. Two measurements per plot were taken and then averaged to calculate the chain reduction. Differences between mean values were tested for significance by ANOVA, with level of poaching and rolling as factors as a randomised complete block design with rolling as split plot.

**Results** Poaching affected herbage mass on the first harvest post damage but there was no difference in cumulative DM yields. After the imposition of treatments, herbage mass was significantly reduced for the BD treatment (Table 1). Cumulative DM yields to the end of August were not different between treatments and tiller density was not affected by poaching or rolling. Average depth of hoof prints were 3.57, 4.83 and 5.83 cm deep ( $P < 0.001$ ) for BR, ID and BD, respectively. Surface roughness, expressed as a percentage of chain reduction, was 2.52, 6.17, 8.37 and 12.73 % ( $P < 0.001$ ) C, BR, ID and BD, respectively. Surface roughness was greater in areas that had not been rolled (5.89 %) compared to areas that had been rolled (2.97 %;  $P < 0.001$ ).

**Table 1** Dry matter yield (kg DM/ha) and perennial ryegrass tiller density (m<sup>2</sup>) of the four treatments Control (C), Barely damaged (BR), Intermediately damaged (ID) and Badly damaged (BD), and for the two sub-treatments Rolled (R) and Not rolled (NR).

	Grazing	C	BR	ID	BD	P value	R	NR	P value	SED
DM yield (Kg DM/ha)	1	2,304 <sup>ab</sup>	2,402 <sup>a</sup>	2,095 <sup>b</sup>	1,620 <sup>c</sup>	<0.001	2,042	2,169	n.s.	153.7
	2	1,215	1,320	1,369	1,351	n.s.	1,309	1,319	n.s.	56.6
	3	2,192	2,332	2,472	2,509	n.s.	2,361	2,392	n.s.	123.3
	4	1,488 <sup>a</sup>	1,558 <sup>a</sup>	1,587 <sup>ab</sup>	1,706 <sup>b</sup>	0.0432	1,564	1,606	n.s.	69.2
	5	2,038	1,983	2,156	2,145	n.s.	2,086	2,075	n.s.	121.1
Tiller density (PRG/m <sup>2</sup> )	1	7,985	8,276	8,050	8,106	n.s.	8,126	8,082	n.s.	734.9
	2	7,700	7,498	7,485	7,817	n.s.	7,646	7,604	n.s.	712.4

SED = Standard error of the difference; <sup>abc</sup> values in the same row not sharing a common superscript are significantly different; PRG=Perennial rye grass.

**Conclusions** Previous studies have reported reduced pasture production following a single intensive poaching event (Drewry *et al.*, 2008). However, in those studies, the soil type was silt loam or clay. Conversely, in the present study, the soil type was a free-draining, acid brown earth with sandy-to-loam texture. Herbage on this soil may be more resilient to damage than herbage on soils with higher clay content. Hence, the lack of treatment effect in the present study may have been due to soil texture. This study indicates that perennial ryegrass swards on sandy-to-loam soils overcome a single event of substantial treading damage in spring. A similar experiment is being repeated in a heavier textured soil.

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## The effects of dairy cow weight on selected soil physical properties related to compaction

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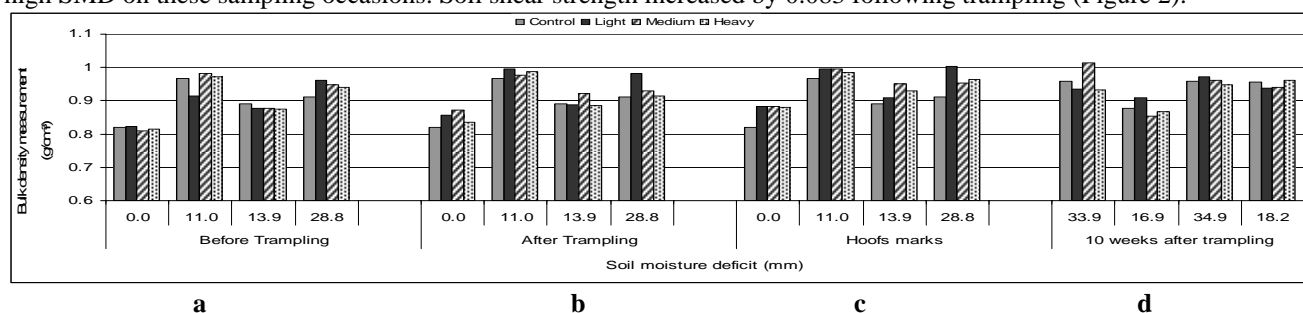
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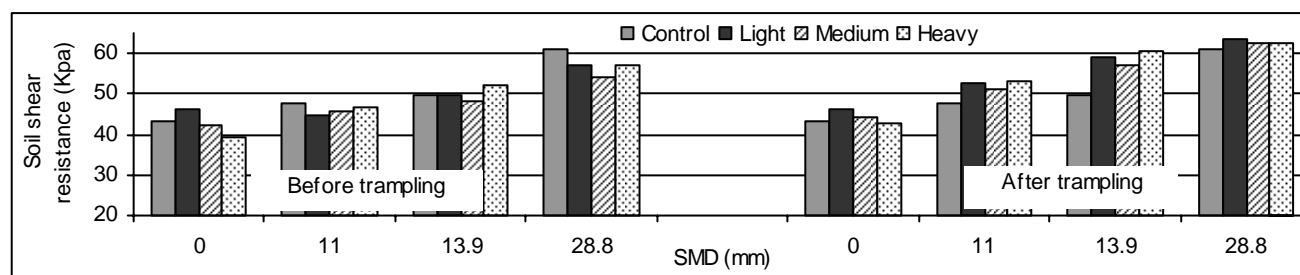
**Introduction** Compaction occurs as a result of traffic on the soil surface, e.g. tractors, machinery, cattle trampling. In grazing systems, animal treading can affect soil physical properties and in some circumstances, such as critical soil water contents can result in soil compaction and reduction or loss of soil porosity. Excessive compaction has damaging consequences for agriculture and the environment, such as reduced plant growth, reduced infiltration rates and increased runoff potentials (Gifford *et al.*, 1977). Changes in soil bulk density can be used to determine the occurrence of compaction as a result of surface activities. An increase in bulk density following treading is an indication of compaction. The objective of this study was to investigate the effect of treading by dairy cows of different weights on soil physical properties on a free draining soil at a range of soil moisture deficits (SMD).

**Materials and methods** An experiment was undertaken at Teagasc Moorepark, Fermoy, Co. Cork on a free draining soil type. The experiment was a 4 x 4 factorial arrangement, with four replicates per treatment. The treatments were SMD (SMD = 0.0, 11.0, 13.9 and 28.8 mm) and four dairy cow weights representative of Holstein Friesian (545 kg +/- 20 kg), Jersey X Friesian (478 kg +/- 20 kg), Jersey (389 kg +/- 20 kg) or no cow (0 kg). Soil moisture deficit was estimated using the model developed by Schulte *et al.*, 2005. At the target SMD, 2 cows per treatment were walked up and down the assigned plots five times in each direction. The size of the front left hoof on each cow was measured and taken to represent that of the four hooves on the cow. Bulk density, total porosity, gravimetric and volumetric water content, penetration resistance and soil shear strength were measured, using standard methods, before and after treading. Data relating to bulk density and soil shear strength are presented here. Bulk density measurements were repeated 10 weeks after the trampling event to assess soil recovery.

**Results** There was a significant effect (<0.001) of SMD and trampling on bulk density (Figure 1). The effect of cow weight was approaching significant (0.0599). Bulk density increased by 0.024 following trampling, and was 0.048 greater in the hoof marks. Ten weeks after trampling the soil bulk density was 0.04 higher than before trampling, this is explained by the high SMD on these sampling occasions. Soil shear strength increased by 0.083 following trampling (Figure 2).



**Figure 1** The effect of four cow weights (0, 389, 478, 545 kg) on soil bulk density at four SMDs (0.0, 11.0, 13.9, 28.8 mm) before trampling (a), after trampling in non-hoof marks area (b), after trampling in hoof mark areas (c) and 10 weeks after trampling (d).



**Figure 2** The effect of three cow weights on soil shear strength at four SMDs (0.0, 11.0, 13.9, 28.8 mm) immediately before and after trampling.

**Conclusions** Bulk density and soil shear strength increase significantly with increasing SMD. Soil bulk density and soil shear strength were similar for all cow weights at the four SMDs. No significant differences between the three cow weights on bulk density and soil shear strength measurements were found.

**Acknowledgements:** This project is part funded by the Research Stimulus Fund 2005 – RSF05-201 administered by DAFF.

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## The effect of co-blending water treatment residual with manure on the concentrations of soluble phosphorus in surface runoff

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**Introduction** Water treatment residual (WTR) is a waste product from the purification of potable water whereby chemical products (Aluminium [ $\text{Al}_2(\text{SO}_4)_3$ ] or iron salts ( $\text{FeCl}_3$ )) are included for coagulation of suspended sediment and dissolved organic carbon. Such residues have high levels of unutilised amorphous Al or Fe and could be used as an amendment to lower manure water soluble P (WSP). The aim of this study was to 1) characterise WTR phosphorus absorption maxima (P<sub>max</sub>) (2) determine the WTR capacity to lower WSP in cattle manure in the laboratory and the optimum time required for co-blending WTR with manure and 3) determine if runoff phosphorus (P) is lowered when WTR is pre-mixed with manure and surface applied in the field.

**Materials and methods** WTR from Carron Hill water treatment works, Co. Armagh was dried and ball-milled to  $<150\mu\text{m}$ . Phosphorus sorption isotherms were generated by batch equilibrium to determine WTR P<sub>max</sub>. WTR (25 g WTR/L) was shaken with standard P solutions (ranging from 0 to 3.5 g P/L, as  $\text{KH}_2\text{PO}_4$ ) at 150 rpm for six days at 20°C. The isotherm data were fitted to the linearised Langmuir model (Olsen and Watanabe, 1957) and a P<sub>max</sub> value was determined. In the laboratory, WTR was added to dairy slurry (2.5g WSP/kg) at 36, 72 and 144 g/kg. Samples were incubated in triplicate at 10°C and removed from the incubator at 10, 24, 48, 72 and 108 hours. Samples were homogenised, centrifuged and analysed for residual WSP by the colorimetric method of Murphy and Riley (1962). For the field runoff experiment, slurry was mixed with WTR at two rates (100 and 250 g/kg) and after four days was surface applied at 50 m<sup>3</sup>/ha to 0.5 m<sup>2</sup> grassland plots. A control slurry received no WTR amendment. Simulated rainfall was applied for 30 minutes at 40 mm/hr, two days later. Total runoff was collected and analysed for total P (TP) and dissolved reactive P (DRP). Logarithmic transformations (log base 10) were performed on all P concentrations and runoff volumes and differences in flow-weighted mean P concentrations between slurry treatments were assessed by analysis of variance.

**Results** A P<sub>max</sub> of 82.6 g P/kg was calculated for the WTR. This was much higher compared to the P<sub>max</sub> reported for 18 WTRs in the US, 10.4 to 37.0 g/kg (Dayton and Basta, 2005). In the laboratory experiment it was only with the highest WTR rate that WSP was found to be substantially reduced (Fig. 1). At 108 hours, WSP was reduced from 2.15 to 0.61 g P/kg, approximately 70%. Applications of WTR were further increased in the field runoff experiment to maximise the potential of reducing runoff P concentrations. However, although runoff P concentrations in runoff from WTR co-blended manures were lower than the control manure no significant difference was found in either P concentrations or runoff volume. A possible reason for this may be the increased manure dry matter which prevented thorough mixing.

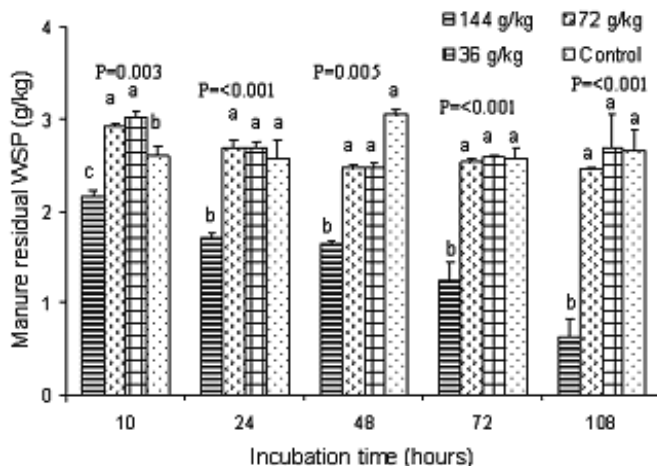


Figure 1 Rate of WSP sorption by WTR in dairy manure

Table 1 Effect of WTR on Flow weighted mean P concentrations and runoff volume

Manure treatment g WTR/kg manure	TP ——mg P/L——	DRP	Runoff mm
Control	3.7	3.0	0.3
100	2.7	2.4	0.4
250	2.0	1.9	0.9
<i>P</i>	NS	NS	NS

NS, not significant

**Conclusions** This is the first study to assess the effect of co-blending WTR with manure on P concentrations in runoff. To date only WTR applications in soil incorporation and as buffer strips have been investigated. Given that WTR may be a stronger sorbing material in Northern Ireland than in the US further work is required to optimise the rate of WTR rate in slurry as well as temperature and moisture conditions during incubation to realise the full potential of WTR co-blended manures as a best management practice.

**Acknowledgements** The authors gratefully acknowledge funding from DARDNI and the Gibson Institute.

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## The effect of dairy cows grazing in early spring on phosphorus loss in overland flows post first and second grazing events

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**Introduction** Early season grazing has a number of benefits on dairy farms, including reducing silage requirements, reducing the quantity of slurry in stores, and encouraging good sward development. However, soil moisture levels can be high in early spring, thus increasing the risk of soil surface damage. This, combined with the removal of herbage by grazing cows may increase the risk of phosphorus (P) export to waterways via overland flow pathways. In view of the contribution of P to eutrophication of waters in Northern Ireland, early spring grazing may present a risk. Thus, the aim of this experiment was to examine the effect of grazing intensity in spring on P losses via overland flow.

**Materials and methods** Four treatments (Table 1) were examined in a randomised block design experiment. Treatments were imposed during the first grazing in the spring (23<sup>rd</sup> February), and comprised swards being ungrazed (UG), lightly grazed (LG) or heavily grazed (HG). A second grazing event (-G) took place six weeks later (6<sup>th</sup> April). A fourth treatment (UG-UG) was not grazed during either grazing event. Sixteen plots (each measuring 3 x 7m) were established in four blocks on a permanent pasture on a north facing slope (average slope 6.5%). Blocks were separated by buffer strips, and were enclosed within an area measuring 35 x 38 m. Pre-grazing sward heights for the first and second grazing events were 5.2 cm and 6.9 cm respectively. This area was grazed by ten lactating dairy cows (average liveweight, 650 kg) fitted with excreta collection bags. During the first grazing event the ten cows had access to the LG- and HG- plots for a period of 90 minutes, before being removed. Cows were returned to the site later on the same day and had access to the HG- plots for a further 90 minutes. Following the first grazing event average sward height was 4.6 cm and 4.0 cm for LG- and HG- plots respectively. During the second grazing event cows had access to the UG-G, LG-G and HG-G plots for 120 minutes with plots grazed to a mean residual sward height of 3.0 cm. The soil was a Brown Earth clay loam of Olsen P content 39.2 mg/l (Index 3). Average volumetric soil moisture content was 63% and 56% for the first and second grazing events respectively. Overland flow was generated at 2 and 16 days post grazing using two portable rainfall simulators. Rainfall was simulated at a rate of 40 mm/hr (return period >1 in 50 yrs). Runoff was generated for a 30 minute period and collected in 3 x 10 minute discrete samples. Soluble reactive phosphorus (SRP), total soluble phosphorus (TSP) and total phosphorus (TP) were determined using standard colorimetry techniques. Particulate phosphorus (PP) was inferred as the difference between TP and TSP. Differences between each of the four treatments were analysed by ANOVA using SPSS software, with each of the first and second grazing events analysed separately. Significance of differences between treatments were determined by Least Significant Difference.

**Table 1** Treatments imposed during the first and second grazing events

Treatment	First Grazing (23.02.09)	Second Grazing (06.04.09)
UG-G	Ungrazed	Grazed
LG-G	Light Grazing	Grazed
HG-G	Heavy Grazing	Grazed
UG-UG	Ungrazed	Ungrazed

**Results** Average P concentrations measured in overland flow are presented in Table 2. Treatment had no effect on SRP or TSP losses during either the first or second grazing events ( $P>0.05$ ). During the first grazing event light grazing (LG) had no significant effect on either TP or PP losses, compared to the ungrazed treatments ( $P>0.05$ ), while losses were higher with the heavy grazing (HG) treatment ( $P<0.05$ ). When swards were grazed in early April, TP and PP losses were higher ( $P<0.001$ ) in treatments which had been grazed in early season (LG-G and HG-G), compared to those that were not (UG-G). The numerically higher TP and PP losses recorded in all treatments during the second grazing event reflects a higher volumetric soil moisture levels (68.1%) at the time when runoff was generated, a consequence of a period of heavy rainfall post grazing.

**Table 2** Average P concentration ( $\mu\text{g/l}$ ) in overland flow during the first and second grazing events

	First grazing event						Second grazing event					
	UG-G	LG-G	HG-G	UG-UG	S.E.M.	sig.	UG-G	LG-G	HG-G	UG-UG	S.E.M.	sig.
Soluble Reactive P	172	150	188	154	19.6	NS	132	127	139	106	11	NS
Total Soluble P	222	217	249	197	21.3	NS	185	176	192	153	11	NS
Total P	673 <sup>a</sup>	835 <sup>ab</sup>	993 <sup>b</sup>	634 <sup>a</sup>	82.5	0.05	1363 <sup>a</sup>	1984 <sup>b</sup>	2373 <sup>b</sup>	1035 <sup>a</sup>	182	0.001
Particulate P	450 <sup>a</sup>	618 <sup>ab</sup>	744 <sup>b</sup>	437 <sup>a</sup>	81.3	0.05	1168 <sup>a</sup>	1805 <sup>b</sup>	2103 <sup>b</sup>	867 <sup>a</sup>	175	0.001

**Conclusions** Heavy grazing in early spring increased the risk of P losses (TP and PP), while 'well managed' light grazing had no effect on P losses. However, there was evidence of a carry-over effect, with early spring grazing resulting in increased P losses during a later grazing event, although these losses were measured at a time when soil moisture levels were high.

**Acknowledgements** DMcC acknowledges receipt of a DARD postgraduate studentship. Study funded by DARD and AgriSearch.

## Assessing groundwater denitrification under two contrasting land uses in South-East Ireland

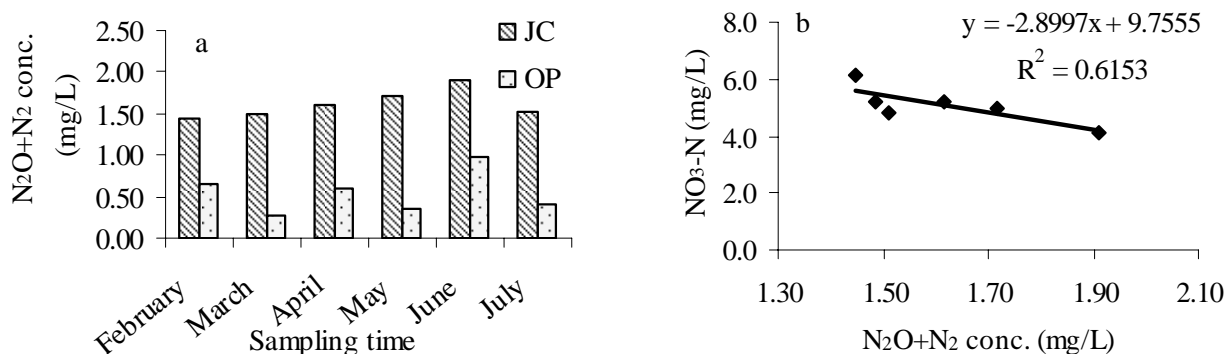
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**Introduction** Denitrification is focused upon as the dominant nitrate attenuation process in groundwater zones. A major concern for the implication of denitrification is that this process does not only serve as a natural pathway for excess  $\text{NO}_3^-$  attenuation but its intermediate product  $\text{N}_2\text{O}$  is a potent greenhouse gas (IPCC, 2007). The implementation of the Nitrates Directive and the Kyoto Protocol in Ireland has identified the considerable need for robust scientific data on the fate and transport of nitrogen (N) in agricultural systems. The aim of present work was to investigate the denitrification capacity and  $\text{N}_2/(\text{N}_2\text{O}+\text{N}_2)$  ratio within the shallow groundwater zone.

**Materials and methods** A groundwater monitoring network was established in Johnstown Castle (JC) grazed grassland and Oak Park (OP) tillage farm (100 km far from JC). The aquifer geology in the shallow groundwater was sand and gravel intermixed with clay and sand, and dense gravel with clay bend in Johnstown and Oak Park, respectively. Seven piezometers (5 cm ID) and 2 m screen sections were installed 3.5 to 5.5 m below ground level. Groundwater was sampled monthly using a bladder pump following the USEPA low flow sampling procedures (*In situ* Inc. USA). DO, pH, temperature, turbidity, electrical conductivity and redox potential were measured during sampling using a multiparameter probe. Groundwater samples were transported at 4°C to the laboratory and analysed, using standard methods, for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ -N, TOC, Total N, Na, K, Ca, Mg, Fe, Zn,  $\text{SO}_4^{2-}$ , and Cl<sup>-</sup>. Dissolved groundwater  $\text{N}_2\text{O}$  was analysed by degassing groundwater in sealed serum bottle (160ml) using high purity He (water: He=3:1) and the headspace gas was analyzed on a Varian gas chromatograph (Reay *et al.*, 2003). The quantity of groundwater  $\text{N}_2$  was estimated from  $\text{N}_2/\text{Ar}$  ratios, measured using Membrane Inlet Mass Spectrometer.



**Figure 1** Mean monthly  $\text{N}_2\text{O}+\text{N}_2$  conc. over the sampling period in Johnstown Castle (JC) and Oak Park (OP) (a); and correlation between dissolved  $\text{N}_2\text{O}+\text{N}_2$  and  $\text{NO}_3-\text{N}$  concentrations (b).

**Results** Total denitrification was higher in JC than in OP (Fig. 1a). High temporal variation of total denitrification were observed in both land uses systems, giving the highest and lowest concentrations of dissolved  $\text{N}_2\text{O}+\text{N}_2$  in June and February, respectively in JC. In contrast, OP showed the highest and lowest denitrification in June and March, respectively. The estimated loss of  $\text{NO}_3-\text{N}$  by denitrification was 24.30 and 4.80% in JC and OP, respectively. The  $\text{N}_2/(\text{N}_2\text{O}+\text{N}_2)$  ratio ranged from 0.96 to 0.99 in JC and 0.59 to 0.98 in OP. The  $\text{NO}_3-\text{N}$  concentrations over the whole period showed a strong negative correlation with the total denitrification indicating the transformation  $\text{NO}_3-\text{N}$  to  $\text{N}_2\text{O}-\text{N}$  and  $\text{N}_2$  (Fig. 1b).

**Conclusion** Grazed grassland showed a higher  $\text{NO}_3^-$  removal capacity through denitrification process than the tillage farming system. A higher percentage of denitrification in the tillage site is  $\text{N}_2\text{O}$  rather than  $\text{N}_2$ , which could be indirectly contributing to greenhouse gas emissions upon discharge to surface water. Groundwater denitrification appeared to be an important mechanism in reducing  $\text{NO}_3^-$  in groundwater zone.

**Acknowledgement** This research is financially supported under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture and Food, Ireland.

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## Laboratory study of a denitrification system using a permeable reactive barrier

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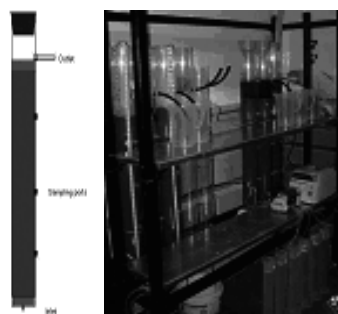
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**Introduction** Point-source nitrate ( $\text{NO}_3$ ) contamination of shallow groundwater can result in  $\text{NO}_3$  plumes of high concentration. Pressure to clean these waters under the EU Water Framework Directive (WFD) has seen the need for *in situ* remediation technologies. One such technology is a Permeable Reactive Barrier (PRB). A PRB comprises a layer of carbon (C)-rich material positioned perpendicular to the direction of flow of contaminated groundwater. In this study, the objective was to build laboratory-scale PRBs to determine the best reactive media to be used in a field-scale study to treat  $\text{NO}_3$ -rich water. This paper presents the finding from two reactive media – (1) lodgepole pine woodchips and (2) cardboard – that have been loaded with  $\text{NO}_3$ -amended water for a period of greater than 130 days. Future work will test new materials and will address the production of greenhouse gas (GHG).

**Materials and methods** 100mm-diameter x 1m-deep acrylic columns were constructed in a temperature-controlled room operated at a temperature of  $10^\circ\text{C}$ . Each column comprised a 1.5cm-deep 'water tank' (built using a fine metal mesh) at the base to allow uniform distribution of influent water into the column. 0.8m-deep reactive media rested on top of the metal mesh. The columns were sealed at the top with rubber stoppers. Influent water was applied at the base of each column using a peristaltic pump (operated continuously) and the water exited the column via a 1cm-diameter tube positioned just above the reactive media surface. This mode of operation was in accordance with existing work in this area (Hunter & Shaner 2009; Moon *et al.* 2008; Rocca *et al.* 2007; Saliling *et al.* 2007; Volokita *et al.* 1996) and prevented the occurrence of preferential flow pathways that may occur if the system was loaded from the surface. Rubber septum stoppers were positioned at 20 cm-intervals along the side of the columns to enable water samples to be collected (Figure 1).



**Figure 1** Columns set-up

Two reactive media were used in this study: lodgepole pine woodchips (LPW) (~14-20 mm in size) and cardboard (CB) (cut in squares of ~25mm). Both sets of columns were constructed in triplicate ( $n=3$ ). The LPW were placed in the columns in alternating 30mm-deep layers with soil from the proposed study site. The average initial porosity was ~33%. The CB columns were constructed in a similar manner to LPW columns. Here, the average initial porosity was ~41%. All 6 columns were covered with black plastic. Prior to operation, all columns were loaded with tap water, loaded at a rate of  $19\text{--}22.5 \text{ mm d}^{-1}$  (to establish background C and nutrient release), after which they were seeded with ~1L of bulk fluid containing heterotrophic bacteria from a wastewater treatment plant and loaded with  $\text{NO}_3\text{--N}$  solution at a concentration of 20 mg/L. Almost daily water samples exiting the columns were analysed for: pH, chemical oxygen demand (COD), ammonium ( $\text{NH}_4\text{--N}$ ),  $\text{NO}_3\text{--N}$ , nitrite ( $\text{NO}_2\text{--N}$ ) and ortho-phosphorus ( $\text{PO}_4\text{--P}$ ).

**Results** The results are presented in Table 1. The LPW and CB columns have been working for more than 150 and 130 days, respectively, and continue to produce effluent  $\text{NO}_3\text{--N}$  concentrations below 0.0076mg/L. Student t tests were utilized in the data analysis, and a P-value greater than 0.05 was obtained, indicating no statistical difference between the columns (LPW and CB). This represents a removal of almost 100%. Effluent COD from both sets of columns continues to remain high (>572 mg/L), indicating an abundant C source in the media. Effluent  $\text{NH}_4\text{--N}$  from both columns is high (>3.85mg/L), indicating N release from the media.

**Table 1** Average results from the Lodgepole pine woodchips (LPW) and Cardboard (CB) columns.

	pH outlet	COD		$\text{NH}_4\text{--N}$		$\text{PO}_4\text{--P}$		$\text{NO}_3\text{--N}$ inlet		$\text{NO}_3\text{--N}$ outlet	
		mg/L		mg/L	stdev	mg/L	stdev	mg/L	stdev	mg/L	stdev
LPW	7.37	572	4.8	2.2	0.20	0.17		21.35	0.93	0.008	0.015
CB	7.16	911	3.9	2.5	0.10	0.16				0.005	0.013

**Conclusions** The results from this study indicate that lodgepole pine and cardboard are good media for use in PRBs. Both media have produced effluent  $\text{NO}_3\text{--N}$  concentrations below 0.0076mg/L. This represents a removal of almost 100%. Effluent pH, COD and  $\text{PO}_4\text{--P}$  concentrations have continued to remain stable.

**Acknowledgements** The authors gratefully acknowledge funding from the Department of Agriculture and Food under the National Development Plan 2007-2013.

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## The addition of chemical amendments to dairy cattle slurry for the control of phosphorus (P) in runoff from grasslands

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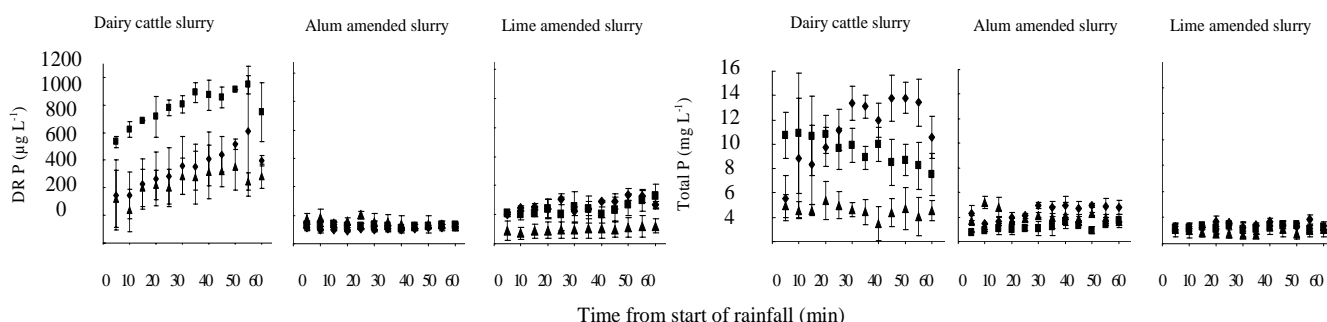
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**Introduction** Runoff from grassland pastures and meadow fields following slurry application can result in incidental P and suspended sediment (SS) losses, and has the potential to transport nutrients to surface water where fields are in close proximity to surface waters (Volf *et al.*, 2007). This may result in eutrophication of rivers and fresh water lakes. ‘Incidental P loss’ is the term given to P losses that occur shortly after manure application. Dairy cattle slurry is an excellent source of nutrients and land spreading is the most efficient means of disposal. It is critical that manure is spread effectively and efficiently to minimise P losses. Chemical amendment of slurry for the control of P is not currently considered a mitigation method in Ireland. However, the Water Framework Directive (WFD) recommends research and development of new pollution mitigation measures to achieve a 2015 target of ‘good status’ waters. The aim of this work was to identify potential amendments to control P in runoff following land spreading of dairy cattle slurry. The effects of amendments on gaseous emissions from slurry and effects on metals in runoff were not determined in this experiment. The objective of this work is to examine – at laboratory scale - the effect of chemical amendments on dissolved reactive phosphorus (DRP) and total phosphorus (TP) loss from grassland following land spreading of dairy cattle slurry.

**Materials and methods** Intact grassed sods were cut in 300-mm-wide by 600-mm-long by 80-mm-deep sods using a spade and placed on plastic-covered boards. The sods were then trimmed and placed end-to-end in 2m-long flumes. Molten candle wax was used to seal any gaps along the flume. Replicated experiments (n=2) were conducted within 10 days of soil collection. Homogenized dairy cattle slurry samples were collected in 10-L drums and transported to the laboratory and stored at 4°C until testing commenced.

A runoff experiment was designed to compare the nutrient effects of chemical amendment of dairy cattle slurry subjected to low-energy rainfall with an intensity of 11.5 mm hr<sup>-1</sup>. All sod samples were approximately at field capacity before the treatment commenced. Surface runoff samples were collected in 5-min-intervals once runoff began. Each rainfall simulation comprised 3 successive 1-hr rainfall events at time zero (Rainfall 1), after a 1-hr interval (Rainfall 2) and after a 24-hr interval (Rainfall 3) to determine the effect of storm interval on the effectiveness of chemical amendments in reducing P loss via surface runoff. Four treatments were examined in the rainfall simulator: (i) dairy cattle slurry (the study control); (ii) alum-amended slurry; (iii) and lime-amended slurry. Dairy cattle slurry and the amended slurries were applied to the soils in the flumes at a rate approximately equal to 26 kg TP ha<sup>-1</sup>. Alum (0.68:1 Al: TP stoichiometric rate) and lime (10:1 Ca: TP) were added to the slurry immediately prior to land spreading. Immediately after collection, runoff water samples were filtered (0.45µm) and analysed colorimetrically for DRP using a nutrient analyser (Konelab 20, Thermo Clinical Labsystems, Finland). Unfiltered runoff water samples were frozen at -20°C for TP analysis using acid persulphate digestion. All samples were tested in accordance with the Standard Methods (APHA, 2005).

**Results** Alum addition resulted in best reduction in DRP in runoff, lowering the mean flow-weighted concentration (MFWC) of DRP by 87% during Rainfalls 1 and 2, and by 66% during Rainfall 3. Lime addition reduced the DRP MFWC by 67% during Rainfall 1, 76% during Rainfall 2, and by 77% during the Rainfall 3. Lime was best at reducing TP losses from the flume. Lime reduced MFWC of TP loss by an average of 85% and alum reduced TP loss by 53%.



**Figure 1** Dissolved reactive phosphorus, suspended sediment, total phosphorus and particulate phosphorus for dairy cow slurry, alum amended dairy cow slurry and lime amended dairy cow slurry. (Rainfall event 1 (♦), Rainfall event 2 (■) and Rainfall event 3 (▲))

**Conclusion** Alum and lime reduced losses of DRP and TP for each of the 3 rainfall events. Alum resulted in the greater reduction in DRP in runoff however it was not as effective as lime at reducing TP. Work is required to examine long term effects of treatment.

**Acknowledgements** Authors wish to express gratitude to Teagasc for funding this project through Teagasc Walsh Fellowship.

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## Effect of cultivation of clover based grassland on N losses to groundwater on a clay-loam soil

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**Introduction** Implementation of Nitrate Directive (91/676/EEC) in Ireland has constrained farmers to lower losses of nitrogen (N) to water. Cultivation of long-term permanent grassland increases the rate of mineralization of soil organic N and thus promotes N losses via leaching and denitrification. The objective of this experiment was to examine the impact of cultivation of permanent grassland on losses of N to water on a clay-loam soil (42% clays in the upper horizon) at Solohead Research Farm (52°51'N, 08°21'W).

**Materials and methods** The effect of cultivation of grass-clover swards in three dairy production systems on losses of N to ground water was studied over a one year period (August 2008-August 2009). Monitoring was conducted in a randomised complete block design with 2 factors. There were two cultivation treatments: (i) cultivated and reseeded grass-clover swards [cultivated] and (ii) permanent grass-clover swards [uncultivated]. There were six replicated plots of both cultivated and uncultivated swards within each of three systems. The systems of dairy production compared had: (i) a mean calving date of 17 February, stocking density of 2.15 cows/ha, receiving 90 kg/ha annual fertilizer N input; (ii) a mean calving date of 17 February, stocking density of 1.6 cows/ha, receiving no fertilizer N input and (iii) a mean calving date of 16 April, stocking density of 1.6 cows/ha, receiving no fertilizer N input. During the summer of 2008 five sampling units constructed from plastic pipe were installed in each plot (> 1ha). The depth of the wells ranging from 0.8m to 2.4m below ground level was based on ground water (GW) depth. Wells with screen openings on the lower 0.20 m of the pipes, covered by a cotton filter were sealed with bentonite on the soil surface. Sampling was conducted 18 times, fortnightly during the winter drainage period and after periods of high rainfall during other times of the year. All the resident water was removed from the wells and wells were allowed to recharge for two hours before sampling. The water samples from each plot were bulked. Concentrations of total oxidised N, nitrite N, ammonium N were determined by Aquakem 600A. The total N concentration was determined by LECO CN 2000 analyser. Meteorological data were recorded at the meteorological station located at the farm. Effective drainage during experimental period was calculated using model of Schulte *et al.* (2005). Nitrogen losses for each treatment were quantified. Concentrations of nitrate N, organic N and ammonium N were subjected to ANOVA (SAS Institute, 2009) examining the effects of milk production system, cultivation, sampling date and all their interactions.

**Results** Total rainfall for the period from August 2008 to August 2009 was 1364 mm and effective drainage was 705.5mm. Nitrate N concentrations were well below maximum admissible concentration (MAC; 11.3mg dm<sup>-3</sup>; Table 1). There were no effect of cultivation and dairy production systems on the concentrations of nitrate N, ammonium N and organic N in the shallow GW. Most of water samples (>0.97) had organic N and ammonium N concentrations exceeding the limits for Kjeldahl N (organic N + ammonium N) for drinking water (1.0 mg /L) defined by (EC 80/778). There were no differences in the quantities of ammonium N and organic N lost from the cultivated and uncultivated treatments. The annual quantity of nitrate N lost from the cultivated treatment were almost double (P<0.05) that from the uncultivated treatment. Organic N losses represented by far the largest proportion (>0.7) of the total N leached from the cultivated and uncultivated grass-clover swards.

**Table 1** Mean NO<sub>3</sub>-N, Organic-N and NH<sub>4</sub>-N concentrations (mg dm<sup>-3</sup>) and total annual losses of N to groundwater

	Concentrations of N (mg dm <sup>-3</sup> )			Total loss of N (kg N ha <sup>-1</sup> year <sup>-1</sup> )		
	nitrate N	ammonium N	organic N	nitrate N	ammonium	organic N
Uncultivated	0.356	0.213	2.543	2.127	1.401	15.029
Cultivated grassland	0.371	0.245	2.747	4.102	1.571	15.565
Significance	NS	NS	NS	P<0.05	NS	NS
SEM	0.068	0.042	0.176	1.218	0.303	1.519

**Conclusions** There were no effect of cultivation and system of dairy production on the concentrations of nitrate N, ammonium N and organic N in the ground water. The quantities of nitrate N lost by leaching were lower than reported in a previous study (Humphreys *et al.*, 2008) This may be largely attributed to high rainfall, wet soil conditions and relatively high soil temperatures during summer months, which promoted denitrification of nitrate in this heavy clay loam soil. In the present study, organic N was by far the largest pool of N lost from the soil to ground water.

**Acknowledgement** This study was funded by the Department of Agriculture, Fisheries and Food (Project RSF07-511)

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## Treatment of dairy soiled washwater using a woodchip filter

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**Introduction** Soiled water is produced on dairy farms through the washing-down of milking parlours and holding areas and contains nutrients and other constituents that pose a potential threat to water quality if not managed correctly. In Ireland, soiled water is generally applied to land, but the risk of nutrient loss to surface and ground waters from land application has attracted increased attention and legislation in the form of the EU Nitrates Directive and Water Framework Directive. Treatment and re-use of soiled water may negate some of these risks. This study examines the potential of woodchip filters to treat soiled water.

**Materials and methods** Laboratory filter units were constructed from 300 mm diameter plastic piping filled with woodchip. A stainless steel frame held the units in position so that soiled water could be applied to the top and effluent collected and sampled at the base. Sitka Spruce (*Picea sitchensis*) logs were de-barked and chipped (10-50 mm). There were three filter unit heights (0.5, 1 and 1.5 m) and two suspended solids (SS) loadings (1 % (S1) and 3 % (S3)). However, total nitrogen (TN) concentrations were low for S1, at an average of 235 mg/l. S3 gave an average TN concentration of 519 mg/l, which is close to the average found in a farm survey. There were three replicates, giving a total of 18 units. Fresh manure was dried down on-site and, once received in the laboratory was reconstituted with water and used as the influent. Soiled water was applied in equal volumes of 0.67 l three times daily (hydraulic loading rate was 28 l/m<sup>2</sup>/d). A 100 ml sub-sample of effluent was collected three times weekly after four hours drainage. Units were sampled for 277 days for S1 and 197 days for S3. Samples were analysed within 24 hours of collection for chemical oxygen demand (COD), SS, filtered and unfiltered TN, NH<sub>4</sub>-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N and PO<sub>4</sub>-P. Grab samples of woodchips, from different heights along the filter columns, were ground down and analysed for C using the CHN test before and after the experiment. NH<sub>3</sub> gas was trapped in HCl for one hour periods over a week and analysed using a Konelab Aquakem discrete spectrophotometric analyser. Statistical analysis was carried out using two-way factorial with replication using Statistical Analysis Software (SAS).

**Results and Discussion** Woodchip filters achieved considerable decreases in SS, TN and COD concentrations (Table 1). Over 99% of SS were removed for all heights at both loading rates ( $p > 0.05$ ), indicating that woodchip is an effective physical filter medium. A build up of solids on the top of the filters further indicated and aided filtration. There were significant decreases in the level of influent TN versus effluent TN, ranging from an 88% decrease for the 0.5m columns in S1 to 92% for the 1.0m and 1.5m columns. For S3, the percentage removal rates for TN were 93%, 92% and 93% for the 0.5m, 1.0m and 1.5m columns, respectively ( $p > 0.05$ ). A comparison between the unfiltered and filtered concentrations of TN for the influent and effluent indicated that filtration was the main treatment mechanism. The C content of the woodchip decreased from 49% to 45%, indicating that C in the woodchip may have contributed to biological nitrogen removal. Circumstantial evidence from the literature indicates that the woodchip can adsorb some nitrogen through physico-chemical absorption (Bolan et al., 2004). Nitrification is indicated by the increase in NO<sub>2</sub>-N and NO<sub>3</sub>-N. Analysis of NH<sub>3</sub> gas indicated that there was no significant volatilisation. Percentage removal rates of COD for S1 were 96% for all three heights and for S3 removal rates of 98% for the 0.5m columns and 97% for the 1.0m and 1.5m columns were observed ( $p > 0.05$ ). COD removal likely also occurred primarily due to filtration but filters were aerobic so biological oxidation of organic compounds is also possible.

**Table 1** Mean water quality parameters (mg/l) ( $\pm$  standard deviation) for wood-chip filter influent and effluent loaded at 1 % SS (S1) over a period of 277 days and 3% SS (S3) over a period of 197 days.

	Influent		Effluent					
	S1	S3	S1			S3		
			0.5m	1.0m	1.5m	0.5m	1.0m	1.5m
COD	12167 $\pm$ 1882	34418 $\pm$ 4982	523 $\pm$ 164	526 $\pm$ 115	518 $\pm$ 152	795 $\pm$ 160	999 $\pm$ 161	941 $\pm$ 145
TN	235 $\pm$ 56	542 $\pm$ 192	27 $\pm$ 12	24 $\pm$ 10	19 $\pm$ 9	40 $\pm$ 10	43 $\pm$ 11	40 $\pm$ 12
NH <sub>4</sub> -N	3.69 $\pm$ 0.49	0.98 $\pm$ 0.06	7.15 $\pm$ 4.56	6.54 $\pm$ 4.01	5.97 $\pm$ 3.58	6.69 $\pm$ 4.33	6.90 $\pm$ 4.49	7.27 $\pm$ 4.68
NO <sub>2</sub> -N	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.52 $\pm$ 0.94	0.61 $\pm$ 0.98	0.26 $\pm$ 0.39	0.13 $\pm$ 0.17	0.08 $\pm$ 0.13	0.09 $\pm$ 0.1
NO <sub>3</sub> -N	0.00 $\pm$ 0.00	0.54 $\pm$ 0.15	4.07 $\pm$ 3.97	3.20 $\pm$ 4.54	1.92 $\pm$ 2.90	2.23 $\pm$ 2.25	2.44 $\pm$ 3.22	1.76 $\pm$ 2.19
SS	10000 $\pm$ 0.00	30000 $\pm$ 0.00	6.77 $\pm$ 5.30	6.54 $\pm$ 6.79	4.83 $\pm$ 3.83	9.23 $\pm$ 5.52	8.56 $\pm$ 6.10	6.32 $\pm$ 3.74

**Conclusions** Woodchip was shown to be an effective filter medium for decreasing the concentrations of COD, SS and nutrients in dairy soiled washwater at two different loading rates.

Filtration, adsorption, nitrification, biological oxidation and biological removal may all have contributed to the treatment process.

The effect of filter unit depth on filter performance was found to be negligible.

**Acknowledgements** This study was funded by the Research Stimulus Fund of the Department of Agriculture, Fisheries and Food.

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European Communities (Good Agricultural Practice for Protection of Waters) Regulations 2009, SI 101 of 2009

## The effect of soil test P on export of P in overland and macropore flow from a tillage rotation

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**Introduction** The export of phosphorus from grassland has been researched over several years but losses from tillage at field scale have not been recorded in Ireland. The phosphorus loss pathway from land to surface water is primarily by overland flow (OLF) but some P loss is in macro-pore flow (MPF) which is soil water flowing through cracks and wormholes to field drains. The object of this experiment was to monitor total P (TP) loss from a cereal rotation in a tillage farming system.

**Materials and methods** The trial was set up on free-draining silty sandy loam underlain by glacial sands and gravels. Three plots, 1, 2 and 3 with areas of 0.7, 1.0 and 1.0 ha respectively had gradients of 0.5 to 6 % towards a line of surface drains connected to V-notch flow meter/samplers measuring overland flow, one monitor set for each of the three plots. Eighteen macro-pore samplers, each with a straight 0.075 x 1.2 m tube with a water sampler close to the bottom and installed in the soil at 45°, were installed on a grid pattern in the three plots. Overland flow was recorded continuously from the 1/1/2005 to 31/12/2007 but MPF sampling took place at weekly/fortnightly intervals during January to April and October to December each year. The main treatment in this trial, soil test P (STP) was determined from soil samples. Only limited statistics were used on account of data variability. Comparisons used annual-average and plot-average for most parameters. In regression, a similar number of mean values were used on each side. Mean values could not be calculated for MPF data due to incomplete data so the median and mode were used. Total number of samples for STP, OLF, TP, MPF and sediment were 54, 1141, 267, 146 and 285 respectively.

**Results and discussion** The target soil P index values of 2, 3 and 4 in plots 1, 2 and 3 respectively were not achieved as plot 3 reached soil P index 3 only. Significant overland flow was expected from this site due to observations of flooding in the previous years. However total flow averaged only 13.8 mm y<sup>-1</sup> compared to over 100 mm y<sup>-1</sup> at sites fifty miles away. The range in annual OLF exceeded 300% over the period of the trial (Table 1) while the range in annual rainfall was only 25%. This illustrates that even moderate increases in rainfall volume and intensity, predicted under climate change could give rise to large increases in flooding.

**Table 1** Annual values for STP and overland flow parameters compared

Year	Rainfall (mm)	STP (mg l <sup>-1</sup> )	OLF (mm y <sup>-1</sup> )	TP (mg l <sup>-1</sup> )	TP Load (kg ha <sup>-1</sup> y <sup>-1</sup> )	Sediment (kg ha <sup>-1</sup> y <sup>-1</sup> )
2005	731	5.9	5.4	1.01	0.04	92.3
2006	911	5.4	17.5	0.64	0.11	117.3
2007	842	7.1	18.1	0.60	0.18	109.0
Average(+/-SEM)	828	6.13+/- 4.5	13.8+/-130	0.75+/-0.19	0.11+/-0.0032	106+/-6300

The TP recorded at this site averaged 0.75 mg l<sup>-1</sup> for the three plots. Its trend over time was downward which corresponds with the upward trend in OLF over the same period and indicates dilution as the process controlling TP (Table 1). It was expected that when the soil was fallow in winter 2006 and spring 2007, TP would have increased but this effect was not evident, being masked perhaps by variation in overland flow. Total P Load at an average of 0.11 kg ha<sup>-1</sup> y<sup>-1</sup> was low compared to the average for Irish Agriculture, 0.4 kg ha<sup>-1</sup> y<sup>-1</sup> (Tunney *et al.*, 1998) and most European catchments 0.2 to 2 kg ha<sup>-1</sup> y<sup>-1</sup> (Kronvang *et al.*, 2005). Sediment volume was also low at 106 kg ha<sup>-1</sup> y<sup>-1</sup> when losses of the order of 1000 kg ha<sup>-1</sup> y<sup>-1</sup> are not uncommon in the USA. In regression analysis, OLF was found to be fairly closely related to TP, P load and sediment. (R<sup>2</sup> = -0.62, 0.53, 0.67 respectively) but less significance was found in the relationship between the main treatment STP and TP (R<sup>2</sup>=0.35). Macropore samplers were erratic in that they produced samples on only some days and not always the same sampler. This limited the analysis of TP in MPF that could be performed so the median (1.59 mg/l) and mode (0.82 mg/l) were calculated but not the mean. These values are higher than the corresponding statistics for TP in OLF (median; 0.34 mg/l, mode; 0.1 mg/l) suggesting that TP in MPF is higher than TP in OLF. This was expected as TP accumulates in macropores.

**Conclusions** The quantity of overland flow measured on this site was low compared to other sites. While this is good news for tillage in Ireland, the shortage of flood data limited the accuracy of the trial. The risk of pollution from this site was low but it was still possible to delineate tentative relationships between OLF and some other parameters but the relationship between STP, the main treatment, and TP was poor. The technique that was used for sampling MPF needs to be improved if TP loads are to be calculated. Overall the absence of pollution from this site suggests that tillage land is not a major source of P in surface water but more information is required to confirm this.

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## Are cows more likely to lie down the longer they stand?

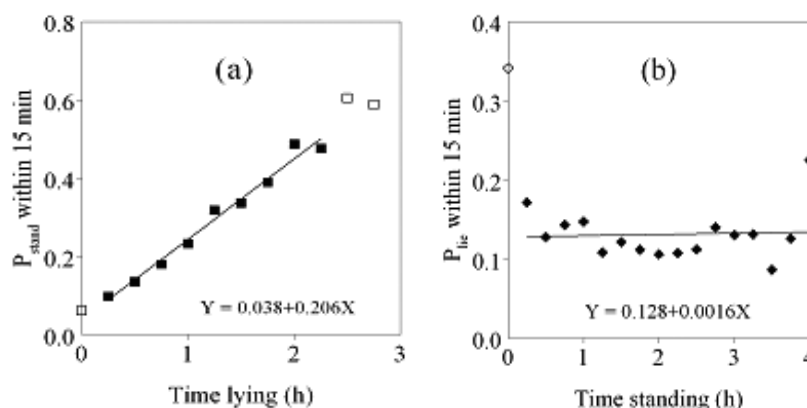
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**Introduction** Information on (changes in) standing and lying behaviour can be used for oestrus detection and early diagnosis of health problems, to evaluate welfare consequences of changes in housing and management and to investigate the underlying animal motivation for these behaviours. A data set on lying and standing behaviour was collected from cows with IceTag™ sensors (IceRobotics, South Queensferry, UK) fitted to their legs. Our aims were (a) to investigate whether this behaviour was bouted, (b) to estimate bout criteria if required and (c) to test the hypotheses that (i) the probability of cows standing up would increase with lying time and (ii) the probability of cows lying down would increase with standing time.

**Materials and methods** Data were obtained from IceTag™ sensors fitted to 10 late-pregnant indoor-housed beef cows for periods up to 16 days. During part of the experiment, video recordings were made to validate the sensor records. The sensors produced one record per cow per min with an estimate of the percentage of standing and lying time, from which uninterrupted standing and lying episodes were calculated. The structure of standing and lying behaviour was investigated first by analysing log-survivorship plots and frequency distributions of (log-transformed) standing and lying episode lengths. The probability of cows standing up ( $P_{\text{stand}}$ ) in the next 15 min at lying time  $t$  was calculated as  $1 - (\text{the number of lying bouts} > t + 15 \text{ min} / \text{the number of lying bouts} > t \text{ min})$ . The same method on the basis of standing bout lengths was used to calculate the probability of cows lying down ( $P_{\text{lie}}$ ) within 15 min. Effects of lying time on  $P_{\text{stand}}$  and of standing time on  $P_{\text{lie}}$  were estimated using linear regression. Only probabilities based on at least 100 observations were included in the regression analyses to avoid effects of data points based on few observations only.

**Results** A total of 10,814 lying episodes were recorded. Analyses of the (cumulative) frequency distributions of (log-transformed) lying episode lengths suggested that standing bouts were interrupted by an excessive number of short lying episodes (i.e.  $< 4$  min). Comparison of IceTag™ records with video recordings showed that lying episodes  $> 4$  min did correspond with lying behaviour, but episodes  $< 4$  min did not (these tended to occur e.g. when a cow was displaced at a feeder). In contrast, short standing episodes recorded by the sensors did correspond to actual standing behaviour. Lying and standing bouts were, therefore, calculated by ignoring all lying episodes  $< 4$  min. This decreased the number of episodes by 88%, but it had only minor effects on estimated total daily lying time ( $\sim 3\%$ ). The mean individual daily lying time varied from 7.9 to 15.4 (mean 10.0, SE 0.7). Individual mean daily lying time varied from 10.2 to 13.0 h (mean 11.6, SE 0.33 h). The probability of cows standing up increased linearly with lying time (Fig. 1a), as hypothesized. The probability of cows lying down was, however, entirely unaffected by standing time (Fig. 1b), which contradicted our hypothesis. Disaggregation of the data in subsets showed that the absence of any effect of standing time on  $P_{\text{lie}}$  was not caused by the pooling of data obtained during the day and the night or across individuals with different behavioural strategies.



**Figure 1** The probability of cows standing up ( $P_{\text{stand}}$ ) within 15 min in relation to time lying (a) and the probability of cows lying down ( $P_{\text{lie}}$ ) within 15 min in relation to time standing (b). Regression lines were fitted to the data indicated by the solid symbols. The regression line in graph (a) was highly significant ( $R^2 = 0.98$ ,  $P < 0.001$ ,  $\text{RSD} = 0.021$ ). The regression line in graph (b), however, was not ( $R^2 = 0.003$ ,  $P = 0.83$ ,  $\text{RSD} = 0.033$ ).

**Conclusions** Sensors can give relevant information on cows' standing and lying behaviour but the type of sensor used here recorded an excessive number of short lying episodes which must be adjusted for. Determination of a bout criterion that distinguishes between actual lying bouts and sensor settings suggesting short lying episodes but caused by other factors, such as sudden leg movements, then allows a meaningful interpretation of the data. The increase in the probability of cows standing up with lying time was as expected. Cows were, however, not more likely to lie down the longer they were standing, thereby refuting our second hypothesis. This suggests that the increase in motivation to lie down that has been observed after lying deprivation (Metz 1985; Munksgaard *et al.*, 2005) may have limited relevance for cows that are not deliberately lying-deprived.

**Acknowledgements** SAC receives support from Scottish Government, Rural and Environment Research and Analysis Directorate.

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## Factors affecting dairy cow preference to be indoors or at pasture

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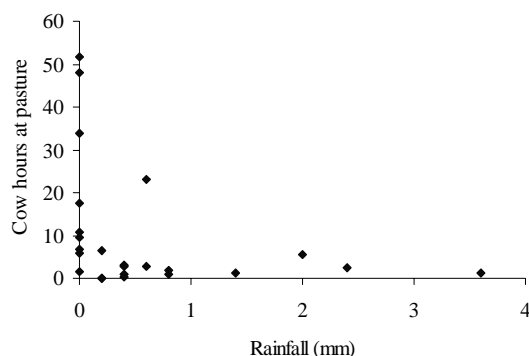
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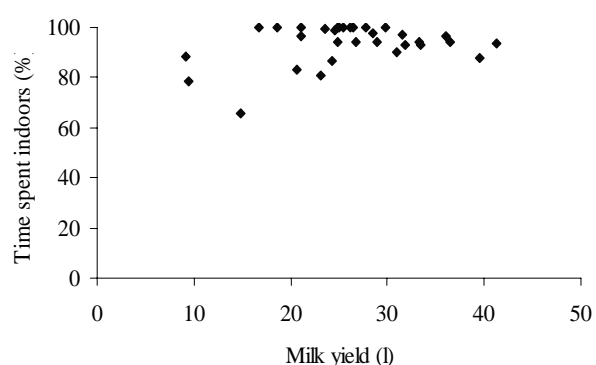
**Introduction** Indoor housing and pasture can have both positive and negative effects on the welfare of dairy cows, and environmental conditions can influence the location that dairy cows prefer. For example Krohn *et al.* (1992) reported pasture to be the preferred lying place for dairy cows during the summer months, but preference shifted towards indoor straw housing with deep bedding in the winter. However, it is unclear whether high yielding dairy cows have a preference for indoor housing or pasture and how environmental conditions and cow factors influence their choice. The aim of the study reported here was to determine whether high genetic merit dairy cows have a preference to be indoors or on pasture and to assess which environmental factors influence their preference.

**Materials and methods** The study was conducted between May and August 2008 with 24 high genetic merit Holstein dairy cows in mid to late lactation. Twice a day after milking, cows were given the choice of going to pasture (0.56 ha) or to a cubicle house. They were then free to move between the two until the next milking. Indoors, a total mixed ration (TMR) (DM basis: 32% grass silage; 25% maize silage; 22% concentrate blend (GLW Feeds, Loughborough, UK); 17% whole crop wheat; 2% seed hay; 2% molasses) was available *ad libitum*. At pasture, sward dry matter (DM) was maintained between 1800 and 3000 kg DM/ha measured using a rising plate meter. The study had three experimental periods, during each of which eight cows received an eight day training period followed by an eight day study period. Cows had at least two weeks prior experience of each location. A video camera was used to record time spent indoors and at pasture. To determine the environmental factors influencing the cow's decision, weather conditions were recorded indoors and at pasture using a Davis Vantage PRO2 weather recorder (Hayward, California, USA). Milk yield was recorded daily and each cow was given a body condition score (BCS), lameness score and were weighed on days one, eight and 16 of the study. One sample t-tests were used to analyse time spent and linear regressions were used to analyse factors affecting preference.

**Results** When given a choice the cows spent 91.9% ( $\pm 2.33$ ) of their time indoors, which was significantly different from 100% ( $P=0.001$ ), 50% ( $P<0.001$ ) and 0% ( $P<0.001$ ). Time spent indoors was influenced by environmental conditions. Rainfall influenced preference ( $P=0.015$ ) (Figure 1), with cows spending more time indoors on days when it rained. Relative humidity outdoors influenced preference ( $P=0.045$ ), as did relative humidity indoors ( $P=0.004$ ). When the relative humidity was low indoors ( $\leq 70.2\%$ ) and outdoors ( $\leq 78.7\%$ ) the cows spent more time at pasture (0.4 vs. 2.7 hours for high vs. low relative humidity indoors respectively; 0.7 vs. 1.7 hours for high vs. low relative humidity at pasture respectively). Average temperature indoors ( $P=0.985$ ) and average temperature outdoors ( $P=0.742$ ) had no effect on preference. Of the animal factors, milk yield affected preference ( $P=0.005$ ) (Figure 2), with high yielding cows spending more time indoors than low yielding dairy cows. There was a tendency for BCS to influence preference ( $P=0.058$ ). Cows with a high BCS score ( $> 2.7$ ) spent more time at pasture (1.5 vs. 0.9 hours for high vs. low BCS respectively) compared to cows with a low BCS ( $\leq 2.7$ ). Lameness ( $P=0.41$ ) and liveweight ( $P=0.77$ ) had no effect on preference.



**Figure 1** Number of hours the cows spent at pasture over 192 h



**Figure 2** Percentage time spent indoors and at pasture

**Conclusion** Cows expressed a partial preference to be indoors which was influenced by environmental conditions and individual cow factors. It is possible that the higher yielding dairy cows expressed a stronger preference to be indoors as the TMR indoors allowed them to satisfy nutritional demands more easily than grazing at pasture. The difference in the feed provided in each location may have influenced cow preference and requires further investigation.

**Acknowledgements** Genus farm staff provided much-appreciated co-operation throughout the study. Financial support was provided by Silcock Fellowship for Livestock Research, Harper Adams University College and Reaseheath College.

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## Adding straw to a total mixed ration and the method of straw inclusion affects production and eating behaviour of lactating dairy cows

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**Introduction** The inclusion of straw in the diet of lactating dairy cows as a source of physically effective fibre may benefit rumen function and production. By stimulating chewing and rumination, effective fibre should promote a more stable rumen by preventing large variations in rumen pH that can occur when cows consume high levels of readily fermentable carbohydrate (Beauchemin *et al.*, 2008). Whilst the concept of including limited amounts of straw in milking rations is accepted as being nutritionally sound, there is limited scientific proof available (Ferris *et al.*, 2000). Further to this, chop length of the straw as well as the amount included in the ration may be crucial if expected benefits are to be achieved without compromising production or feed efficiency. Our objective was to evaluate the effects of adding straw to a total mixed ration (TMR) and the method of straw inclusion (and resulting differences in straw chop length) on feed intake, eating and rumination activity, rumen pH, milk yield, and milk composition of lactating dairy cows.

**Materials and methods** Nine multiparous lactating Holstein-Friesian cows averaging 43 litres milk/d were used in a replicated 3 x 3 Latin Square design experiment with 3 wk periods. Treatments were a control TMR prepared using a horizontal mixer (C) and two treatment rations containing wheat straw added using a horizontal mixer (horizontal straw; HS) or a vertical mixer that gave a longer straw chop length (vertical straw; VS). The C TMR contained on a dry matter (DM) basis: 37% maize silage, 18% grass silage, and 45% concentrates. For treatments HS and VS straw was added to the C TMR at 4% of ration DM, diluting other ingredients. Measurements were obtained in the last week of each period. Feeding (6 cows) and rumination behaviour (3 cows) was monitored using computerized feed weight and jaw movement recording, respectively and rumen pH (3 cows) was monitored using indwelling probes. Data were analyzed using Mixed Models procedures and a model testing fixed effects of square (when appropriate), diet, period and diet by period interaction and random effects of cow. Orthogonal contrasts tested overall effects of straw and effect of mixer type.

**Results** Measurements of particle size distribution using a Penn State Separator found no difference between the C and HS rations, but a greater percentage of long particles ( $P<0.01$ ), and fewer medium ( $P<0.02$ ) and small ( $P<0.02$ ) particles for the VS ration. Average particle size was 12.2, 11.7, and 14.2 mm for C, HS, and VS rations, respectively. Cows fed the VS ration had lower DM intake (DMI), milk yield, and milk protein concentration and yield than for the C and HS rations (Table 1). Cows fed straw spent more time eating, thus had a lower eating rate compared to C, and these effects were greater for VS than for the HS ration (Table 1). In addition, cows fed straw consumed fewer meals of 4 to 8kg fresh weight, compared to the C ration (3.1 vs. 2.8 meals/d;  $P<0.001$ ). There were no effects of ration on rumination activity or rumen pH, but time below pH 6.0 was numerically lower for HS (5.28 h) compared to C (6.99 h) or VS (7.28 h).

**Table 1** Effects of straw inclusion and method of inclusion on intake (DM) milk yield and composition.

	Ration			SEM	P < <sup>1</sup>		
	C	HS	VS		Ration	Straw	Mixer
Intake (kg)	24.1 <sup>a</sup>	23.4 <sup>a</sup>	22.5 <sup>b</sup>	0.64	0.006	0.006	0.040
Milk yield (kg)	40.8 <sup>a</sup>	40.3 <sup>a</sup>	39.3 <sup>b</sup>	1.71	0.017	0.027	0.043
Fat (g/kg)	36.0 <sup>ab</sup>	35.2 <sup>a</sup>	36.5 <sup>b</sup>	1.78	0.113	0.840	0.041
Protein (g/kg)	32.1 <sup>a</sup>	31.7 <sup>b</sup>	31.1 <sup>c</sup>	0.82	0.001	0.001	0.001
Fat (g/day)	1460	1427	1418	83.1	0.303	0.137	0.735
Protein (g/day)	1288 <sup>a</sup>	1253 <sup>a</sup>	1208 <sup>b</sup>	46.8	0.006	0.006	0.050
Time eating, min/d	237 <sup>a</sup>	261 <sup>b</sup>	291 <sup>c</sup>	14.9	0.001	0.001	0.001
Eating rate, g DM/min	0.096 <sup>a</sup>	0.087 <sup>b</sup>	0.078 <sup>c</sup>	0.0066	0.001	0.001	0.011
Average rumen pH	6.15	6.18	6.14	0.087	0.778	0.827	0.550

<sup>1</sup> Probability of no effect of ration, addition of straw (C vs. HS and VS), or the mixer used (HS vs. VS).

<sup>a, b, c</sup> Values with different superscripts are statistically different ( $P<0.05$ )

**Conclusions** The results of the present study demonstrate that when rations prepared using identical ingredients, in the same proportions, are processed to give differing chop length there are differences in the responses of feed intake and milk production to added straw. Adding straw to a TMR at 4 % of ration DM reduced feed intake and milk yield when the straw was processed to give a longer chop length (VS). These differences in production response to straw incorporation into a TMR can be attributed in part to differences in particle size distribution and subsequent effects on total nutrient intake.

**Acknowledgements** The authors gratefully acknowledge funding from Richard Keenan and Co. Ltd

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## The effect of time of day when dairy heifers are introduced to a group containing mature cows on welfare and performance

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**Introduction** The integration of dairy heifers into the main herd during the post calving period can have negative effects on the heifer's welfare and productivity (Gonzalez *et al*, 2003). This appears to be related to the fact that heifers attain low social status when entering the milking herd and therefore are subjected to increased levels of bullying and aggression (Wierenga, 1990; Knierim, 1999). The aim of this study was to assess if the time of day (i.e. after 'AM' or 'PM' milking) when freshly-calved heifers are introduced to a group containing mature cows influences the heifers' welfare and performance.

**Materials and methods** Twenty-eight Holstein Friesian (HF) heifers were used in this study, with heifers introduced into a group containing mature cows between approximately 24-36 hours post calving. Animals were housed in cubicle pens with solid floors. Two treatments were examined, with heifers introduced either 1) after morning milking (AM: between 06:00-08:00 hours) or 2) after evening milking (PM: between 16:00-18:00 hours). Treatments were replicated five times, with treatments balanced for genetic merit, body weight, condition score and sire. In each replicate, a resident group containing 12 HF cows and 6 non-experimental HF heifers was established at least 7 days prior to the introduction of the first experimental heifer. In replicates 1 to 4, three non-experimental heifers were replaced by heifers in Treatment 1, and three by heifers in Treatment 2. In replicate 5, two non-experimental animals were replaced by heifers in Treatment 1 and two were replaced by heifers in treatment 2. In both treatments, experimental heifers were introduced approximately 30 minutes after the resident group returned from milking (Day 1). The social and exploratory behaviour of each heifer was recorded directly over a 2-hour period immediately after introduction to the group on Day 1. These behaviours were observed for each experimental heifer during 4 x 5 minute continuous observations at 30-min intervals during the 2 hour post-feeding period on one day each week for 1 month post introduction. A sub-category of social behaviours; 'social cohesive' was created for analysis and included the behaviours licking another animal/rubbing heads. The heifers' lying and standing behaviour was monitored by automated data loggers (Tinytag Plus, Re-Ed volt, Gemini dataloggers (UK) Ltd., Chichester, UK). These were fitted for 24 hours on Day 1, and for a continuous 24 hour period each week during the first 4 weeks post-introduction. Milk yield of heifers was recorded daily from day 5 to 35 post-calving. Mean behavioural data for the heifers from each treatment within each replicate was analysed by ANOVA. Individual production data was analysed by REML with PTA values used as covariates.

**Results** On Day 1, animals in the AM treatment received more threats ( $P<0.05$ ) and butts ( $P<0.001$ ) than those in the PM treatment (Table 1). There was a tendency for heifers introduced at AM to be chased more than those introduced at PM ( $P=0.05$ ). Heifers introduced at PM were more socially cohesive than those in the AM treatment ( $P<0.05$ ). No significant treatment effect was found for total hours lying, with heifers in both treatments lying for an averaged 7.5 hours/day ( $P>0.05$ ). During Day 1 heifers in both treatments lay for less than 4 hours/day.

**Table 1** Effect of timing of introduction on heifer behaviour during Day 1 and on performance during the first month post calving

	AM	PM	SED	Significance
<i>Behaviour (frequency/minute)</i>				
Receive threat	0.01	0.00	0.002	*
Receive shoulder	0.02	0.01	0.008	NS
Receive butt	0.16	0.05	0.013	***
Receive chase	0.01	0.00	0.004	NS
Receive nosing	0.05	0.06	0.020	NS
Social cohesive	0.01	0.04	0.009	*
<i>Performance (kg/day)</i>				
Milk yield	25.2	25.9	0.70	NS
Fat plus protein yield	2.02	2.06	0.107	NS

**Conclusions** Introducing heifers to the resident group after PM milking appeared to improve welfare in the immediate post mixing period by reducing levels of aggression to which they were exposed. However, this did not promote increased lying behaviour. There did not appear to be any long term effects of treatment on behaviour or performance.

**Acknowledgements** The authors gratefully acknowledge funding from AgriSearch and DARDNI.

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## The effect of the provision of 'furniture' on the use of a loafing area by continuously housed dairy cattle

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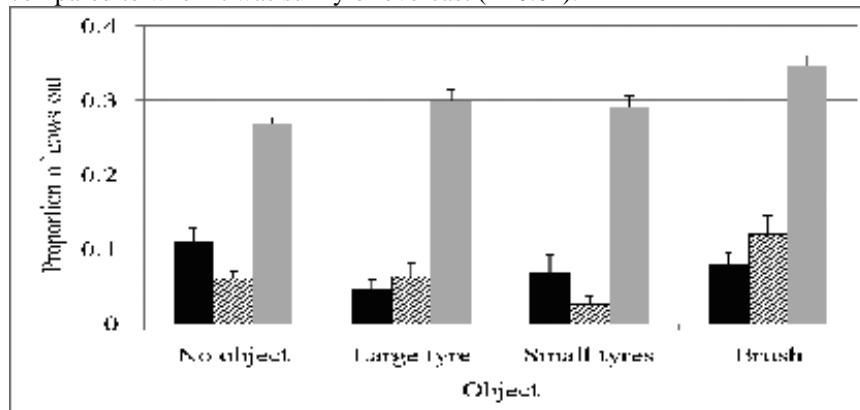
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**Introduction** Traditionally in Great Britain, dairy cattle are housed in the winter, and allowed to graze at pasture during the summer. However, some farmers have moved to a 'continuous housing' system in which the cows are housed throughout the year, or are only put out to grass when in the late stages of lactation or not lactating. This is due to a number of issues including the uptake of robotic milking, an increase in herd size, the need to provide a consistent feed ration to high-yielding dairy cows and the need to control pollution (Van den Pol-van Dasselaar *et al.*, 2002). However, there are a number of public concerns over animal welfare with this management system. As well as concerns about the levels of lameness, there is concern that continuous housing may restrict the performance of natural behaviour (FAWC, 1997). To address this concern, farmers might provide cows with an open exercise or 'loafing' area. The aim of this experiment was to assess the use of a concrete loafing area by dairy cattle, and to determine whether usage was enhanced when 'furniture' such as tyres and brushes was provided.

**Materials and methods** Two groups of 40 lactating dairy cows were used. Cows were housed in a cubicle house, milked twice daily and fed a standard total mixed ration at about 9:00h. Adjacent to the cubicle house was a loafing area which consisted of an uncovered runway leading to 20 x 12m roofed, concreted area. Cows were given free access to the loafing area between 9:00 and 14:40h. Each group was observed for six 5-day blocks (30 days in total). In blocks 1, 3 and 5 there was no furniture in the loafing area, while in Blocks 2, 4 and 6, one item of furniture was provided. The items were a grooming brush, a large tractor tyre standing upright and two smaller, suspended tyres, which were chosen to elicit play or grooming behaviour, rather than providing food or other resources. The order of presentation was randomised over the two groups. Instantaneous scan samples recording the location of all cows were made every 20 min during the period cows had access to the loafing area. Weather conditions (sunny, overcast, raining) were recorded. The observations were divided into three periods: pre-feeding, hour of feeding and post-feeding. Data on loafing area use were analysed using a GLMM with weather, period of day and object as fixed effects, and group, block, day and scan as random effects.

**Results** There was a main effect of period of day and an interaction between period of day and object. Cows used the loafing area more in the post-feeding period than at other times ( $P < 0.001$ ), with 29% of the cows in this area in the post-feeding period, compared to 9% prior to feeding and 7% during the hour of feeding. The interaction indicated that more cows used the loafing area in the post-feeding period when the brush was present ( $P < 0.001$ ; Figure 1). There was also a significant main effect of the weather on the use of the loafing area, with fewer cows using the area when it was raining, compared to when it was sunny or overcast ( $P < 0.01$ ).



**Figure 1** Mean proportion of cows in the loafing area according to object and period of day. The pre-feeding period is shown by the black bars, hour of feeding by the striped bars and post-feeding by the grey bars. Error bars represent SEMs.

**Conclusions** Cows used the loafing area most when feeding was completed. The effect of the furniture was relatively small, and was most evident for the grooming brush. The weather appeared to have a significant effect, with fewer cows going out when it was raining. The results suggest that cows are motivated to use a loafing area in the right weather conditions, and that the addition of furniture may improve the use of that area.

**Acknowledgements** The support of Defra (AW1026) and of M. Jack and M. Bertraud with recording is gratefully acknowledged. SAC receives financial support from Scottish Government RERAD.

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## Influence of bedding substrates on lying and rising behaviour in dairy heifers

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**Introduction** Problems with rising and lying behaviour in cattle result in lesions to the hocks, knees and teats (Mortensen, 1978). Lying behaviour has been used to assess comfort levels of adult cattle (Hultgren, 2001) and has been shown to differ between different bedding substrates (O'Connell *et al.*, 1997). The aim of the present study was to assess the effect of different bedding substrates on lying and rising behaviour of young dairy heifers. This was to determine if there were any differences in apparent comfort, or in potential injurious behaviour, between the different substrates.

**Materials and methods** Twenty-eight Holstein Friesian dairy heifers aged between 10 and 12 months were housed in four groups of 7 animals. The current study was part of a larger trial where each group of heifers was subjected to a preference test to determine their relative preference for different bedding substrates in the cubicles. The substrates that were tested included sawdust, shredded paper, chopped straw and a felt covering (mat). Sawdust and straw were provided to a depth of at least 3 cm and paper to a depth of at least 6 cm. Substrates were replenished as necessary and were placed on top of a felt covering. Each group had access to 14 cubicles and pairs of substrates were compared over a 3 week period in a random order in each group until all combinations had been tested. Prior to the start of each preference test, heifers had a 'forced choice' with each of the two substrates over a 2 day period whereby they only had access to the 7 cubicles containing this substrate. Animals were offered silage *ad libitum* supplemented with concentrates each day during the experimental period. The data for this study was collected from the second forced choice for each substrate for each group. Animals were video recorded (in 72 hour timelapse) during day 1 of the forced contact period and behavioural recordings were made during the following time periods: 07.00-09.00, 12.45-14.45 and 18.00-20.00 hours. In each observation the number of times a heifer rose from a lying position or lay down was recorded, and the time taken to complete these behaviours was also recorded. A binary score was assigned to each rising behaviour (0=Free, fluid movement or short pause on knees, or 1= Long pause on knees, awkward movement of head and neck or abnormal rising sequence). A short pause was determined when the phase where the chest lifted from floor using front knees was less than or equal to 5 seconds. A long pause was when this phase was greater than or equal to 6 seconds (Chaplin, 2000). The proportion of rising incidences that were assigned a binary score of 1 was calculated and used in analysis. Treatment effects were assessed by Analysis of Variance using Genstat Version 12.

**Results** Treatment effects on different lying and rising parameters are presented in Table 1. Treatment had no significant effect on the average number of lying or rising incidences per observation, or on the time taken to lie down or rise from a lying position. Finally, there were no significant treatment effects on the proportion of rising incidences that were given a score of 1 ( $P>0.05$ ).

**Table 1** Effect of bedding substrate in cubicles on lying and rising parameters

	Treatment				SEM	P
	Straw	Felt mat	Sawdust	Paper		
No. lying incidences (per observation period)	9.04	6.00	7.58	5.54	1.207	NS
No. rising incidences (per observation period)	10.96	7.08	10.00	8.25	1.200	NS
Average duration lying (sec)	12.74	11.80	13.47	12.63	1.055	NS
Average duration rising (sec)	6.22	6.22	6.34	7.04	0.342	NS
Proportion of heifers with "1" rising score	0.086	0.129	0.089	0.079	0.0271	NS

**Conclusions** The fact that there were no significant treatment effects on parameters measured suggests two things. Either (1) the behavioural parameters chosen are not a good indicator of comfort in dairy heifers, or (2) these bedding substrates were equally comfortable for heifers. It is possible that differences in apparent comfort of bedding substrates become more obvious as animals get older and heavier.

**Acknowledgements** The authors gratefully acknowledge funding from DARDNI.

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## Estimating a marginal abatement cost curve for greenhouse gas emissions from Irish agriculture using farm-level data

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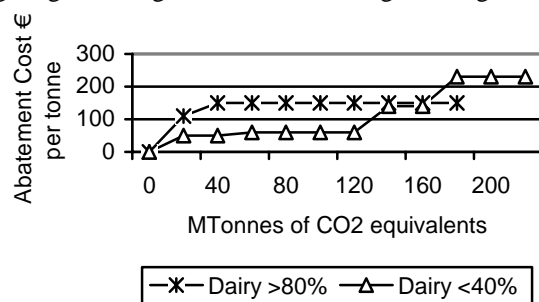
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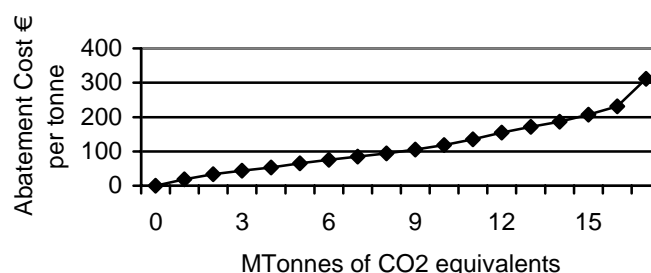
**Introduction** In 2008, the EU made a commitment to achieve at least a 20% reduction in greenhouse gas (GHG) emissions by 2020 compared to 1990 levels (European Commission 2008). In order to achieve this target, each member state (MS) has been set an emissions reduction target for their non-Emissions Trading Sector (ETS). These emissions reduction targets vary by MS, Ireland, Denmark and Luxembourg are faced with a reduction of 20 percent in their non-ETS emissions by 2020, the largest of any MS. Agriculture currently accounts for approximately 40% of Ireland's non-ETS emissions and so the 20% reduction target may have ramifications for Irish agriculture. In order to fully understand the implications of an emissions reduction target for Irish agriculture, it is necessary to first quantify the marginal cost of emissions abatement in Irish agriculture. This paper uses data from the Irish National Farm Survey (NFS) to estimate a marginal abatement cost curve (MACC) for GHG emissions from Irish agriculture.

**Materials and methods** An individual farm-level linear programming (LP) model has been constructed using 2006 NFS data (Connolly *et al.* 2007) and replicated for all of the 1,160 farms in the 2006 NFS dataset. The model consists of a number of traditional farm activities, as well as two forestry activities, two biomass crop activities and a number of alternative abatement technology activities. The returns to each activity are calculated based on each individual farm's price and cost information from the 2006 NFS. The price of outputs sold and inputs purchased are inflated using output and input price projections from the FAPRI-Ireland aggregate level model (Binfield *et al.* 2008). The total GHG emissions for each farm within the model are calculated using activity data from the model and Tier 1 and Tier 2 GHG emissions factors, the approach taken is designed to be consistent with the approach used in the Irish National Inventory Report (EPA 2008). By applying farm-weights from the NFS, each individual farm within the farm-level model is weighted up to the sectoral level. The objective function of this LP model is to maximize the overall discounted gross margin for the agricultural sector. The MACC is estimated by introducing an emissions tax into the model, which is then increased incrementally. This allows for the calculation of the volume of emissions abated at each increment of the emissions tax.

**Results** Figure 1 compares the MACC for two specialist dairy farms, Dairy >80% is a dairy farm where more than 80% of the livestock units (LU) are dairy cows, while Dairy <40% is a dairy farm where less than 40% of the LU are dairy cows. The slope of the Dairy >80% curve is initially steeper due to the higher proportion of dairy cows in the herd which have a higher marginal cost of emissions abatement. Figure 2 below presents the aggregated MACC for Irish agriculture. The curve is initially relatively flat, largely due to the abatement of emissions from the drystock sector where the marginal cost of abating emissions is lower due to the relatively low gross margin earned by these emissions. The slope of the curve steepens due to a greater proportion of emissions from dairy and cereal production being abated. These activities have a higher gross margin and therefore a higher marginal cost of abatement.



**Figure 1** MACC for Example Dairy Farms



**Figure 2** MACC for Irish Agriculture

**Conclusions** The results presented in Figure 1 illustrate the variability that can exist in the marginal cost of emissions abatement both within farms and between farms due to issues related to farm type, production system etc. This variability in the marginal cost of emissions abatement is vital to understanding how alternative emissions reduction targets will impact on Irish agriculture.

**Acknowledgements** The authors acknowledge funding from the Department of Agriculture, Fisheries and Food

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## Risk and the decision to produce biomass crops: a stochastic analysis

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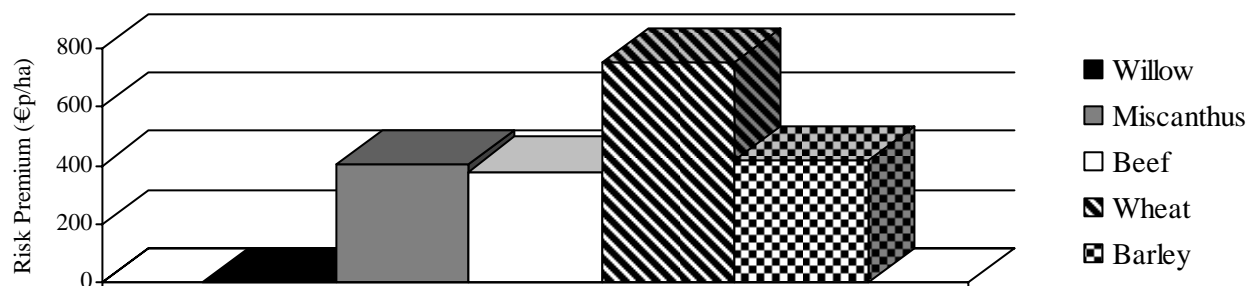
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**Introduction** There is increasing interest in biomass crops as an alternative farm enterprise however given the relatively low uptake of these crops in Ireland, there is limited information concerning the risk associated with their production and its impact on returns. The uncertainty surrounding risky variables such as the costs of production, yield level, price per tonne and opportunity cost make it difficult to accurately calculate the returns to biomass crops. The lengthy production lifespan of these crops may only serve to heighten the level of risk that affects key variables. This analysis aims to measure the degree of risk in the returns from biomass crops, and compare that to the risk faced by conventional agricultural enterprises.

**Materials and methods** In this analysis a stochastic budgeting model, is used to calculate the returns from willow and miscanthus. Stochastic budgeting is an improvement on the traditional deterministic approach as it involves attaching probabilities of occurrence to the possible values of the key variables in a budget, thereby generating the probability distribution of possible budget outcomes. The selected variables to be added stochastically should comprise those that will have the largest effect on the level of risk of the outcome. The stochastic variables included in the model are costs, yields and prices, which Clancy *et al.* (2009) have shown a large effect on the returns from biomass crops. The hierarchy of variables approach was used to calculate the stochastic costs and prices of willow and miscanthus, while the stochastic yields are derived from an empirical distribution of the historical data. The Cumulative Distribution Function (CDF) of the stochastic gross margins (GM) from the biomass crops is used to elicit ten discrete states of potential returns, with each having an equal probability of occurrence. The opportunity cost of land is accounted for through the inclusion of foregone returns from a conventional agricultural activity, such as spring barley, winter wheat or store to finished beef. The estimation of parameters of the probability distribution of these stochastic enterprises GM was empirically based, with National Farm Survey (NFS) data from 1997 – 2006 used to calculate their financial performance. The biomass discrete states and the historical conventional gross margins are used as parameters in a Multivariate Empirical (MVE) distribution to simulate the financial performance of all enterprises over a 16 year planning horizon. The results of this simulation are then compared using their respective CDF and the enterprises are ranked using Stochastic Efficiency with Respect to a Function (SERF).

**Results** SERF uses risk premiums to determine the confidence of decision makers in a particular preferred risky alternative. The risk premium reflects the minimum sure amount that would have to be paid to a decision maker to justify a switch from the dominant alternative to a less preferred option. Figure 1 shows that while farmers would need a substantial risk premium to be persuaded to switch from any conventional enterprise to willow, miscanthus is relatively competitive with all but Winter Wheat. This suggests that a greater level of risk is associated with willow than conventional enterprises, and that miscanthus may be a viable alternative to some of these systems.

**Figure 1** Risk Premiums for each enterprise relative to willow



**Conclusions** Uncertainty regarding the returns from biomass has manifested itself in a reluctance of farmers to enter production, and this may be a key factor in the low planting rates to date. This analysis found that accounting for risk underlined the results of the baseline economics from Clancy *et al.* (2009), who found that under given assumptions and costings, miscanthus has a greater level of return than willow. The results from this analysis tell a similar tale with miscanthus having a lower risk premium than willow. The value of the risk premium required to entice farmers to switch to miscanthus production is significantly less than that required for willow, suggesting a greater level of risk is associated with willow than with miscanthus.

**Acknowledgements** The authors gratefully acknowledge funding from the Research Stimulus Fund, DAFF

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## Analysis of distribution system in beef marketing

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**Introduction** Nowadays the marketing of both livestock and meat is a complex process. The reflection of consumer preference for meat back through the marketing system to the original producer is highly dependent upon a national marketing strategy. The marketing concept holds that the key to achieving organizational goals consists in determining the needs and wants of target markets and delivering the desired satisfactions (Cross and Savell, 1994). In this study we tried to investigate the possible distribution paths of beef marketing, also comparing consumer preferences with producers and distributors. The possible correlations among various variables were also tested.

**Materials and methods** In order to have an adequate sample size, the region of capital of Iran, Tehran was selected as target population in the year 2008. Random sampling was applied for field study and information was collected using designed questionnaires. Of five statistical populations, 60 producers, 17 industrial slaughterhouses, 104 wholesalers, 700 retailers and finally 100 consumers, were sampled. The samples were finally divided into two study groups where consumers in one group were separated from producers, processors and sellers as another group and these two groups were compared and evaluated for 21 different variables such as effect of processing units, management of official distribution, refrigerating system, sanitation, slaughter quality, local sale centres, chain stores, governmental support, proper packing, delivery costs, imported frozen beef, content of fat, etc. Two statistical methods, Chi-Square test for testing the significance of differences between two groups and T-test for testing the magnitude of correlation coefficients among different outputs were applied. The four known distribution paths are shown in Figure 1.

path	description	percent
1	producer → slaughterhouse → wholesaler (associations) → retailer → consumer	٪35
2	producer → slaughterhouse → wholesaler (processing units) → retailer → consumer	٪14
3	producer → dealer → slaughterhouse → retailer → consumer	٪15
4	producer → dealer → slaughterhouse → wholesaler → retailer → consumer	٪36

**Figure 1** Four distribution paths in beef marketing

**Results** In 51% of known distribution paths, dealers played the main role while processing units had 14% participation in distribution. The remaining 35% was under control of beef associations. Price difference of beef between two paths, one including dealers and another without dealers is 7.6% of final sale price for producer and 53.33% of total marketing costs. In paths 3 and 4, the difference in final price is imposed to consumer while in paths 1 and 2, this difference shows the changes of price among final distributors in four possible paths. Statistical analysis showed significant differences between two groups for some variables like governmental support, management of official distribution, refrigerating system, sanitation, content of fat, proper packing and imported frozen beef. Also 29% of consumers and 34% of producers agreed with local sale centers while 71% of consumers and 66% of producers agreed with distribution via processing units. In general 68.5% of samples agreed with distribution via processing units and it was believed to be the appropriate system of beef distribution.

**Conclusions** According to final results, some solutions may correct the current systems and increase the efficiency of marketing. Some local sale centers can be established which have sale representatives in local small marketplaces. Distribution must be exclusive to these centers. In the lack of processing units these local centers may improve the distribution. In the presence of processing units, some points like packing the beef in proper packages or producing low fat beef must be concerned. Also it is recommended to have diversity in packing and sizes according to consumers demand. The small local markets must be exclusive representatives of processing units, The quality of livestock production and slaughter must be under intensive supervision.

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# The impact of spring grazing and harvest date on the total forage costs of an integrated grazed and conserved perennial ryegrass sward

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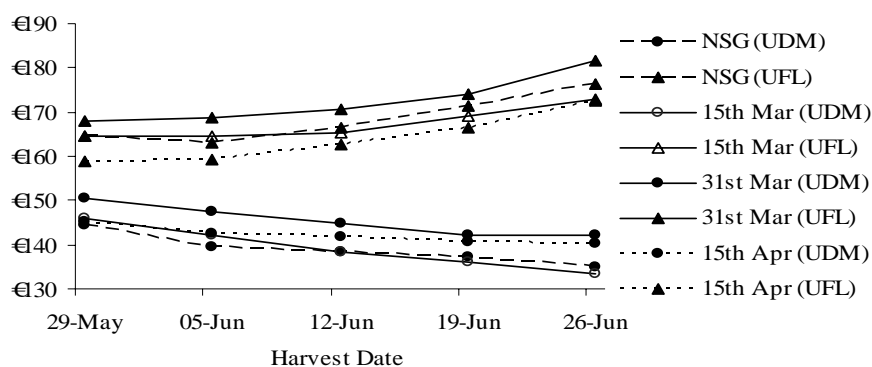
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**Introduction** Feed cost is the greatest variable cost incurred by livestock farms and thus, management of feed cost can impact greatly on the profitability of such farms. Grass, both as a grazed feed or conserved as silage or hay, is the primary feed source on Irish livestock farms. Although these grazed and conserved grass feeds are produced within an integrated grassland management system they are typically costed separately for the purposes of feed cost evaluation (Finneran *et al.*, 2009; Keady *et al.*, 2002). The objective of this study is to model the cost of total annual herbage production from a perennial ryegrass sward which is both grazed (GG) and conserved for grass silage (GS) within an integrated grassland production system.

**Materials and methods** A static agro-economic simulation model; the Grange Feed Costing Model (GFCM; Finneran *et al.*, 2009) was used to model total annual herbage output and total annual feed cost (TFC) of a sward both grazed and harvested for a single grass silage crop under 20 individual management scenarios. The baseline scenario was a perennial ryegrass sward receiving 200 kg N ha<sup>-1</sup> and yielding 12.2 t dry matter (DM) ha<sup>-1</sup> annually under a rotationally grazed system. Data from six years grass growth plot studies at Teagasc, Grange Beef Research Centre was used to predict weekly GG output. Grazed grass DM yield was reduced appropriately to account for the periods closed for GS production, including the first and second week post harvest. GS production data from Grange (O'Kiely, 2001) was used to predict GS DM yield and dry matter digestibility (DMD). In order to calculate utilised dry matter (UDM) production, a mean utilisation rate of 750 g kg DM<sup>-1</sup> was applied for GG across the grazing season, whilst a harvesting and conservation efficiency value of 854 g kg DM<sup>-1</sup> was applied for GS. Both GG and GS were fertilised and managed as specified by Finneran *et al.*, (2009). Four spring treatments were evaluated; not spring grazed (NSG) and grazing until 15<sup>th</sup> March, 31<sup>st</sup> March and 15<sup>th</sup> April before closing for grass silage production. Five GS harvest dates at weekly intervals between 29<sup>th</sup> May and 26<sup>th</sup> June were modelled. DM yields and DMD at harvest for each of the spring grazed silage crops were calculated using co-efficient for the impact of spring grazing on yield and digestibility derived from O'Riordan *et al.*, (1998) and Humphreys and O'Kiely (2006). Net energy (UFL) was calculated from the DMD value at feed-out.

## Results

Total UDM yield as simulated by the GFCM was greatest for those scenarios where GS comprised the greatest proportion of total DM. UDM yield ranged from 9.2 t ha<sup>-1</sup> (closed 15<sup>th</sup> April, harvest 29<sup>th</sup> May) to 11.9 t ha<sup>-1</sup> (closed 15<sup>th</sup> March, harvest 26<sup>th</sup> June). Total UFL output was greatest with a 15<sup>th</sup> March closing; 12<sup>th</sup> June harvest. Mean UFL content in the annual UDM ranged from 0.770 kg UDM<sup>-1</sup> (closed 15<sup>th</sup> March, harvest 26<sup>th</sup> June) to 0.914 (closed 15<sup>th</sup> April, harvest 29<sup>th</sup> May). TFC declined with later GS harvest date for all closing date options on a UDM basis, reflecting the increase in total DM output with increased silage yields (Figure 1). However TFC increases with later harvest on a UFL basis due to the lower mean UFL content of total herbage when a high yield; low DMD GS comprises the majority of the total herbage production. Spring grazing generally decreased TFC on a UFL basis. However TFC was increased by spring grazing followed by closing on 31<sup>st</sup> March because the yield of high UFL spring grass grazed was insufficient to offset the subsequent depression in silage yields and hence total annual UFL output.



**Figure 1** TFC of total annual herbage produced: €/t UDM<sup>-1</sup> and €000 UFL<sup>-1</sup>

**Conclusions** The GFCM analysis indicates that maximisation of mean UFL content in total annual herbage production can result in the lowest TFC for a grazed and harvested perennial ryegrass sward. This is best achieved through efficient grazing of high UFL spring grass and achieving moderate yielding, high UFL silage harvests. TFC can also be reduced on a UDM basis through later GS harvests and higher silage yields with a lower mean UFL content, which may be appropriate depending on the livestock system.

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## Nutrient management efficiency in Ireland – A data envelopment analysis of specialist dairy and tillage farms

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**Introduction** Within the constraints of the EU Nitrates and Water Framework Directives, controlling and managing nutrient transfers to water from excessive fertiliser use on agricultural land is a significant environmental policy challenge. This paper assesses whether there is room to reduce nitrogen and phosphorus fertiliser application by exploring the extent of their over application using data envelopment analysis methodology.

**Materials and methods** Data envelopment analysis (DEA) is a deterministic approach to efficiency measurement. It measures the relative efficiency of a decision making unit (DMU) by comparing relative inputs to outputs. DEA establishes the most efficient DMU's and compares all others to the most efficient. The method uses linear programming to place a non-parametric frontier over the data. This frontier consists of the most efficient DMUs and all other DMUs are measured by their relative distance to this frontier as a measure of their level of efficiency. Analysis was undertaken using the software package DEAP (Coelli *et al.*, 1998).

The main data source employed in this analysis is the Teagasc National Farm Survey (NFS) 2008. The NFS is collected annually as part of the Farm Accountancy Data Network requirements of the European Union. A farm accounts book is recorded on a random representative sample of farms throughout the Republic of Ireland. The sample is weighted to be representative of farming nationally across Ireland. In the 2008 NFS survey 1,102 farmers were surveyed representing 104,800 farmers nationally (Connolly *et al.*, 2009).

This paper concentrates on specialist dairy and tillage farms. These agricultural systems are the most intensive and may pose the greatest risk in terms of managing nutrient transfer from agricultural land to water courses. The analysis was undertaken and stratified by land use potential of soils (Gardiner and Radford, 1980). Output for specialist dairy farms was measured in litres of milk per hectare and the inputs examined were nitrogen (N) and phosphate (P) fertiliser usage  $\text{kg ha}^{-1}$  as well as feedstuffs, labour and other variable costs. Output for specialist tillage farms was measured in the form of gross output in  $\text{€ ha}^{-1}$  and the inputs examined were again nitrogen and phosphate fertiliser usage  $\text{kg ha}^{-1}$ , labour and other variable costs.

**Results** Analysis indicates that specialist dairy farmers on good soils tended on average to overuse fertiliser to the greatest extent at  $51.2\text{kg N ha}^{-1}$  and  $4.1\text{kg P ha}^{-1}$ . Average cost saving on fertilisers of  $\text{€}4.25 \text{ ha}^{-1}$  could be achieved by operating at the benchmark standard as set by other cohort farmers in the sub-sample. This figure declined to between  $\text{€}33.09 \text{ ha}^{-1}$  and  $\text{€}16.83 \text{ ha}^{-1}$  for those on average and poor soils, respectively, as shown in Table 1. Over application of N was  $21.4\text{kg ha}^{-1}$  and  $9.6\text{kg ha}^{-1}$  and average excess P was  $2.3\text{kg ha}^{-1}$  and  $1.6\text{kg ha}^{-1}$  for specialist dairy farms of average and poor soils, respectively. Results for specialist tillage farms on good soils indicate over application compared to the benchmark of  $20.7\text{kg N ha}^{-1}$  and  $3.5\text{kg P ha}^{-1}$ . This declined to  $16.3\text{kg N ha}^{-1}$  and  $4.5\text{kg P ha}^{-1}$  for average soils. Potential cost savings for specialist tillage farms compared to the benchmark was approximately  $\text{€}34 \text{ ha}^{-1}$  to  $36 \text{ ha}^{-1}$  as illustrated by Table 1.

**Table 1** DEA analysis of over application of N and P on specialist dairy and tillage farms.

Farm System	N	N ( $\text{Kg ha}^{-1}$ ) Over application	P ( $\text{Kg ha}^{-1}$ ) Over application	Potential cost saving* ( $\text{€ ha}^{-1}$ )
Specialist Dairy - Good Soils	146	51.2	4.1	74.25
Specialist Dairy - Average Soils	91	21.4	2.3	33.09
Specialist Dairy - Poor Soils	17	9.6	1.6	16.83
Specialist Tillage - Good Soils	88	20.7	3.5	36.47
Specialist Tillage - Average Soils	14	16.3	4.5	34.82

\*Average prices from the CSO (CSO, 2009)

**Conclusions** Results demonstrate considerable inefficiency in the utilisation of N and P fertilisers across specialist dairy and tillage farms. Consequently, there is potentially an opportunity for inefficient producers to reduce costs on N and P fertilisers without affecting output by adopting similar practices to those of the most efficient benchmark farms. Potential cost savings on average ranged from  $\text{€}17 \text{ ha}^{-1}$  to  $\text{€}74 \text{ ha}^{-1}$ . Such reductions have the potential to deliver a win-win situation by reducing the risk of nutrient leaching and diffuse pollution from agricultural land while improving economic margins.

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## The impact of preservation method of lucerne forage on the digestibility of high grain racing or complete competition diets for horses

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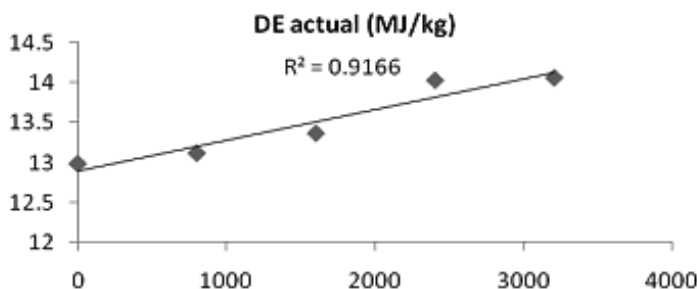
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**Introduction** Previous research has shown that controlled fermented lucerne products have higher DE values, and other potential benefits, relative to dry lucerne chaff. Racing diets and high energy compound competition feeds for horses are typically formulated with higher levels of grain, which are associated with many problems in horses, including gastric ulceration, poor digestion, acidosis and gastric discomfort. Lucerne is associated with reducing the incidence of ulcers (Andrews *et al.*, 2005; Nadeau, 2006), and feeding controlled fermented lucerne can increase intake time, improving the throughput of forage over a longer period of time. Lucerne contains a larger proportion of soluble carbohydrates (Fonnesbeck, 1968), making it more digestible than grass-based versions. Horses have been shown to retain lucerne in their gut for longer than oat straw, increasing overall diet (especially grain) digestibility (Cuddeford *et al.* 1995). Combining these factors, feeding lucerne ensures a more consistent supply of forage through the gut and, when fed alongside grain-based feed, improves the overall diet digestibility, as it maintains gut fill and optimises fermentation in the hind gut as well as diluting the potential negative impacts of starch overload and microflora imbalances. The following trial examined if feeding controlled fermented (CF) Lucerne in replacement for hay resulted in improvements in energy digestion when fed alongside either high grain racing diets or competition feeds for horses, on overall energy digestibility.

**Materials and methods** Ten thoroughbred-type non-racing horses were used to determine the effect of substituting CF lucerne (Fiber Pro, Fiber Fresh Feeds Ltd, Reporoa, New Zealand) for meadow hay chaff, in different proportions, alongside either a high grain racing diet (50% barley, 50% oats plus vitamin and mineral premix, with 33% DE from forage) or a commercial competition diet (Dunstans Sport Horse mix plus vitamin and mineral premix with 50% DE from forage). Diets were formulated, to deliver the same amount of energy per day, based on a 500 kg horse not in work (NRC, 2007). Each horse received each of the forage-diet combinations for a seven day period during the trial in a latin square design, where CF lucerne increasingly replaced dry hay chaff. At the end of each seven day period, a faecal collection was made to determine energy digestibility. The results were used to identify any relationships between the amount of CF lucerne in the forage component of the diet and the overall energy of the whole diet. Any feed refused was weighed and recorded on a daily basis. Samples of both feed and faeces were analysed for dry matter and gross energy (by bomb calorimetry). Data was analysed by the GLM procedure of Unistat 5.5 (Unistat UK Ltd.).

**Results** For the high grain racing diet, the total DE of the entire diet increased linearly in relation to the amount of CF lucerne in the diet ( $R^2 = 0.9166$  – see fig below). Retained energy (MJ/d) increased by 26% from 69.3 MJ/d (100% hay) to 87.8 MJ/d (100% CF lucerne). Digestible energy supplied by the whole diet increased by 8% from 12.9 MJ/kg (100% hay) to 14.1 MJ/kg (100% CF lucerne).

For the competition feed treatments, a polynomial (order 2) relationship was found for increasing levels of CF lucerne in both total diet DE ( $R^2 = 0.50$ ) and for the digestibility of the competition feed alone (as calculated by subtracting the DE contribution of the forage from the DE of the whole diet;  $R^2 = 0.52$ ). This data suggested that the optimum inclusion of CF lucerne in the diet is when it replaces 45% of the hay by weight, as this gave the highest numeric levels of retained energy, DE and the daily DE contribution of the grain only, when the forage contribution was excluded.



**Conclusions** CF lucerne had a positive impact on the digestible energy of high grain, racing-type diets. This forage could be used to increase the supply of energy to racing horses, and improving overall digestibility may help increase the efficiency of feed utilisation in the horse. For the competition diets such relationships were not as evident, possibly due to the less extreme nature of the feeding regime, with higher forage and lower grain inclusion in the daily ration. Hence the optimal inclusion level for substituting CF lucerne for dry hay chaff was 45%. Both trials gave evidence to different degrees that, as per other reported research, feeding lucerne forages improved the digestibility of high grain diets compared to dry hay chaff.

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## The effect of an encapsulated fatty acid mixture on the fatty acid composition of mares' milk

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**Introduction** Milk production and composition, including fatty acid composition, may change in response to the effects of lactation stage, age, parity and nutrition (Doreau *et al.* 1992; Csapó *et al.* 1995). Fatty acid content, chain length and saturation in mares' milk is thought to be influenced directly by dietary content as little or no hydrogenation of unsaturated fatty acids is believed to occur prior to absorption in hind gut fermenting animals (Hoffman *et al.*, 1998). The aim of the study was to compare the different fatty acid concentrations in mares' milk prior to and post inclusion of a fatty acid mixture and to evaluate how long it takes for the fatty acid profile of the milk to change.

**Materials and methods** 16 adult lactating thoroughbred mares maintained at pasture and at least 4 weeks post-foaling were included in the study. Prior to entering the study, all mares underwent a complete physical, haematological and biochemical evaluation as well as faecal analysis for parasites to assess their general health, with any animal showing physical and laboratory evidence of disease excluded from the study. All mares were then weighed and treated with the encapsulated fatty acid mixture (40g/day orally) for a total of 2 weeks. Approximately 30 mls of milk was collected from each mare prior to treatment and then after 1 and 2 weeks on treatment. Milk samples were stored at -20°C until transported to the laboratory to be analysed. Milk fatty acids were prepared using methyl ester according to the method outlined in Amer *et al.* (1995) and the results were expressed as grams lipid/100 ml milk and % of total fatty acids. Data was analysed using a one-way repeated measures ANOVA. Milk data was analysed taking into account the stage of lactation and the period at pasture for each individual mare as these factors can influence the fatty acid content of the milk.

**Results** 16 mares were included in the study with the average post-foaling days for the mares prior to entering the study  $50.6 \pm 6.9$  SEM (range 16-114). The level of fatty acids present in the milk varied widely, with higher levels of long chain fatty acids being observed in comparison to short/medium chain fatty acids in all milk samples analysed. The encapsulated fatty acid mixture treatment resulted in increased levels of saturation, especially within the medium chain fatty acids (Table 1). The levels of unsaturated fatty acids observed remained consistent or decreased during the treatment period (Table 2). The observed increases in the medium chain saturated fatty acid content resulted in a subsequent decrease in long chain unsaturated content expressed as % of total fatty acids with the exception of C18:3. The numerical increase in C18:3 may be a result of the mares being at pasture resulting in an increased source of unsaturated long chain fatty acids.

**Table 1** Effect of treatment and time on saturated fatty acid content of mares milk (% of total fatty acids)

	Total Fat (g/100ml milk)	C4	C8	C10	C12	C14	C16
Prior	2.5	0.05	2.6	5.7	6.1	5.9	19.6
Week 1	2.1	0.08	2.9	6.6	7.7	7.3	20.1
Week 2	1.9	0.06	2.7	5.7	6.4	6.3	19.7
P		NS	NS	0.08	<0.01	0.02	NS

**Table 2** Effect of treatment and time on unsaturated fatty acid content of mares milk (% of total fatty acids)

	Total Fat (g/100ml milk)	C15:1	C16:1	C17:1	C18:1	C18:3	C20:1	C20:2
Prior	2.5	0.15	0.40	0.55	19.7	18.2	0.37	0.30
Week 1	2.1	0.14	0.27	0.56	16.9	18.6	0.36	0.22
Week 2	1.9	0.17	0.37	0.55	18.5	20.8	0.35	0.25
P		0.05	<0.05	NS	<0.05	NS	NS	0.06

**Conclusions** From the results obtained in this study it could be concluded that encapsulated fatty acids within the diet have the potential to enrich the mares' milk, even after only 1 week of supplementation. Reductions were observed in some of the fatty acids due to the modifications achieved. Fatty acids, especially short chain fatty acids, have been shown to be the preferred energy substrate for microbiota and may have a role in preventing certain types of colitis (Scheppach, 1994). Since intestinal microbiota are essential for the development of a healthy and stable intestinal tract/immune system in immature animals, modification of the fatty acid components of the mares milk may have the potential to impact foal health.

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## Quantitative comparison of pressure distribution exerted by different numnahs beneath the saddle of a ridden horse

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**Introduction** Equine lameness and back pathologies are significant welfare issues and may be linked to saddle-fit during ridden exercise. Previously 74.3 % of animals presenting orthopaedic back pathology were shown to be concurrently lame and conversely, 41.1 % of horses diagnosed with primary lameness exhibited back pathology (Landman *et al.*, 2004). Meschan *et al.*, (2007) defined optimum saddle fit as those that transmitted lowest overall force and distributed force without generating pressure peaks. Incorrectly fitting saddles exert pressure peaks, for example overlying the *longissimus dorsi* in the caudal third of the contact. Numnahs are soft, cushion structures placed beneath the saddle to help dissipate the pressure of the saddle and rider over the horse's back, away from the spinal area. They have been demonstrated to increase overall back pressure in a qualitative preliminary study (Harman, 1994). The present study determines quantitatively the effect upon pressure distribution between different numnahs using a pressure sensor mat.

**Materials and methods** Four geldings (horses 1 to 2; 15 years, range 13 to 20 y) of different breeds and height (154 cm, range 151.2 to 161.2 cm) with no recent history of back pain were used. Horses were maintained on a daily ridden exercise programme, a regular shoeing regimen and had an annual teeth check by a veterinary surgeon. The experiment was performed on a single day. One experienced rider (weighing 69 kg) rode all horses. Back pressure data were recorded from each horse walking a figure of eight pattern twice on a uniformly concrete floor. The numnahs used were a standard saddle cloth, poly pad, sheepskin, half sheepskin, gel pad or ridden bareback. A randomised 4 x 6 design was used so that each horse was measured wearing each numnah. A general purpose saddle was placed on top of the numnah. A pressure mat (PX100 model, Xsensor Technology Corporation Calgary, Canada) was placed beneath the numnah and saddle. The pressure mat consisted of 2400 individual piezo-electric sensors in a 60 x 40 grid pattern. Each sensor generated an individual reading every 1/8 second. The pressure mat was connected to a hand-held data logging computer. The mean, minimum and maximum pressures were calculated for each sensor on the pressure mat. Sensors which did not contact the saddle or numnah and thus received no pressure measurement were excluded from the data set giving a final grid of 52 x 36 sensors for all calculations. ANOVA was used to compare pressure between horses and within horse between numnah, for each sensor. Resulting P-values generated for each sensor in the grid were designated a colour to identify areas which were significantly different at either  $P < 0.05$ ,  $P < 0.01$  or  $P < 0.001$ . These analyses were performed for mean, maximum and minimum pressure data.

**Results** All horses when ridden bareback showed areas of peak mean pressure upon the back occurred away from the spine but pressure of 20,000 - 40,000 mmHg were concurrently applied directly to the spine (Figure 1). When ridden in a saddle and numnah the areas of peak mean pressure lay away from the spine (30,000 to 40,000 mmHg) and pressure exerted directly on the spinal area was low (0 to 10,000 mmHg; the lowest pressure category; Figure 2). These observations were also true when considering maximum and minimum pressure values. Horse-dependent variation was apparent; a number of sensors were significantly different between horses, even when lowering the significance value to  $P \leq 0.001$  (Figure 3). Fewer sensors showed a significant difference in a numnah-dependent manner (Figure 4); however, almost all areas which showed significantly different values of  $P \leq 0.001$  were adjacent to the spine.



**Figure 1** Mean pressure distribution exerted upon the back when ridden bareback, using horse 1 as an example



**Figure 2** Mean pressure distribution exerted upon the back when ridden in a standard saddle cloth, using horse 1 as an example



**Figure 3** Areas of significantly different mean pressure between 4 ridden horses (■  $P < 0.05$ , ■  $P < 0.01$ , ■  $P < 0.001$ )



**Figure 4** Areas of significantly different mean pressure between 5 numnahs (■  $P < 0.05$ , ■  $P < 0.01$ , ■  $P < 0.001$ )

**Conclusions** The present study quantitatively demonstrated that the saddle and numnah dissipated pressure away from the spine compared to bareback riding. Some areas of the back exhibited pressure variation between numnahs but the effect of horse resulted in more areas of variation illustrating the importance of fitting saddlery specifically to an individual animal, rather than relying on a 'one type fits all' application of numnahs and/or saddles. The quantitative use of pressure sensor mats enables accurate assessment of the equine back; software to improve statistical analysis would further aid interpretation and pave the way for further studies. The interpretation of between-horse back pressure variation requires further research

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## Influence of cat owners' feeding practice and attitude towards obesity on body condition of cats

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**Introduction** Several authors have reported obesity as one of the most common malnutrition disorders in cats (Anderson 1973, Crane 1991, Markwell *et al.*, 1994, Russell *et al.*, 2000). Successful prevention and treatment of the problem requires understanding and participation of cat owners. The majority of previous studies have investigated risk factors for obesity, but paid little attention to the feeding practices and attitudes of cat owners towards obesity. In addition to understanding the consequences of obesity, and the dietary and physiological risk factors, it is equally important to understand the opinions, attitudes and behaviour of cat owners. Therefore, the aim of this study was to investigate feeding practices, cat owners' attitude towards obesity in cats and their influence on the body condition of cats.

**Materials and methods** In this study, 139 cat owners, who were clients at a veterinary practice in Chelmsford (Essex, UK), with a total 201 cats, were interviewed by a questionnaire. The questionnaire comprised of three sections. The first section gathered data about the gender of cats, age, breed, body weight (actual or estimated), health status, places cats spent most of their time, whether or not they hunted, whether or not cats were fed elsewhere, level of physical activity of the cats, their living environment and existence of other animals in the household. Section two had questions pertaining to feed and feeding regimen. For instance, types and brands of cat food provided, reasons for the choice of food, frequency of feeding, methods of dish-out, amount of foods offered, adherence of owners to feeding instructions on the labels, frequency of feeding treats and milk, types and amount of milk offered, and whether or not the amount of food is adjusted. The third section gathered information about the cat owners. The information gathered included; frequency of playing with cats, experience of cat ownership, frequency of visiting veterinary practices, and amount of money spent on the cat food per week, owner's perception of body condition of their cat, owner's attitude to and knowledge of obesity. In addition, data on the owner's age, gender, height, weight, and the number of people in the household was also gathered. The body condition of the cats were categorised on a five-point scale (5 = obese and 1 = very thin) (Thatcher *et al.*, 2000) by the cat owners and the veterinarian, independently. The data collected were analysed using the chi-square test to determine whether any associations existed between each factor and the body condition scores of cats. Spearman's rank correlation test was applied to assess the strength of relationship between variables, e.g. between the amount of money spent on cat food and the body condition score of the cat etc.

**Results** Of the 198 cats that were condition scored by their owners; 20%, 14%, 37%, 23% and 5% were classified as thin, underweight, optimal, overweight and obese, respectively. In comparison, body condition scores assessed by veterinarians revealed that cat owners underestimated condition scores in 34% of the cat owners surveyed. All cats in this study were fed commercial cat food by their owners. The majority were fed both dry and moist cat foods, either twice a day or on demand. The majority of cat owners did not follow cat food manufacturers' feeding instructions on the label and believed that cats can regulate their food intake. The factors which were found to have significant association with body condition score were the cats' age ( $p < 0.05$ ), whether or not the cats hunted ( $p < 0.05$ ), whether or not the cats were fed elsewhere ( $p < 0.01$ ), the level of physical activity of the cats ( $p < 0.05$ ) and the age of cat owners ( $p < 0.01$ ). Most cat owners had a negative attitude towards their animals' obesity, stating that obesity had a negative impact on health of cats. However, some owners (10 of 140 respondents) thought that problem of excessive weight would not happen to their cats. Owners of overweight cats were more likely to feel that it was not easy to manage weight loss treatment in their cats than owners of under and optimal weight cats ( $p < 0.01$ ). Cats with owners younger than 60 years were more likely to be of optimal weight than those owned by the over 60 age-group ( $p < 0.01$ ). In general, cat owners younger than 30 years of age tended to adhere to manufacturers feeding instructions more than owners in older age groups ( $p < 0.01$ ). No statistically significant association was observed between the age of cat owner and the amount of money spent on cat food. Male (32 out of 38) owners were more likely to give their cats treats than female (82 out of 157) owners ( $p < 0.01$ ).

**Conclusion** This study confirmed that there is a lack of knowledge and awareness of obesity in cats amongst their owners. To prevent and reduce the incidence of obesity in cats more information should be provided effectively to cat owners and individual weight management protocols should be organised by professionals.

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## The effect of a novel complementary feedstuff on canine faecal consistency and odour

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**Introduction** Poor faecal consistency, malodour and excessive or erratic volume are common conditions in companion animals and can present unpleasant situations for owners and handlers. The ratio of water to solids in faeces is an important determinant of faecal consistency but does not always result in poor faecal consistency (Wenzl *et al.*, 1995). Gastrointestinal tract function is also implicated in poor faecal consistency and nutritional intervention is one approach to management of the problem (Hickman 1998). The aim of the current study was to determine owner perceptions of the efficacy of a novel complementary feedstuff on dog faecal quality and a variety of canine parameters.

**Materials and methods** Twenty-one dogs, aged between 1 and 12 years, with body weights ranging from 2.5 to 40.0kg were used in the study. The dogs were randomly assigned to two treatments (feedstuff and control) in a standard cross-over design with a 21 day feeding period. The complementary feedstuff, a mixture of short, medium and long chain fatty acids all of which are encapsulated to reach the hind gut, was included at levels proportionate to the weight of each dog (0.3g/kg). Fresh water was available at all times. Faecal characteristics (consistency, volume, odour, colour and ease of pick up) and coat shine, breath odour and flatulence were scored by owners at weekly intervals throughout the trial, using a 5 level likert scale questionnaire design. Fresh faecal samples were collected for microbiological analysis after 21d. Microbial counts were obtained by plating out decimal dilutions of samples prepared in maximum recovery diluents. Standard media were used throughout. All statistical analyses were performed by ANOVA, using Genstat.

**Results** The current study demonstrated significant differences ( $P < 0.05$ ) in all parameters evaluated within the questionnaire and Enterobacteria levels between the two treatments determined through microbiological analysis tended to be different ( $P < 0.10$ ).

**Table 1** Treatment effects on owner perception on fresh faecal quality and other canine parameters

Parameter	Pre treatment (control)	Treatment	Post treatment (control)	P
Coat shine* (1-5 ≡Dull-Shiny)	3.1	3.6	3.6	0.003
Breath odour* (1-5 ≡Weak-Strong)	3.7	2.1	3.5	<.001
Feed intake* (1-5≡Low-High)	3.0	3.3	3.2	0.018
Flatulence* (1-5≡Never-Frequent)	2.8	2.5	2.7	0.003
Faecal Volume* (1-5≡Little-A lot)	1.9	2.4	2.1	<.001
Faecal colour (1-5≡Light-Dark)	3.7	2.7	3.6	<.001
Faecal odour (1-5≡Weak-Strong)	4.1	2.2	3.8	<.001
Faecal consistency(1-6≡Firm-Liquid)	4.1	3.1	3.7	<.001
Faecal pick up (1-5≡Easy- Difficult)	3.3	2.1	2.6	<.001

\*16 dogs included

**Table 2** Effect of treatment on faecal bacterial concentration (in log colony forming units per g)

Parameter	Treatment	Control	P
TVC	7.68	7.64	0.418
Entero	6.31	6.63	0.075
Lactics	7.36	7.58	0.345
Cl_perf	7.45	7.42	0.874
Total anaerobes	8.27	8.35	0.838

**Conclusion** Inclusion of a novel complementary feedstuff in canine diets decreased breath odour, flatulence and faecal odour in the dogs. Owners also recorded a significant improvement in coat shine, faecal consistency and ease of faecal pick up but there was a significant increase in faecal volume and feed intake. The feedstuff reduced Enterobacteria in the faeces, which may associate with a change in gut health and function.

**Acknowledgements** This study was conducted within a Knowledge Transfer Partnership between Queens University Belfast, the Agri-Food and Biosciences Institute and Devenish Nutrition Ltd.

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## Behavioural and vocal responses in *Canis Lupus* in response to transposed playbacks

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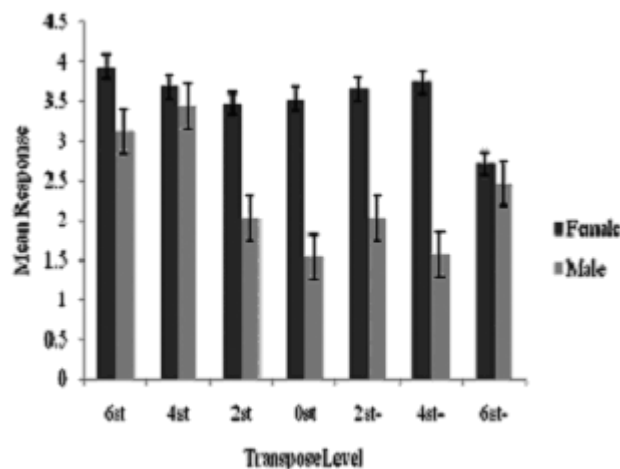
**Introduction** Stimulating wolves (*Canis Lupus*) to get initial howls of choruses can be a method of detecting information about pack identity (Harrington, 1989) and give estimates of numbers within the pack to help decide on conservation status. This study examined the effect of pitch of transposed playbacks on wolf behavioural and vocal reactions and whether age or gender affected the response.

**Materials and methods** Responses of six wolves from two packs (North American and Juvenile packs) at the Wolf Conservation Trust, Berkshire, UK were recorded 10 seconds after ending playback of a pre-recorded howl track (Wolf Howl Animal Preserve LLC, Mississippi, America). Each track was transposed using Ableton Live 7.0 and ranged from 6st- (very low pitched) to 6st (high pitched) at intervals of 2st. Four different tracks of 45 seconds were used in a repeated measures design, played either high to low, or low to high, weekly over an 8 week period. A 10 minute refractory period was left between each track to allow the wolves to return to normal behaviours. Responses were graded using the following scale. 1=no response, 2=eye contact, 3=ear twitching, 4=head up/noticeable looking, 5=sniffing, 6=get up, 7=movement towards the sound or purposefully away, 8=whimper/yip/bark or whine, 9=short or quiet howl, 10=long or loud howl. Data were analysed using Minitab® v15 ANOVA GLM.

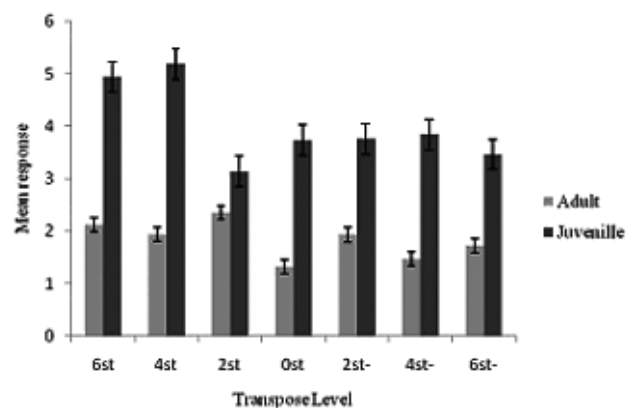
**Results** Transpose level did not have a significant effect on the behavioural and vocal responses of the wolves (Table 1). However gender ( $P<0.001$ ; Figure 1) and age ( $P<0.001$ ; figure 2) had significant effects.

**Table 1** Effect of transpose level on response

	Transpose level							Pooled SEM
	6st	4st	2st	0st	2st-	4st-	6st-	
Behavioural response (least square means)	3.53	3.56	2.75	2.53	2.84	2.66	2.59	0.380



**Figure 1** Effect of gender on response



**Figure 2** Effect of age on response

**Conclusions** Gender and age had pronounced effects on whether wolves would respond to playback experiments. Females responded better than males to the playback tracks, in contrast to the findings of Harrington and Mech (1979) where alpha males demonstrated the greatest response. Younger wolves appeared to respond better than adults which may be helpful in determining breeding numbers in a chosen area. This could be due to juveniles being more inquisitive, active and having better hearing (Fogle, 1990). Individual differences in response may be dependent on the context of the sound and this should be taken into account when choosing the meaning of the tracks chosen to use. The findings indicate that tracking and locating wolves in the wild is possible using playbacks; and gender and age affect response, however the optimal pitch to use needs to be investigated further.

**Acknowledgements** Dr. Darren Juniper and Kirsty Kliem, University of Reading, for advice and help on the completion of the statistical analysis and staff at the Wolf Conservation Trust, Berkshire, UK for help and loan of the wolves.

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## Ring-tailed Lemur (*lemur catta*) behaviour and nutrition at Cotswold Wildlife Park

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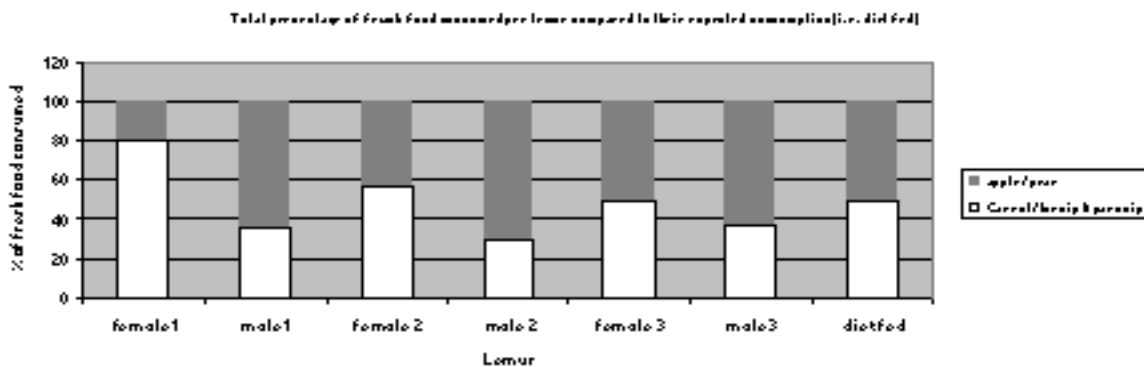
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**Introduction** Walk-through enclosures have initiated the debate as to whether humans are a form of enrichment or stressful excitement with results of behaviour studies arguing each side. Mallapur *et al.* (2005) noted the long term effect of visitors caused a 30% increase in abnormal behaviours but Simpson (2004) contradicts this by observing a positive effect of visitors to red-ruffed lemurs in a walk-through enclosure. Captive diets are formulated to provide a balanced diet but when scatter feeding a group it is difficult to determine whether each animal is eating a correct ratio of feed. The objective of this study was to evaluate the welfare of a troop of Ring-Tailed Lemurs (*lemur catta*) in a walk-through enclosure at Cotswold Wildlife Park (CWP).

**Materials and methods** Having established an ethogram of behaviours (ranging from foraging and grooming through to aggression or playing), the nine (5 male, 4 female) lemurs were observed for 3 hours a day (0930-1030 no visitors; 1130-1230 visitors present and 1400-1500 visitors present until 1430) using scan sampling. Scan sampling is a method where the behaviour of each animal is observed every minute for the trial period. Hence every minute it was noted down how many lemurs were in an area and what behaviour they were doing. Results were compared throughout the observation period and to previous studies completed on these animals (Kent, 2008; Williams & Litten-Brown, 2008). The nutritional observations were focussed on four adults and two juveniles (3 male, 4 female). The males and females were observed separately for am and pm feeds (n=11). At each feeding time a balanced diet (43% apple and pear; 57% carrot/turnip and parsnip) was fed, observations were made at the feeding time to ascertain if each individual received this balanced diet – results were compared to the expected diets. The data was analysed with General Linear Model in Minitab® 15.1.1.0.

**Results** Lemur behaviour was significantly affected by weather ( $P < 0.001$ ) but not by time or visitor presence whereas enclosure use was affected by weather, time and visitor presence (data not shown). For example the lemurs spent approximately twice as long resting when it was raining compared to overcast or sunny weather. Interestingly no lemur consumed the correct ratio of fresh food as they were expected to with their diet formulation with males and females showing different food preferences.

**Graph 1** A graph to show the total percentage of fresh food consumed per lemur during AM study sessions in comparison with their expected consumption



**Conclusions** Williams and Litten-Brown (2008) concluded the walk-through enclosure at CWP provided positive enrichment, this study several months later would tend to agree due to behaviour being similar to that found in the wild (Sussman, 1977). However the lemurs were exhibiting unnatural behaviour toward visitors such as jumping on prams looking for food. The whole of the enclosure was utilised with the lemurs moving from one end to the other in a particular pattern each day. Lemurs received their am & pm feeds indoors where it was obvious one female had dominance over what the others ate, as is the norm in lemur social hierarchy (Sussman, 1977). The findings have implications in attempting to ensure that all animals receive a balanced diet.

**Acknowledgements** The authors would like to thank Cotswold Wildlife Park for their assistance in this study.

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## Seasonal effects on energy requirements in young cats in a temperate environment

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**Introduction** Recently, Bermingham *et al.* (*Bri J Nutr accepted*) carried out a metanalysis of the available data concerning the energy requirements in cats. Obesity levels and related illnesses (e.g. diabetes, joint problems etc.) are increasing in companion animal populations (Scarlett *et al.*, 1994; German 2006), so it is important that the true energy requirements of cats are established. The National Research Council (2006) highlighted the energy requirements for cats at high (36°C) or room temperatures (25°C), however, no information exists on energy requirements in temperate climates, such as those experienced in New Zealand where cats typically have outdoor access all year round. Therefore, this study aimed to determine the difference in maintenance energy requirements of healthy young cats (c. 2 years of age), housed in metabolic cages, in an outdoor environment during summer and winter compared to cats kept in similar cages in an indoor environment during the same two periods.

**Materials and methods** Eight mixed-sex, neutered cats (c. 2 years of age) were housed in individual outdoor pens (80cm x 80cm x 110cm) for 5 weeks (1 week adaptation, 4 week trial) during summer (SO; 18.5 ± 0.5 °C) and winter (WO; 8.5 ± 0.4 °C). Eight full siblings of the first group were kept in similar indoor pens at the same time during summer (SI; 22.4 ± 0.3 °C) and winter (WI; 17.8 ± 0.2 °C). Cats were kept in visual contact with each other, and were familiar with the pens. The cats were fed *ad libitum* AAFCO tested, commercially available fresh wet food daily, had fresh water available at all times and were exposed to natural light regimes. Energy expenditure and body composition were determined using doubly-labelled water over 12 days from the beginning of week 4 of each trial period. During the same two 12 day periods activity monitors (Actical®, Mini Mitter Company Inc, Oregon, USA) were fitted to the collars of two cats (one indoor, one outdoor). Data were analysed using a Repeated Measures procedure of SAS (version 11) and results are reported as mean and standard error of difference (SED).

**Results** Season had no effect on the bodyweight of the cats kept either inside (SI: 3.97 vs WI: 4.02 (SED 0.15) kg; P>0.05) or outside (SO: 3.94 vs WO: 4.04 (SED 0.15) kg; P>0.05). The activity levels of the cats were also similar in both groups between summer (SI: 2272 vs SO: 2469 (SED 178.9) counts/hr) and winter (WI: 2385 vs WO: 2385 (SED 197.0) counts/hr). However, there was a strong seasonal rhythm (P<0.001) of food intake in the cats housed in the indoor environment, with higher intakes observed in the summer (SI: 297.5 vs WI: 258.2 (SED 7.5) kJ/kg BW/d). In contrast, cats kept in an outdoor environment showed more uniform feed intake between the two periods (SO: 299.2 vs WO: 286.2 (SED 7.5) kJ/kg BW/d; P<0.05), but significantly higher intakes (P<0.001) during the winter period. The cats in this study had higher energy requirements than those reported in the literature (213.8 kJ/kg BW/d; Bermingham *et al.*, 2009) which may reflect differences between cats kept outside in temperate climates compared to cats studied in indoor environments. Results on energy expenditure and body composition are being completed and will also be reported.

**Conclusions** An effect of housing was observed in winter when animals housed outdoors consumed 28.0 kJ/kg BW/d more food than their siblings housed indoors. Activity levels and bodyweight remained constant in both groups throughout this period, suggesting that this difference in intake reflected a difference in maintenance energy requirements over the five week winter period. Interestingly, energy intake was higher in summer in both groups (housed inside and outside) compared to winter. This finding may reflect the increased insulatory capacity of the winter versus summer coat, although actual energy expenditure data when it becomes available will begin to answer these questions.

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## Bovine tuberculosis: herd-level surveillance of *Mycobacterium bovis* genotypes in Northern Ireland (2003-2008)

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**Introduction** Bovine TB is a respiratory disease caused by *Mycobacterium bovis* infection. It is the most complex and difficult multi-species, endemic disease facing government, the veterinary profession and the farming industry in the UK (Reynolds 2006). The maintenance and spread of infection within and between populations of cattle and populations of wildlife is poorly understood, with investigations hampered by difficulties in accurately defining epidemiologically-linked cases. The recent development of *M. bovis* genotyping schemes, based on recording DNA repeat copy number variation (variable number tandem repeats or VNTRs) offers highly-discriminating tools for investigating the spread of *M. bovis* (Skuce *et al* 2005, Smith *et al* 2006). Systematic application of *M. bovis* genotyping in conjunction with tracing of cattle movements and wildlife surveillance can be expected to provide valuable insights into disease source and spread. The objective of this study was to investigate, by means of structured herd-level surveillance, the genotype diversity, distribution and structure of the current *M. bovis* population.

**Materials and methods** New, culture-confirmed bovine TB herd incidents were enlisted on a rolling 365 day period (2003-2008). *M. bovis* was isolated and confirmed using standard procedures. The first (disclosing) *M. bovis* isolate per herd was subjected to genotyping (Skuce *et al* 2005). VNTR profiling, spoligotyping, nomenclature, reference isolates and quality control were as described (Skuce *et al* 2005). Animal-level data (movements and test records) were derived from the Animal and Public Health Information System (APHIS, Houston 2001). *M. bovis* isolates were geo-referenced to their final herd using MapInfo Professional v7.5. To investigate potential associations between *M. bovis* genotype and location and inter-annual differences in frequency, contingency tables were analysed using Chi-square tests with Monte Carlo simulation and by test for trend statistics.

**Results** 175 genetically-distinct *M. bovis* genotypes were identified in 8,630 isolates from 6,609 herds. On average 73 genotypes were identified every year, with 29 genotypes present in all 6 years. *M. bovis* genotypes showed striking geographical localisation and significant association ( $P < 0.001$ ) to regions. However, genotypes were also trans-located significant distances from their normal 'home range'. Whilst the frequency of most *M. bovis* genotypes was relatively stable over the survey period, significant differences ( $P < 0.001$ ) were observed for some genotypes in years 2003 to 2008, indicating that the *M. bovis* population was not entirely static. Despite regions being dominated by geographically-localised genotypes, significant and exploitable (in outbreak investigation) local diversity was still present.

**Conclusions** Significant genotype diversity was disclosed in the sampled Northern Ireland *M. bovis* population. The population was highly geographically structured, with different *M. bovis* genotypes tending to cluster significantly in distinct regions. This suggested that most sources tended to be local and relatively stable. Whilst the frequency of most *M. bovis* genotypes was relatively stable over the survey period, expansion and contraction of some genotypes was evident. *M. bovis* genotyping, in conjunction with comprehensive cattle movement databases and wildlife surveillance, offers a powerful tool for investigating bovine TB source, maintenance and spread. The population structure and the performance characteristics of *M. bovis* genotyping support its use to answer detailed epidemiological questions of direct policy relevance.

**Acknowledgements** The authors gratefully acknowledge funding from the Department of Agriculture and Rural Development for Northern Ireland (Project DARD0407) and helpful discussions with Noel H Smith (VLA Weybridge).

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## ***In vitro* screening of various forages for anthelmintic activity on *Haemonchus contortus* eggs**

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**Introduction** Helminth control programmes, based on improvement of farm management and regular anthelmintic treatment, are often impracticable in developing countries due to relatively high price of modern anthelmintics for the smallholder. Medicinal plants may become good alternatives for modern synthetic anthelmintics in smallholder farms if their efficacy can be proved scientifically under controlled studies (Satrija *et al.*, 2001). The objective of the present study was to assess the *in vitro* anthelmintic potential of fifteen tropical forages usually given by farmers as goat feeds, that contain active compounds, in exerting their anthelmintic effects against *Haemonchus contortus* eggs

**Materials and methods** Fifteen plants used as goat feeds by local farmers in Yogyakarta, Indonesia, were screened for *in vitro* anthelmintic activity by measuring the inhibition of the hatching of *Haemonchus contortus* eggs. The plant materials were screened for the presence of water soluble total tannin. The *in vitro* anthelmintic potential of the 15 tropical forages was assessed using aqueous infusions (50%, w/v) of the plant material. Adult female parasites of *Haemonchus contortus* were collected from the abomasums of goats obtained from the abattoir (Daryatmo *et al.*, 2008). Female worms were separated from males, transferred to phosphate buffer saline (PBS) solution (pH 7.2) and incubated at room temperature for 24h. The worms were removed from PBS after 24h and ova laid by them were collected by sedimentation using slow centrifugation. Between 50 - 100 eggs were transferred into petridisks filled with 5 ml of each infusions. The experiment was replicated three times for each infusion. Unhatched eggs were then counted under a dissecting microscope with  $\times 40$  magnification. The positive and negative controls were Albendazole and phosphate buffer saline, respectively. The values obtained were analysed using one way analysis of variance and the LSD test at the 0.05% significance level.

**Results** Tannin contents of the 15 plants ranged from 0.34 – 2.89% of DM (Table 1). The aqueous infusions of the plants inhibited egg hatching by 53.42 - 79.15% (Table 1). The two most potent infusions using egg hatch assay were those of *Manihot esculenta* Crantz and *Artocarpus heterophyllus* leaf in a decreasing order of potency.

**Table 1** Tannin contents of 15 plants and their inhibition (as aqueous infusions) of hatching of *Haemonchus contortus* eggs.

No	Treatment	Water soluble total tannin (% of DM)	Eggs not hatching (%) <sup>1</sup>
1	Albendazole	-	96.15 <sup>a</sup>
2	<i>Manihot esculenta</i> Crantz	2.89	79.15 <sup>b</sup>
3	<i>Artocarpus heterophyllus</i>	2.49	78.72 <sup>b</sup>
4	<i>Swietenia mahagoni</i>	1.23	74.10 <sup>c</sup>
5	<i>Sesbania grandiflora</i>	1.22	71.39 <sup>cd</sup>
6	<i>Ficus benyamina</i>	1.85	69.93 <sup>de</sup>
7	<i>Albizia chinensis</i>	1.19	67.64 <sup>e</sup>
8	<i>Ceiba petandra</i>	1.69	67.47 <sup>e</sup>
9	<i>Carica papaya</i>	1.52	66.21 <sup>e</sup>
10	<i>Acacia</i> spp.	2.10	65.55 <sup>e</sup>
11	<i>Eugenia aquea</i>	1.63	65.12 <sup>ef</sup>
12	<i>Leucaena leucocephala</i>	1.27	61.84 <sup>f</sup>
13	<i>Gliricidea sepium</i>	0.60	61.62 <sup>f</sup>
14	<i>Bauhinia malabarica</i>	0.41	61.48 <sup>f</sup>
15	<i>Dalbergia latifolia</i>	0.84	57.60 <sup>g</sup>
16	<i>Musa paradisiaca</i>	0.34	53.42 <sup>h</sup>
17	Phosphate Buffer Saline (PBS)	-	17.78 <sup>i</sup>

<sup>1</sup>Means of three measurements; within a column, means with different superscripts are statistically different (P<0.05)

**Conclusions** The results indicate that the 15 plants tested showed promising anthelmintic activity. These properties, and their potential as animal feeds, supports their use by farmers in traditional animal health care. Further controlled *in vivo* experiment studies are required to identify possible negative effects on the performance of the animals before any plant can be recommended for save use.

**Acknowledgements** Gadjah Mada University Yogyakarta and DP2M Ministry of Education Indonesia is kindly acknowledged for support from “Hibah Penelitian untuk Mahasiswa Program Doktor” scheme and Hibah Kompetensi Batch II, Tahun 2009.

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## Utilisation of cassava leaf and carica papaya leaf as feeds and anthelmintics for goats

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**Introduction** Cassava is an important agricultural product harvested in the dry season of Indonesia. The national production of cassava was about 19.90 tonne/ha in 2007, increasing to 21.99 tonne/ha in 2009. The main product of cassava is tuber for human food; farmers use the leaf and tuber skin for animal feeds, especially for cattle and goats. Previous research has shown that cassava leaf could be used as a protein supplement for goat production. Young carica papaya leaf is mainly used for human food, but more mature leaf may be used as an animal feed. Although its production is lower than cassava, papaya leaf has a high protein content (19.9%, DM basis). A major problem concerning animal production in the rural areas of Indonesia is parasite infestation. Farmers have difficulty in facing the problem, due to the high price of anthelmintic drugs. Dietary tannins may have an anthelmintic effect. Kustantinah *et al.* (2008) reported that older cassava leaf has a higher tannin content (2.42%) than the younger leaf (1.72%), while carica papaya leaf contains 1.52% of total tannin. The aim of this study was to assess the potential of cassava and papaya as sources of feed and, as anthelmintics for goats.

**Materials and methods** Eighteen individually housed female Bligon goats were used in this study. The animals, which had not been previously treated with anthelmintic drugs and were naturally infected, were divided into three groups (n=6). One group (Control) was offered only grass *ad libitum*. A second group (Treatment I) was offered, *ad libitum*, a mixture of 70% grass and 30% cassava leaf, and the third group (Treatment II) offered a 70% grass and 30% (DM basis) papaya leaf mixture, *ad libitum*. The feeds were offered for 6 weeks, with total collections (feed, refusals and faeces) carried out over the final 10d. Data measured were feed intake, nutrient digestibility, liveweight (LW) gain and, faeces collected directly from the rectum on days 0, 15, 30 and 45 of the feeding period. The number of worm eggs and *Coccidia* oocyst were counted. The data obtained were analysed using analysis of variance and the LSD test at the 0.05% significance level.

**Results** The daily dry matter intakes for Treatment I, Treatment II and Control were 3.53%, 3.54% and 3.30% of LW, respectively. Intakes of organic matter, and crude protein were also increased by supplementation with cassava or papaya leaves, as was the average daily LW gain (Table 1). For Treatments I and II, counts of worm eggs and *Coccidia* oocytes progressively decreased (respective slopes of regressions for treatment I were -291.67,  $P>0.05$  and -325.00 ( $P>0.05$ ) and for treatment II the slopes of regressions were -170.00 ( $P<0.05$ ) and -714.17 ( $P<0.05$ ); whereas the counts increased (slope of regressions were: 216.67 ( $P<0.05$ ) and 246.67 ( $P>0.05$ ) for goats on the Control treatment (Table 2).

**Table 1** Mean nutrient intakes and LW gain in goats receiving grass and cassava or papaya leaf

Nutrient intake	Treatment I	Treatment II	Control
Organic matter (g/kg LW)	30.59±0.51 <sup>a</sup>	30.21±0.59 <sup>a</sup>	28.80±0.76 <sup>b</sup>
Crude Protein (g/kg LW)	5.26±0.10 <sup>a</sup>	5.06±0.12 <sup>b</sup>	4.20±0.10 <sup>c</sup>
Crude fibre (g/kg LW)	8.13±0.14 <sup>a</sup>	7.43±0.12 <sup>b</sup>	8.15±0.21 <sup>a</sup>
Total digestible nutrient (%)	59.14±1.50 <sup>a</sup>	60.92±1.88 <sup>a</sup>	55.81±3.76 <sup>b</sup>
Average daily LW gain (g)	38.51±0.59 <sup>a</sup>	39.08±0.44 <sup>a</sup>	27.01±0.69 <sup>b</sup>

**Table 2** Worm egg and *Coccidia* counts (means± S.E) in faeces from goats receiving grass and cassava or papaya leaf (no./g faeces)

Treatment	Days since beginning of experimental feeding			
	0	15	30	45
Worm egg count:				
Control	217±147	267±163	533±240	850±259
Treatment I	1250±1445	542±562	475±555	300±298
Treatment II	700±547	500±494	400±421	167±108
<i>Coccidia</i> count:				
Control	208±201	283±380	300±354	1025±1948
Treatment I	1108±1267	233±140	108±89	67±51
Treatment II	2108±3589	1108±1757	142±106	50±44

**Conclusion** Supplementation of grass with the leaf of cassava or carica papaya both increased nutrient intakes and had an anthelmintic effect as observed by decreasing faecal EPG and *Coccidia* oocyte counts.

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## IgE and interleukin-5 production and faecal dry matter in parasite-resistant Australian sheep

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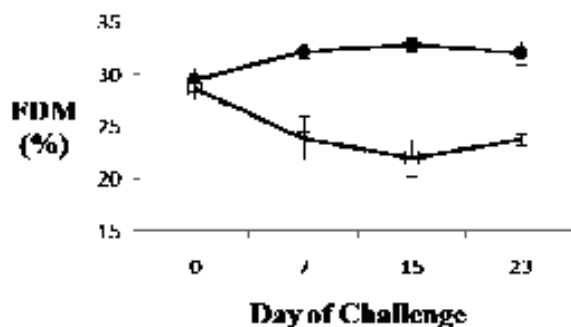
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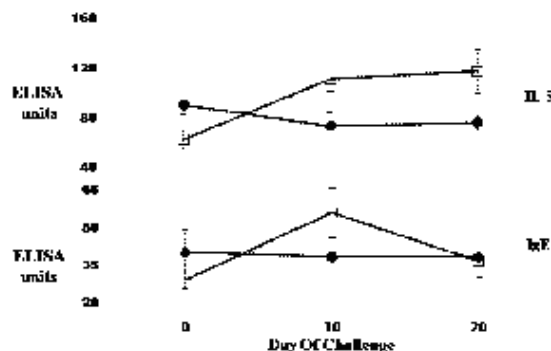
**Introduction** Scouring (diarrhoea) is a major problem for sheep producers as it leads to a build up of faecal material on the wool around the breech (dags), predisposing the animal to flystrike. Scouring occurs when the consistency of faeces is fluid with a low percentage of dry matter, and is associated with ingestion of parasitic nematode larvae such as *Trichostrongylus colubriformis*. We have previously shown that challenging parasite-resistant sheep with nematode larvae results in a reduction in faecal dry matter despite very few worms establishing (Williams *et al.*, 2009). This suggests that some component of the immune response is responsible for scouring in resistant animals. In this experiment we investigated two such components, *T. colubriformis*-specific IgE and the cytokine interleukin-5 which is largely responsible for proliferation of inflammatory cells such as eosinophils. We hypothesised that IgE and IL-5 in serum would be increased when resistant sheep were challenged with *T. colubriformis* larvae and this would be accompanied by a reduction in faecal dry matter.

**Materials and methods** The sheep used in this study were from a parasite-resistant Merino line owned by the Department of Agriculture and Food Western Australia. Sheep from this line are selected for parasite resistance on the basis of low faecal egg counts (FEC) following a natural parasite challenge. FEC and dag scores are regularly monitored on all sheep in this flock. Ten 2-year old rams were selected that all had low FEC but high dag scores in the field. These were then treated with anthelmintic and housed indoors. 5 rams were then dosed daily for three weeks with 1000 *T. colubriformis* larvae. 5 rams were unchallenged controls. Each week, at least ten grams of faeces was collected from each sheep, weighed and then dried in an oven at 90°C to determine the percentage of faecal dry matter (FDM). Serum was collected from all rams before challenge commenced and then at 10 and 20 days after. *T. colubriformis* specific-IgE was measured using a sandwich ELISA. IL-5 was measured according to the method of Doligowska *et al.* (1999). Results were expressed in ELISA units, where 100 units represented the absorbance at 412 nm of a positive control standard (high-reading sera) run on every plate. Differences in FDM, IgE and IL-5 between challenged and control sheep over the course of the experiment were determined using a mixed model analysis in SAS version 9.1 with group and week as fixed factors.

**Results** 7 days after challenge commenced FDM was reduced in the challenged sheep compared to the pre-challenge period and was also lower than in the control sheep ( $P < 0.01$ ). At 14 and 21 days after challenge FDM in the challenged sheep did not differ from day 7 but remained lower ( $P < 0.01$ ) than in the control sheep (Figure 1). IgE increased ( $P < 0.05$ ) in the challenged sheep ten days after challenge commenced but fell to baseline levels by day 20 (Figure 2). IL-5 also increased ( $P < 0.05$ ) in the challenged sheep at 10 days after challenge commenced but did not increase further at day 20. IL-5 tended to decrease in the control sheep (Figure 2).



**Figure 1** FDM (means  $\pm$  sem) in control (●) and challenged (□) rams during larval challenge



**Figure 2** IL-5 and IgE concentrations in serum (means  $\pm$  sem) in control (●) and challenged (□) rams during larval challenge

**Conclusions** Some sheep that are resistant to parasites may scour as a result of the immune response to newly ingested larvae. IgE and IL-5 were increased during larval challenge which may indicate a role in this immunity, although serum levels may not fully indicate the role that they play at the gut mucosal level. It is of interest to note from these results that the marked reduction in FDM in the challenged sheep occurred within 7 days which tends to coincide with the peak in IgE and IL-5 production we observed. Therefore, it could be tentatively postulated that these mechanisms may be responsible for some of the scouring seen in resistant sheep. IgE may be responsible for releasing mast cell-derived mediators such as histamine that contribute to diarrhoea, even after the initial increase in IgE has returned to baseline levels.

**Acknowledgements** We are very grateful to Phil Stein, Novartis Animal Health Australia, for supplying the infective larvae and to Susan McClure, CSIRO Livestock Industries, Armidale, Australia for the ovine IgE monoclonal antibody.

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## Effect of maternal exposure to nematode parasites in ewes on performance and parasite resistance in their lambs

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**Introduction** There is some evidence to suggest that exposure of ewes to parasites reduces weight gain in their lambs (Zaralis *et al.*, 2008), although these effects are not observed when exposure is limited to late pregnancy only (Leyva *et al.*, 1982). However, maternal exposure to parasites throughout pregnancy may affect off-spring performance and its resistance and resilience to parasites, as this may program offspring to better cope with parasite infections (Kristan, 2002). Here, we assessed effects of exposure of ewes to abomasal nematode parasites on ewe and lamb performance and on lamb resistance to the same parasites.

**Materials and method** Thirty-two 4-5 year old housed Mule ewes were either trickle infected with 10,000 *Teladorsagia circumcincta* infective larvae (I, n=16) or sham infected with water (C, n=16) from one week before synchronised mating with Suffolk rams until parturition (day<sub>0</sub>). Ewes weighed (69.9±0.9kg) on day<sub>-158</sub> and were commercially fed throughout. At scanning (day<sub>-88</sub>), 2 single-, 9 twin- and 1 triplet-bearing ewes were selected from each experimental group. Ewes were drenched at lambing to terminate the parasitic infection with Levacide (levamisole) and Oramec (ivermectin), and their lambs were trickle infected from day<sub>38</sub> until day<sub>77</sub> with 2,000 *T. circumcincta* larvae. Lambs had access to creep feed from day<sub>31</sub> onwards and were weaned on day<sub>66</sub>. Ewes were weighed on day<sub>-88</sub>, and ewes and lambs were weekly weighed from lambing onwards. Ewe faecal egg counts (FEC, in eggs per gram, epg) were assessed during pregnancy to monitor the infection; lamb FEC were assessed twice weekly between day<sub>56</sub> and day<sub>77</sub>. FEC were transformed via log(FEC+1) for statistical analyses, and reported as back-transformed means with a 95% confidence intervals range. Obtained data were averaged per lamb reared, and were analysed using ANOVA, with ewe body weight at day<sub>-158</sub> as covariate for ewe performance, lamb birth weight as covariate for lamb performance, and a repeated measure analysis for lamb FEC.

**Results** The number of barren ewes were similar in the C and I treatments (2/16 and 3/16, respectively). Mean FEC of I ewes gradually increased from 1 (1-2) epg on day<sub>-84</sub> to 129 (66-251) epg on day<sub>0</sub>. All FEC were 0 on day<sub>18</sub>. I ewes lambed on average 1.4 days later than C ewes (s.e.d. 0.66; P<0.05), but reared similar number of lambs (1.9±0.1). Table 1 shows that I ewes were lighter than C ewes during pregnancy and at parturition. This difference was smaller by day<sub>31</sub> due to weight loss from C ewes, and disappeared by day<sub>66</sub> due to a higher weight gain of I ewes. Ewe exposure to parasites did not affect mean lamb birth weight, but reduced lamb weight gain until day<sub>38</sub> and increased lamb weight gain during lamb exposure to parasites. Ewe exposure did not affect lamb FEC, which increased from 25 (22-29) to 242 (223-263) epg on day<sub>62</sub> and averaged 116 (105-128) epg on day<sub>77</sub>.

**Table 1** Effect of maternal exposure to parasites on ewe and lamb performance and lamb faecal egg counts (FEC).

	Control (C)	Infected (I)	s.e.d.	P-value
Ewe body weight (kg)				
day <sub>-88</sub>	70.1	65.8	1.00	<0.001
day <sub>0</sub>	70.3	65.1	1.29	<0.001
day <sub>31</sub>	68.5	65.3	1.91	0.114
day <sub>66</sub>	68.4	68.4	1.29	0.991
Ewe body weight gain (g/d)				
day <sub>0-31</sub>	-45	16	63.5	0.342
day <sub>31-66</sub>	15	109	42.5	0.039
Lamb body weight (kg)				
day <sub>0</sub>	5.0	5.1	0.37	0.887
day <sub>38</sub>	19.7	18.2	0.60	0.020
day <sub>73</sub>	35.3	35.2	1.15	0.923
Lamb body weight gain (g/d)				
day <sub>0-38</sub>	382	342	16.4	0.020
day <sub>38-77</sub>	372	412	17.1	0.031
Lamb FEC (log(epg+1))				
mean (day <sub>56-77</sub> )	1.95	1.98	0.087	0.805

**Conclusion** These data support the view that expression of immunity to parasites in immune ewes is nutritionally expensive and that body reserves may be sacrificed over reproductive effort to account for this. The reduced body reserves may be the basis for the reduced lamb weight gain observed during the immediately post lambing period. The data are consistent with the view that maternal exposure to nematode parasites may increase offspring resilience rather than resistance to infection with the same parasite.

### Acknowledgements

SAC receives support from the Scottish Government, Rural and Environment Research and Analysis Directorate.

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## Resistance to gastrointestinal nematodes during the periparturient period is sensitive to specific amino acid deficiency

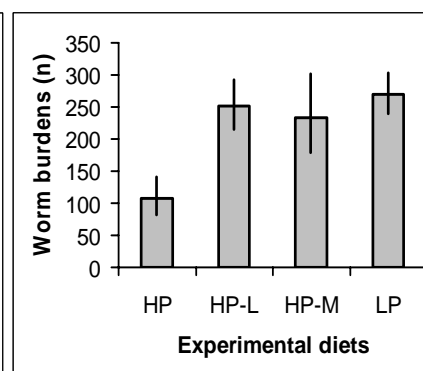
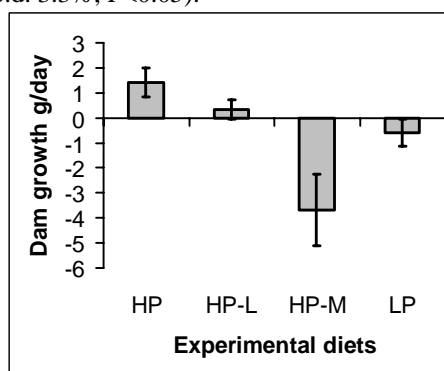
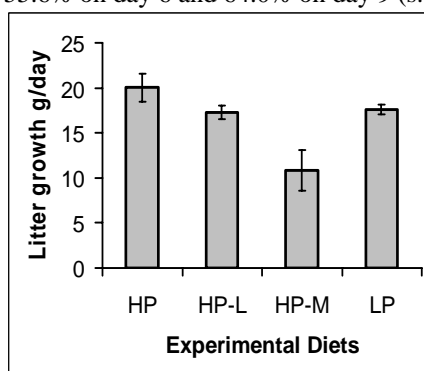
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**Introduction** Periparturient resistance to parasites in previously immune mammals often breaks down, resulting in elevated levels of parasitism. The underlying periparturient relaxation in immunity (PPRI) may have a nutritional basis (Coop and Kyriazakis, 1999); immune rats, re-infected with *Nippostrongylus brasiliensis* given access to low crude protein (CP) diets showed an augmented degree of PPRI compared to their high CP counterparts (Jones *et al.*, 2009; Sakkas *et al.*, 2009). As host responses to dietary CP can be seen as responses to essential amino acids, we assessed sensitivity of PPRI to reduced availability of two essential amino acids, i.e. leucine and methionine. Leucine is relatively abundant in proteins of up-regulated immune responses in response to CP supply (Houdijk and Athanasiadou, 2003), and supplemental methionine has been shown to improve resistance to *N. brasiliensis* (Cummings *et al.*, 1986).

**Materials and methods** Forty rats were infected with 1600 *N. brasiliensis* larvae prior to mating, and upon parturition, allocated to one of four feeding treatment groups, balanced for parturition body weight (n=10). Diets LP and HP were formulated to supply 150 and 250 g CP per kg, respectively. LP protein was methionine-enriched casein, and HP was made by including purified amino acids at levels found in methionine-enriched casein at the expense of starch/sucrose. Diets HP-L and HP-M were identical to HP but without extra leucine and methionine, respectively. The iso-energetic diets were fed at ~90% of metabolizable energy intake on *ad libitum* fed diets (Jones *et al.*, 2009). Parturition litter size was adjusted to 12 pups and dams were re-infected with 1600 *N. brasiliensis* larvae on day 2 of lactation. Dams and litters were weighed daily to estimate litter and dam growth (using linear regression) until either on 6 or 9 days post infection when worm burdens (number and sex) were assessed as a proxy for the degree of PPRI. Worm burdens were log-transformed prior to statistical analysis, and reported as backtransformed means with 95% CI. Results were analysed using ANOVA through REML. Main effects of feeding treatments are reported as interactions with sampling time were not significant.

**Results** Figures 1, 2 and 3 shows that feeding treatment affected litter growth, dam growth and worm burdens, respectively ( $P < 0.005$ ). HP litters grew faster than LP and HP-L litters, which in turn grew faster than HP-M litters. In a similar fashion, HP dams had higher weight gains than LP and HP-L dams, which in turn grew faster than HP-M dams. HP dams had lower worm burdens than LP, HP-L and HP-M dams, whilst worm burdens for the latter three groups did not differ. Worm burden composition was affected by time only; across feeding treatments, female worm percentage was 55.6% on day 6 and 64.0% on day 9 (s.e.d. 3.3%;  $P < 0.05$ ).



**Figure 1** Litter growth

**Figure 2** Dam growth

**Figure 3** Worm burdens

**Conclusion** The response in litter gain to increased protein supply suggests that protein was limiting for LP dams. As expected, (Coop and Kyriazakis, 1999), LP dams had consequently higher worm burdens than HP dams. The responses to the deficiency in leucine and methionine may suggest that the underlying PPRI is sensitive to the reduced availability of these specific amino acids. Further studies using this model may be required to identify if the responses observed are the consequence of feeding imbalanced protein *per se* and could lead to identification of an optimal amino acid composition required to reduce the degree of PPRI.

**Acknowledgements** This work was funded by the Biotechnology and Biological Sciences Research Council (BBSRC). Mr Panagiotis Sakkas is grateful to the Hellenic State Scholarship Foundation for the provision of a postgraduate scholarship.

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## Asymptomatic carriage of *Clostridium difficile* PCR ribotype 078 in pigs

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**Introduction** *Clostridium difficile* has been reported to be the most common cause of neonatal enteritis in pigs in the United States (Songer and Anderson 2006) and there are a number of reports of its presence in pigs in Europe (Nagy and Bilkei 2003, Songer and Anderson 2006, Debast *et al.* 2009). In contrast, it has been observed in previous experimental work at the University of Leeds pig farm that the incidence of asymptomatic *C. difficile* carriage in pigs is common in young pigs but rare in pigs at slaughter age (Brunton *et al.*, 2009). The aim of this study was to observe the incidence of *C. difficile* carriage in piglets at weaning through to 18 weeks of age. We hypothesised that asymptomatic *C. difficile* carriage would be high in pigs at weaning, but would decrease with age. Our second hypothesis was that the addition of a therapeutic level of an antibiotic, phenoxymethyl penicillin, to the pigs diet would increase the incidence of *C. difficile* carriage. Antibiotic treatment is a risk factor for the onset of *C. difficile* associated infection (CDI) in humans (Bignardi 1998).

**Materials and methods** Thirty-six piglets from six litters were distributed across the two treatments. Pigs were allocated across six pens (six pigs in each) so that pens were balanced for litter, gender and weight (average weight = 7.7 kg  $\pm$  0.93 SD). All pigs were fed on a standard commercial weaner diet from 4 to 7 weeks of age, after which a standard grower diet was introduced until week 12. This grower diet contained therapeutic levels of phenoxymethyl penicillin (0.2 kg/tonne, prescribed to combat *streptococcus suis* infections) for pigs in 3 of the pens, whilst the other 3 pens were fed the base diet with no antibiotics. From week 12 onwards all pigs were fed a standard commercial finisher diet containing no antibiotics. Faecal samples were collected from all 36 pigs at 4 weeks (weaning), and then at 7, 10, 12, 13 and 18 weeks of age. The health of the animals was monitored throughout the study. DNA was extracted from each of the faecal samples using a phenol:chloroform based method. *C. difficile* DNA was detected using the polymerase chain reaction (PCR) with probes specific for the *tcdA* gene which encodes the toxin A. PCR results were analysed using the chi squared test including Yates' correction for two categories of data. PCR positive samples were cultured in an attempt to isolate *C. difficile*. Ribotyping of the isolates was carried out by the *Clostridium difficile* Ribotyping Network (CDRN) based at the Leeds General Infirmary, Leeds, West Yorkshire. A cytotoxin assay was also carried out on all samples in which the *tcdA* gene had been detected using PCR.

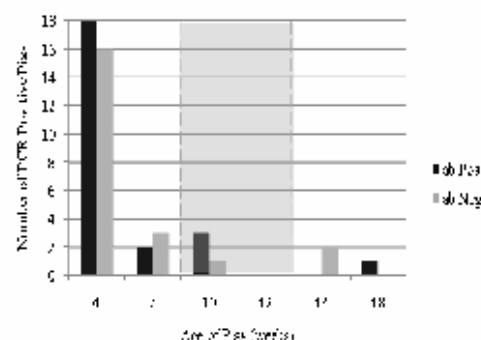
**Results** At week 4, all of the treatment group (18/18) and 16/18 of the control group screened positive for *C. difficile*. All animals were asymptomatic and there was no statistically significant difference between the two groups. By week 7, the number of pigs that were positive for *C. difficile* had decreased significantly for both groups to only 2 positive samples for the treatment group and 3 positive samples for the control group ( $P < 0.001$  and  $P < 0.01$  respectively) (Figure 1). The number of positive samples remained low for the rest of the trial. There was no significant difference between groups across the trial. Of the 46 samples that were screened as positive by PCR, 13 yielded *C. difficile* when cultured. Eight of the 13 were from pigs in the treatment group, and the remaining 5 were from pigs in the control group. All 13 isolates were ribotyped and found to be the PCR ribotype 078. None of the samples demonstrated significant cytotoxic effects.

**Conclusions** We have demonstrated that on the UK farm studied asymptomatic carriage of *C. difficile* is common, but short-lived in piglets. The inclusion of phenoxymethyl penicillin in the pigs' diet was found to have no effect on the carriage of *C. difficile*. The observations of this study are contradictory to reports from North America and parts of Europe, where *C. difficile* is claimed to cause symptomatic illness in young pigs (Songer and Anderson 2006, Debast *et al.* 2009). The phenomenon of asymptomatic carriage which decreases with age has been observed in other species, including humans and rabbits, but has not before been demonstrated in pigs. Further work is required to determine the reasons for this phenomenon, and to understand why *C. difficile* is a problem in North America, but not in the UK.

**Acknowledgements** The authors wish to thank Professor Mark Wilcox and his group for their assistance with the culture and ribotyping of isolates, and cytotoxicity assays. Lucy Brunton was supported by a BPEX PhD studentship.

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**Figure 1** The number of pigs screened as positive for *C. difficile* by PCR at each time point, on diets containing either therapeutic levels of penicillin (black bars) or no antibiotic (grey bars). N = 18 pigs for each treatment. Antibiotic was administered between weeks 7 and 12, as indicated by the shaded area on the graph.

## Development of infection models to assess subclinical disease in pigs through the use of acute phase proteins as markers

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**Introduction** Sub-clinical disease is a major contributor to a lower than expected pig performance, economic losses and reduction in pig welfare. Currently there are no markers to objectively assess the presence and extent of sub-clinical disease in live pigs, which hampers the development of strategies to overcome the consequences of subclinical disease, including increased environmental burdens. Acute phase proteins (APP) are components of the innate immune response and their response to infection pathogens is generic rather than pathogen specific (Eckersall 2000); for this reason they have the potential to characterise pig disease status. Here we describe two studies which aimed to establish infection models to assess the presence of sub-clinical disease in young pigs through changes in their APP profiles.

**Materials and methods** In Experiment 1 (Exp1), 24 8-week old male pigs were used (live weight (LW) 22.16±0.44 kg) and were either sham infected (n=2 controls for each infection protocol) or trickle infected (n=4) with one of the following pathogens: *Enterotoxigenic E. coli* (ETEC) and *Brachyspira pilosicoli* (models for local intestinal disease; *per os* dose: 10<sup>8</sup> cfu/pig/day) and *Haemophilus parasuis* and *Listeria monocytogenes* (models for systemic disease; doses: *per os* 10<sup>4</sup> and subcutaneously 10<sup>9</sup> cfu/pig/day respectively). In Experiment 2 (Exp2), 18 4-week old male pigs were used (LW 8.45±0.8 kg) and were infected with either 10<sup>9</sup>, 10<sup>8</sup> and 10<sup>6</sup> cfu/pig/day of ETEC, *B. pilosicoli* and *H. parasuis* respectively (n=4) or were sham infected controls (n=2 controls for each infection protocol). Bacterial challenge was mixed with the food for ETEC and *B. pilosicoli*, whereas it was administered intranasally for *H. parasuis*. Animals were challenged 3 times per week for either two or one weeks for Exp1 and Exp2 respectively. Blood samples were obtained at regular intervals to monitor the response on 3 APP (haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA)). Food intake was recorded daily; weight gain, health scores, bacteria excretion and temperature were monitored at regular intervals. The two studies were analysed separately, using a one-way ANOVA which included APP results prior to infection as covariate. Contrast statements were used to compare each infection group to the control (Genstat 9).

**Results** In Exp1, subclinical disease was evident in *L. monocytogenes* infected pigs, with an increase in their rectal temperature (P<0.001) and a reduced, although not significantly different, LW gain (Table 1). None of the other infected pigs showed any signs of subclinical disease throughout the experiment. All measured APP were significantly upregulated in *L. monocytogenes* infected pigs (Table 1), whereas they were not affected by infection in any of the other groups. In Exp2, ETEC infected pigs showed a reduced, although not significantly different food intake and LW gain. This was accompanied with a significant increase in Hp and SAA. There was no effect of infection on performance or APP profiles in the remaining infected pigs. The consequences of infection were evident only during the first week of the infections.

**Table 1** Effects of infection with different pathogens on log-transformed APP, feed intake (FI) and LW gain (LWG) in growing pigs during the first week of infection. Superscripts denote significant difference from the control at P<0.05; <sup>(1)</sup> pooled across all controls as results were not affected by sham-infection protocols

	Exp1					Exp2				
	Hp g/L	CRP mg/L	SAA mg/L	FI g/day	LWG g/day	Hp g/L	CRP mg/L	SAA mg/L	FI g/day	LWG g/day
Control <sup>(1)</sup>	0.09	2.10	0.37	1290	880	0.09	2.14	0.81	503	434
ETEC	0.09	2.19	0.19	1234	842	0.21 <sup>d</sup>	2.32	1.23 <sup>e</sup>	433	300
<i>B. pilosicoli</i>	0.08	2.12	0.16	1250	820	0.13	2.12	0.66	488	429
<i>H. parasuis</i>	0.07	2.13	0.43	1210	923	0.04	2.37	0.94	527	472
<i>L. monocytogenes</i>	0.32 <sup>a</sup>	2.63 <sup>b</sup>	2.11 <sup>c</sup>	1197	772	NA	NA	NA	NA	NA
s.e.d.	0.03	0.07	0.19	58.5	124	0.04	0.15	0.19	112	147

**Conclusions** *L. monocytogenes* infection resulted in systemic effects (pyrexia), upregulation of all three APP measured and limited penalties in performance. These make it a good candidate model for assessing the use of APP as markers for subclinical, systemic infection in pigs. ETEC challenge resulted in local infection (no pyrexia) and upregulation of two APP and these, in combination with studies where penalties in performance were confirmed (Wellock *et al.* 2008), make it a good model for assessing the use of APP as markers for subclinical localised infection. Four-week old animals were considered better candidates than 8-week old pigs to test these models at the tested infection doses. The lack of evidence for subclinical disease from *B. pilosicoli* and *H. parasuis* infections under the protocols tested prohibits their use as models to study subclinical pig infections at this stage.

**Acknowledgements** This research was financially supported by Pfizer, JSR Genetics Ltd, ABN, BPEX and QMS with match funding from the Scottish Government, through the Sustainable Livestock Production LINK programme.

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## The potential of hyperspectral imaging for the measurement of meat quality

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**Introduction** The application of near infrared spectroscopy to predict meat tenderness has been recently reviewed by Prieto *et al.*, 2009. To obtain representative sampling, NIR equipment suitable for measurement of relatively large areas of the muscle is required. Marbling fat within the muscle is not equally distributed and thus sampling different areas of the muscle can result in different spectra depending on the amount of marbling fat in the sampled area. Hyperspectral imaging instead provides the potential to identify and measure spectra for specific regions of interest. Applications to several food quality applications are reviewed by Gowen *et al.*, 2007. For beef, NIR hyperspectral imaging has the potential to identify independent spectra relating to subcutaneous fat, marbling fat and lean. The aim of this study was to identify and characterise the spectra from different regions in the beef foreb joint and within the longissimus dorsi (LD) muscle between marbling fat and areas of lean devoid of visible marbling.

**Materials and methods** A random selection of 150 foreribs were obtained 2 days post slaughter from a commercial abattoir and transported to the laboratory. A slice approximately 25 mm thick was removed from the caudal surface of the forerib and an image of the exposed surface taken within 2 minutes of cutting. Images were taken using an instrument described by Millar *et al.*, 2008, incorporating a SWIR spectral camera (Specim, Oulu, Finland) with a cooled 14 bit HgCdTe detector and N2SE spectrograph. The system was configured to image a 200mm line spanning the full width of the joint with a spatial resolution of 320 pixels, and 256 spectral bands with a wavelength range of 900-2500nm. The sample was moved on a motorised stage and scans were taken at a rate of 50 lines/s to acquire an image of the whole cut surface in a total scan time of 9s. After scanning the freshly cut surface, the meat was allowed to bloom at 4°C for at least 1 hour and scanned again.

Further scans were made to assess the contribution of several factors. The effect of blooming was assessed by scanning selected samples at a larger number of time intervals up to 1 hour. To assess the depth of the sample surface contributing to the measurements, scans were made for thinly sliced samples of lean beef and subcutaneous adipose tissue presented against white and black backgrounds. Scans were also undertaken samples containing clearly identifiable regions of connective tissue to characterise the corresponding connective tissue spectra.

**Results** Figure 1 shows examples of reflection spectra for several tissues. The mean spectrum is shown for a freshly cut lean region of the longissimus dorsi. Further measurements of the same sample at times of up to 1 hour (not shown) showed no strong changes in the NIR spectrum as the sample bloomed, despite clear visible changes in colour. This may be due to the measured spectral range being outside the visible region, however, Moss *et al* (2010) noted changes due to blooming in the NIR region. Spectra are also shown for examples of subcutaneous, intermuscular and marbling fat for a single freshly cut sample. All types of fat are clearly distinguishable from lean tissue facilitating automated classification of the lean and fat regions of each image and determination of representative spectra for each component. In samples where the area of marbling fat was small, the spectra obtained in these regions were intermediate between those of lean and fat. This was partly due to the resolution of 0.6mm used, such that pixels in regions of thin marbling may contain both lean and fat, and also due to the penetration depth of the NIR radiation. As can be seen from Figure 1, the absorbance was greater at longer wavelengths. Tests for samples of known thickness showed measurable penetration to a depth of about 10mm at 1100nm, but minimal penetration beyond 2-3mm for wavelengths greater than about 1500nm.

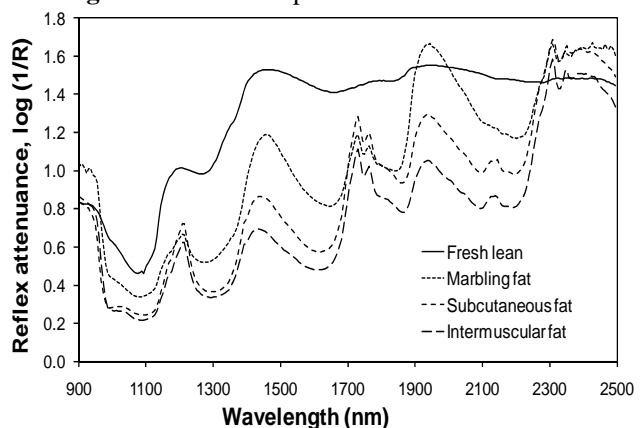
**Conclusion** The results show that hyperspectral NIR imaging has the potential to discriminate between lean and fat tissue. Further work is required to identify how the ability to discriminate between tissues (eg marbling fat & lean) can be developed for prediction models for meat quality.

**Acknowledgements** This work was funded by EBLEX.

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**Figure 1** Reflection spectra for lean and fat tissue





## Predicting carcass cut yields in cattle using routinely collected digital images

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**Introduction** Payment for beef carcasses in the European Union is generally based on a combination of cold carcass weight and classification for carcass conformation and fat. Although, carcass classification in Ireland was originally based on subject assessment by trained personnel, carcass classification is now undertaken in Ireland using authorised classification machines. Since 2005, a copy of two carcass images taken by the VBS2000 mechanical grading machine after slaughter to derive the EUROP conformation and fat grading have been stored in the Irish Cattle Breeding Federation database. The objective of this study was to investigate the potential of using Video Image Analysis (VIA) to predict carcass cut yields.

**Material and methods** Digital images taken at slaughter can be summarised into 428 variables describing linear measurements of carcass dimensions, carcass contour and carcass color measurements. Two datasets with information on detailed carcass dissections as well as carcass images were treated separately: an experimental (n = 346 steers) and a commercial (n = 281 heifers) dataset. In both datasets, four groups of wholesale cut weights were created, in consultation with industry representatives, according to their retail values: Lower Value Cuts (LVC) including fore- and hind shins, flank, ribs, brisket, neck, and lean trimmings; Medium Value Cuts (MVC) comprising of the shoulder and the chuck cuts; High Value Cuts (HVC) including the sirloin and the round cut weights; Very High Value Cuts (VHVC) comprising of the weights of the rib roast, strip-loin, and fillet cuts. In the experimental dataset, total carcass fat weight and total bone weight were also available. For each of the carcass groups, the two datasets were individually split into a calibration and a validation sub-datasets, based on an equal distribution (i.e., similar mean and standard deviation) of the trait under investigation. In the experimental dataset, 232 steers (67% of the steer population) were included in the calibration dataset and 114 steers were included in the validation dataset; in the commercial dataset, the respective numbers were 189 (67% of the heifer population) and 92 heifers. Three alternative prediction models were evaluated within the experimental and commercial dataset separately: 1) model including carcass weight only, 2) model including carcass weight plus EUROP classification for conformation and fat, and 3) model including carcass weight plus VIA parameters. Stepwise regression, principal component analysis, partial least squares, least angle regression and canonical correlations methods were all separately tested to choose which VIA variables best described the calibration dataset. The regression models developed from the calibration datasets were then applied to the validation dataset and the fit assessed. Statistics used to quantify the goodness of fit in the validation dataset included the mean bias, the RMSE, the coefficient of multiple determination of the model ( $R^2$ ), and the correlation between the predicted values and the residuals ( $r_e$ ).

**Results** Of the alternative statistical methods tested, stepwise regression gave consistently the most accurate prediction; the number of predictors in the model varied from 6 to 10 depending on the trait. Across all traits, the model that included carcass weight plus VIA parameters gave the best prediction as evidenced by the greater  $R^2$  and lowest RMSE. The lack of a residual correlation and mean bias not significantly different from zero implies no systematic bias in the predictive ability of almost all regressions. The large difference in accuracy of prediction of LVC between the experimental and the commercial dataset reflects the differences in cutting procedures between the two plants. The lowest accuracy of prediction was for the VHVC; this is consistent with the fact that VHVC includes the fillet positioned inside the carcass, thus hidden from the camera pictures, and the full loin which can also be difficult to appreciate from a side view image due to its flat shape. Other image technologies (cross section analysis, X-ray tomography) provide a more detailed appreciation of the carcass composition, but at a greater cost.

**Table 1** Residual root mean square error (RMSE) and coefficient of determination ( $R^2$ ) in the validation datasets of the experimental and the commercial dataset using stepwise regression models containing carcass weight (CCW), carcass weight and EUROP grading for conformation and fat (CCW + EUROP), and carcass weight and VIA variables (CCW + VIA)

Traits (kg)	Experimental steer dataset						Commercial heifer dataset					
	CCW		CCW+EUROP		CCW+VIA		CCW		CCW+EUROP		CCW+VIA	
	RMSE	$R^2$	RMSE	$R^2$	RMSE	$R^2$	RMSE	$R^2$	RMSE	$R^2$	RMSE	$R^2$
Total meat	11.78	0.91	7.43	0.97	6.77	0.97	11.31	0.68	9.07	0.80	8.00	0.84
Total fat	10.71	0.33	6.67	0.74	6.38	0.77						
Total bone	4.31	0.66	3.38	0.79	3.22	0.81						
LVC	6.92	0.87	6.54	0.89	5.60	0.92	8.32	0.46	7.35	0.57	6.62	0.65
MVC	3.73	0.74	3.36	0.79	2.73	0.86	1.53	0.62	1.43	0.67	1.37	0.70
HVC	6.03	0.75	3.91	0.89	3.27	0.93	3.16	0.68	2.47	0.81	2.16	0.85
VHVC	2.28	0.74	1.74	0.85	1.75	0.84	1.28	0.68	1.20	0.71	1.24	0.72

**Conclusion** Inclusion of VIA variables in prediction models improved the fit to the data compared to including only carcass weight or carcass weight and EUROP classification. VIA technology is fast and non-invasive and VIA classification machines are in all Irish cattle abattoirs with the images routinely stored thus providing a powerful tool for exploiting in beef breeding programs.

# Accuracy of virtual partial dissection by computed tomography as predictor of beef carcass composition

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**Introduction** Dissection is the reference method to measure carcass composition, which is one of the attributes that defines carcass value. Full carcass dissection is an expensive and time-consuming procedure, and therefore only suitable for a limited number of applications such as research trials involving a relatively small number of animals. Partial dissection using a sample joint was one of the first methods to be considered to reduce costs and increase the number of carcasses to be assessed. X-ray computed tomography (CT) is a non-invasive method recently evaluated as a predictor of beef carcass composition. Carcass tissue weights were assessed with  $R^2$  values between 0.96 and 0.97 based on the CT scanning of all primal cuts (Navajas *et al.*, 2009). The aim of this study was to compare the potential of the compositions of a forerib sample joint measured by CT and dissection as predictors of beef carcass composition.

**Materials and methods** Data were recorded on 30 Aberdeen Angus and 43 Limousin crossbred heifers and steers that were slaughtered with average (standard deviation) age and hot carcass weight of 584 (26.2) days and 342 (30.0) kg, respectively. At 48 h after slaughter, left carcass sides were split into 20 primal cuts (Navajas *et al.*, 2009), each small enough to pass through the CT scanner, and vacuum packed. Spiral CT scans (SCTS) were collected for each primal cut. After scanning, carcass side (CS) tissue weights were computed based on the fully dissection of all primal cuts into fat, muscle and bone. The weights of muscle, fat and bone of the foreribs (FR) were predicted by CT based on tissue areas and densities and the thickness of slices in the SCTS: Tissue weight =  $\Sigma$  tissue area  $\times$  slice thickness  $\times$  weighted average density of tissue, where weighted average density =  $\Sigma$  (area  $\times$  tissue density) /  $\Sigma$  area. Tissue densities were calculated for fat and muscle using the equation: Tissue density ( $\text{g}/\text{cm}^3$ ) = (CT tissue density in HU  $\times$  0.00106) + 1.0062 (Fullerton, 1980). Bone density assumed in these calculations was  $1.55 \text{ g}/\text{cm}^3$ . The prediction equations for tissue weights in the CS were estimated from the composition of the FR using general linear models. The models fitted were: (i) simple regression models to analyse the association between the composition of the FR assessed by dissection and CT methods and CS calculated after dissection, i.e.  $\text{CSF}_D = a + b \text{FRF}_{CT} + e$ , where,  $\text{CSF}_D$  is the carcass side fat weight,  $a$  is the intercept,  $\text{FRF}_{CT}$  is the fat weight in the forerib by CT,  $b$  the regression coefficient and  $e$  is the residual error, and (ii) full model fitting breed (B), sex (S), CS weight (CSWT), FR weight (FRWT) and the weights of the other tissues in the FR, as suggested by Fisher (1990). For example, for  $\text{CSF}_D$ , the model was:  $\text{CSF}_D = a + B + S + b\text{CSWT} + c\text{FRWT} + d\text{FRF}_D + f\text{FM}_D + g\text{FB}_D + e$ , where  $\text{FRF}_D$ ,  $\text{FRM}_D$  and  $\text{FRB}_D$  were the weights of fat, muscle and bone of the FR measured by dissection, respectively; and  $b$  to  $g$  were regression coefficients.  $B$  and  $S$  were fitted as classificatory effects. In (ii), for each carcass tissue weight measured, all possible combinations of predictors were fitted separately by method (dissection or CT) using backward stepwise regression. Final model terms were chosen within method. Models were compared using adjusted coefficient of determination ( $\text{adj-R}^2$ ) and root mean square error (RMSE, kg).

**Results** Accuracies of the prediction models are presented in Table 1. Single regression models with the weights of fat, muscle or bone in the FR measured by CT as the only predictors to estimate fat, muscle or bone of the CS showed  $\text{adj-R}^2$  of 0.77, 0.60 and 0.52, respectively. By adding B, S, CSWT and FRWT to FR composition by CT improved the prediction accuracy of carcass fat and muscle weights significantly to  $\text{adj-R}^2$  values of 0.89 and 0.94, respectively, whilst the highest value for carcass

**Table 1** Fitted effects and accuracy of the models for each carcass tissue measured by dissection or predicted by CT

Models & accuracy	Dissection			CT		
	Fat	Muscle	Bone	Fat	Muscle	Bone
(i) Forerib tissue weight as predictor						
Adj- $R^2$	0.71	0.54	0.61	0.77	0.60	0.52
RMSE	3.78	8.18	1.84	3.40	7.64	2.02
(ii) Full model						
Adj- $R^2$	0.87	0.95	0.84	0.89	0.94	0.80
RMSE	2.56	2.82	1.17	2.34	2.89	1.33

bone weight was 0.80. In general, equations derived using CT data had slightly lower  $\text{adj-R}^2$  values for bone but similar or better accuracies for fat and muscle compared to those obtained using the FR composition by dissection.

**Conclusions** Virtual partial dissection by CT using the forerib as sample joint provides accurate predictions of CS tissue weights. It could be considered as a very accurate and fast alternative method to assess beef carcass composition, with minimum depreciation of the primals, that could be very useful for breeding programmes and research studies involving large number of animals, including the calibration of other indirect methods (e.g. *in vivo* and carcass video image analysis). Future cross-validation analysis is recommended.

**Acknowledgements** We are grateful to Scottish Government for funding this work. The support of Scotbeef is gratefully acknowledged.

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## Prediction of beef carcass composition and tissue distribution using ultrasound scanning

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**Introduction** Weight, proportion and distribution of fat and muscle in beef carcasses are of importance in order to maximise economic value and reduce waste. *In-vivo* ultrasound-measured predictors of cattle carcass composition could be of use when taken at the beginning of the finishing period (to allocate feeding groups and levels or for early selection of potential breeding stock), or pre-slaughter (to market-select finished animals, sort carcasses on quality, or feed back information from slaughter animals to breeding programmes). The aims of this study were to investigate the ability of ultrasound-measured fat and muscle depths to predict carcass composition and tissue distribution and to quantify the relative accuracy of predictions using ultrasound measurement taken at the start or end of the finishing period.

**Materials and methods** Live weight (LWT) and ultrasound measurements (muscle depth (UMD) and four fat depths (UFD) over the 3<sup>rd</sup> lumbar vertebra; four UFD measurements over the 12<sup>th</sup> thoracic vertebra) were measured on 52 crossbred steers and 10 heifers at the start and end of the finishing period (average age 482 and 576 d, respectively). Animals were slaughtered in batches, depending on weight and visual predictions of carcass grade, and one carcass side was fully dissected. Stepwise multiple linear regression analyses were performed in Genstat (Payne *et al.*, 2005) to determine the best combinations of predictor traits (LWT, UMD, UFD) to predict: total weights of fat and muscle in the carcass side (CF and CM, respectively), the forequarter (FQF, FQM) and hindquarter (HQF, HQM); CF and CM as proportions of carcass side weight (CF%SD, CM%SD); FQF and FQM as proportions of carcass side weight (FQF%SD, FQM%SD); HQF and HQM as proportions of carcass side weight (HQF%SD, HQM%SD); HQF as a proportion of total fat weight in the carcass side (HQF%F); HQM as a proportion of total muscle weight in the carcass side (HQM%M).

Three models (M1-M3) were tested for each carcass trait that used LWT and informative ultrasound data from: (1) pre-finishing; (2) post-finishing; or (3) pre-finishing and post-finishing. Individual UFD measurements (max. 8, if all contributed towards a reduction in residual standard deviation) were used in the models, rather than averaging UFD measurements at each site. Only LWT and UMD were tested in models to predict muscle traits and only LWT and UFD were used in models to predict fat traits, in order to minimise confounding between fat and muscle traits.

**Results** As expected, higher prediction accuracies (adjusted  $R^2$ ) were generally achieved using measurements taken post-finishing than pre-finishing, although combining both substantially increased adjusted  $R^2$  values (Table 1). Fat weights and proportions (of total carcass side) were predicted with higher accuracy than muscle traits. For muscle traits, weights were predicted more accurately than their proportions, whereas there was an opposite trend for fat traits. The proportion of the carcass side weight consisting of muscle in the forequarter (FQM%SD), in particular, was poorly predicted using all models.

**Table 1** Adjusted  $R^2$  values for each model describing each carcass trait (values >0.05 significantly different from zero)

Carcass trait	M 1 (pre)	M 2 (post)	M 3 (pre + post)	
CF	0.53	0.69	0.84	Muscle weight in the hindquarter expressed as a proportion of total muscle in the carcass side (HQM%M) could be predicted with moderate accuracy using the models including post-finishing measurements, but hindquarter fat weight expressed as a proportion of total fat in the carcass side (HQF%F) could not be predicted accurately.
CM	0.44	0.47	0.58	
CF%SD	0.67	0.71	0.84	
CM%SD	0.33	0.25	0.40	
FQF	0.51	0.63	0.76	
HQF	0.49	0.68	0.81	
FQM	0.38	0.45	0.54	
HQM	0.44	0.47	0.59	
FQF%SD	0.64	0.65	0.79	
HQF%SD	0.64	0.71	0.83	
FQM%SD	0.23	0.11	0.23	
HQM%SD	0.32	0.34	0.49	
HQF%F	0.09	0.03	0.10	
HQM%M	0.08	0.31	0.39	

**Conclusions** Ultrasound tissue depths measured before and after finishing, combined with live weight (M3), can predict fat weights and proportions in beef carcasses and carcass quarters with high accuracy, and muscle weights and proportions with moderate accuracy. Pre-finishing measurements alone (M1) give moderate predictions of composition traits and improve predictions when combined with post-finishing data (M2 vs. M3). The ability of these measurements to distinguish between animals with more of their fat or muscle in one quarter was poor. However, prediction equations specialised for different carcass regions, rather than the whole carcass, may be more appropriate to target certain markets or allow greater flexibility for selection.

**Acknowledgements** SAC receives funding from the Scottish Government.

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## The influence of blooming on the near-infrared spectra of beef

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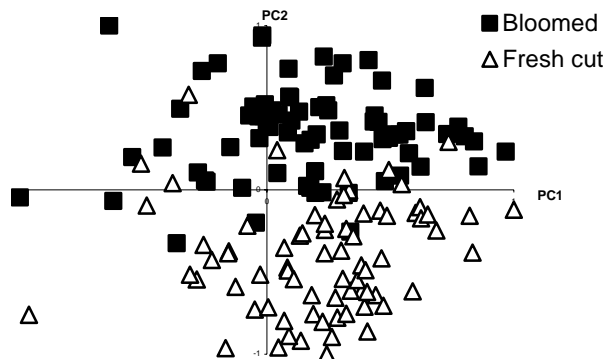
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**Introduction** The use of near infrared reflectance (NIR) for the prediction of meat quality has been reviewed recently (Prieto *et al.* 2009). Factors which may affect the NIR spectra and the prediction developed, include both time of measurement after slaughter, and whether the meat was allowed to bloom (Moss *et al.* 2009a, 2009b) and method of carcass suspension prior to rigor (Ooltra *et al.* 2009). The effect of blooming on the visible spectral region (380 to 780nm) is well known, however, the effect on the NIR region (780 to 2500nm) is not well characterised. In order to use NIR on cut surfaces of beef it is important to know the time course of blooming and the relative changes at different wavelengths in the spectra to aid in selection of wavelengths for prediction equations, such that blooming would have little influence on the prediction model.

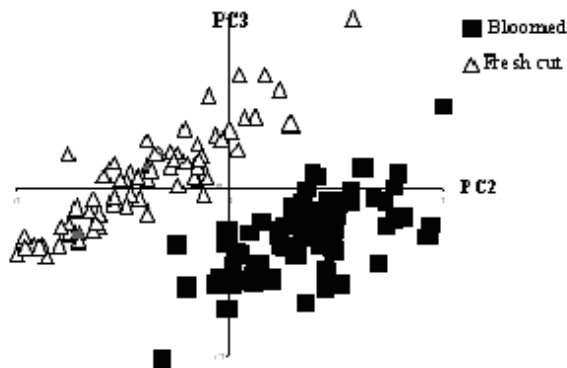
**Method** The NIR spectra were measured on 150 beef sirloins using a prototype beef reflectance probe (Analytical Spectral Devices, Colorado, USA) both immediately after cutting and after 1 hour. Selection of the carcasses (50 bulls, 50 steers, 50 heifers) was as described by Moss *et al.* 2009a).

To determine the difference between freshly cut and bloomed surfaces three statistical approaches were used; paired t test, principal component analysis (PCA) and discriminant analysis (DA). The paired t test and PCA was undertaken at each wavelength over the region 380 to 2250 nm on all samples. The DA, however, was performed on a subset so that the freshly cut and bloomed samples were not from the same animals. The discriminant model was then validated on different set of unrelated spectra.

**Results and discussion** The paired t test showed that in the visible region (380 to 780nm) the wavelengths not statistically significant between freshly cut and bloomed were: 454, 508, 526, 527, 552, 553, 573, 590nm. These wavelengths represent isobestic points for the various myoglobin pigments. Statistically significant differences were found in the NIR region at wavelength regions: 805 – 1193, 1416– 1533, 1710–1783, 1801– 2434nm. The PCA analysis undertaken on the reflectance data from 380 to 2250 nm, showed that 3 components explained 96% of the variation in the data. Separation between freshly cut and bloomed was mainly along PC2 (fig 1), with complete separation between freshly cut and bloomed when PC2 was plotted against PC3 (fig 2). High loading coefficients were found both in the visible region (380 to 780 nm) and in the NIR region between 780 and 1340.



**Figure 1** PCA plot for principal component 1 and 2



**Figure 2** PCA plot for principal component 2 and 3

DA showed 100% discrimination between freshly cut and bloomed samples when performed on data in the visible and NIR regions (up to 1350nm). Above 1350nm it was not possible to obtain a statistically significant discriminant rule based on percentage reflectance data.

**Conclusion** The data shows that although major differences between freshly cut and bloomed spectra are greater in the visible region there are spectral differences in the NIR region. The results of the PCA and discriminant analysis indicate that above 1350nm blooming has less effect on the spectra. Thus prediction models based on wavelength regions between 1350nm and 2250nm will be less influenced by the time from cutting to measurement. Further work is required on the time course of blooming on NIR spectra.

**Acknowledgements** The project was funded by EBLEX

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## Potential of nuclear magnetic resonance spectroscopy to measure biochemical changes during post mortem aging of beef hung by Achilles or pelvic suspension prior to rigor

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**Introduction** During the aging of beef tenderness increases mainly due to the proteolysis of myofibrillar proteins (Koohmaraie & Geesink, 2006). Increases in amino acids, nucleotides and sugars during the post mortem aging period (Koutsidis *et al.*, 2008) are important in relation to flavour development when the meat is cooked. Although a single extraction procedure can be used for amino acids, nucleotides and sugars, different analytical procedures are required for each type of metabolite. <sup>1</sup>H nuclear magnetic resonance (<sup>1</sup>HNMR) has been shown to be an ideal technique to systematically characterise and quantify metabolites in plants (Graham *et al.*, 2009); and has the potential to characterise a wide range of metabolites thus removing the requirement for different analytical techniques. <sup>1</sup>HNMR has been used to investigate the relationship between stress, metabolite profiles and water-holding capacity in pigs (Bertram *et al.*, 2005) but not been applied to beef muscle. The aim of this study was to evaluate the ability of <sup>1</sup>HNMR to characterise the changes in amino acids, nucleotides and sugars during the post mortem aging of beef.

**Materials and methods** Six carcasses were selected at random at a commercial meat plant and one side of each carcass was hung by Achilles tendon (AT) and the other by pelvic suspension (TS). Three days post slaughter the forerib joint was removed and from the *longissimus dorsi* (LD) four 2cm thick slices were taken, placed in bags and vacuum packed. The samples were stored at 2°C for periods of 3, 7, 14 and 21 days after which time they were blast frozen and stored at -80°C. Metabolites were extracted from a 3 g sample of LD using 0.6M perchloric acid according to the procedure of Aliani & Farmer (2005). The neutralized supernatants were lyophilized, then reconstituted in 650 µl of 0.1 M phosphate buffer (pH 7.0), in D<sub>2</sub>O, containing 1 mM of the internal standard sodium trimethylsilyl-2,2,3,3,-tetra deuterio propionate (TSP, Sigma Aldrich, UK). Insoluble material was removed by centrifugation (16,000 g for 15 min), and 600 µl of the remaining supernatant was transferred to a 5 mm diameter NMR tube.

The spectra were recorded in D<sub>2</sub>O on a Bruker AC 300 and 500 (2D (<sup>1</sup>H/<sup>13</sup>C) heteronuclear multiple quantum coherence (HMQC) NMR experiments) MHz spectrometer. Thirty-two transients were acquired. Spectral processing was carried out using ACDlabs NMR Processor v9.0 (ACD labs, Toronto, Canada). Baseline correction was performed manually. Data reduction was carried out by manually binning the spectra and measuring the integral for each bin between 0.80 p.p.m. and 8.70 p.p.m.. The region from 4.75 p.p.m. to 5.00 p.p.m., which contained the signal for the water resonance, was excluded. Analysis of variance was undertaken to determine the effect of carcass suspension method and aging on metabolites measured. Principal component analysis (PCA) was also undertaken to evaluate the relationship between metabolites and period of aging.

**Results** A total of 27 compounds were identified by using a combination of NMR databases, spiking with known pure compounds and 2 dimensional NMR (<sup>1</sup>H/<sup>13</sup>C). The metabolites identified included a range of amino acids, adenine nucleotides and sugars. The two major peaks were lactate and creatine. There were 20 peaks in the NMR spectra which could not be identified and require complementary NMR techniques to aid identification. Fructose could not be clearly identified due to its low level relative to adjoining peaks of ribose and glucose at a much higher level.

There was no statistically significant effect of carcass suspension method on any of the metabolites measured. Three components in the PCA explained 71% of the variation (PC1 45%, PC2 19%, PC3 7.5%). Separation according to ageing periods was mainly along principal component 1 (Fig 1) but followed a diagonal vector from low PC1 & PC2 scores (3 days aging) to high PC1 and PC2 scores (day 21). The PCA loadings showed that longer ageing periods were associated with a number of amino acids, particularly; alanine, phenylalanine, isoleucine and valine. The PCA loadings

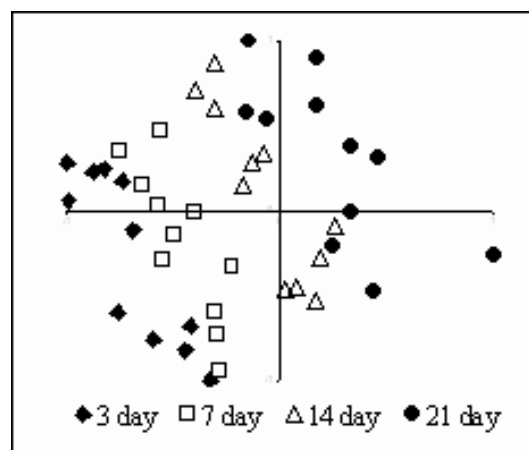
for shorter aging periods were associated with peaks identified as ATP and combination of ATP/ADP/hypoxanthine/inosine, however, the level of these metabolites was low.

**Conclusion** <sup>1</sup>HNMR provides a useful analytical technique for the measurement of polar metabolites in the post rigor period. Further work is required to identify unknown peaks and the rate at which metabolites change during aging of meat.

**Acknowledgement** Stewart Graham was funded by Department of Agriculture and Rural development, Northern Ireland.

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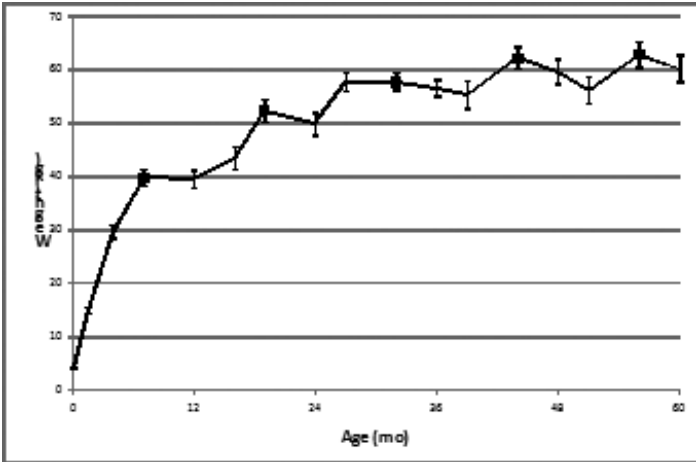
**Figure 1** PCA of metabolites by NMR

A comparison of six models used to describe ewe growth to maturity

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**Introduction** The mature weight of breeding ewes has implications for both the amount of feed required for maintenance/productive processes as well as the likely greenhouse gas emissions, both of which are linked to bodyweight. The growth of lambs has been studied extensively (e.g. Lambe *et al.*, 2006) but reports on ewe growth to maturity are rare. As part of a larger study into the genetics of mature weight in sheep, 4 common and 2 novel growth models were fitted to ewe weight data collected from birth to their 7<sup>th</sup> lambing, to investigate the relationships between growth and mature weight. This paper reports on the efficacy of the models.

**Materials and methods** The records used in these analyses came from a fully recorded flock of 600 ewes selected for a range of objectives over an 18-year period. Animals in the flock were weighed at birth, 6 and 16 weeks of age and then annually at mating, lambing and weaning until after the 7<sup>th</sup> lambing. Lifetime records of ewe weights were available from 1,390 ewes born over a 13-year period. A range of models were fitted to two derivatives of this dataset using the Procedure NLIN in SAS (SAS, 2003). The first dataset comprised all records available for the ewes (ALLWTS) whereas the second dataset comprised the lamb weights plus the annual mating weight (MATWTS). Six 3-parameter non-linear models were fitted to each ewe’s lifetime weights; these were logistic, 3<sup>rd</sup>-order polynomial, Gompertz and Brody equations plus two new models, one based on Hill (1910) and a linear spline model with a knot and two slopes. This spline model was fitted to the data, given the birthweight and mature weight of the ewes. Because all the models had 3 parameters and the same dataset was used for all comparisons, models were compared with using the average RMS.



**Results** The growth of the ewes to maturity had a distinctive pattern (Figure) whereby growth rate up to first mating (7 months) had a different pattern to that between first mating and maturity. The ewes reached mature weight at 44 months of age, on average. The results of fitting the 6 models to the two datasets are summarised in Table 1. Models with a fixed rate of change (Gompertz, Brody, Logistic, Polynomial) had the largest RMS, reflecting the lack of flexibility of their parameters to deal with the two growth phases. This contrasts with the Hill curve which can change both the point of inflection and the slope of the curve and consequently had a better fit. The spline model fits two straight lines and estimates their slopes and the knot where they meet. The mean knot was at 5.08mo

and the two slopes were 228 and 23g/d; effectively fitting one slope during early lamb growth and the second from 1<sup>st</sup> mating to maturity. This model had the lowest RMS. The Brody model predicted the mature weight of the data most closely.

**Table 1** The mean RMS and predicted mature weights from fitting 6 3-parameter growth models to ewes with all weights (ALLWTS), and lambing plus mating weights (MATWTS) (actual mean mature weights 59.8 [ALLWTS] and 62.9kg [MATWTS]).

	ALLWTS RMS (kg <sup>2</sup> )	Predicted wt. (kg)	mature	MATWTS RMS (kg <sup>2</sup> )	Predicted wt. (kg)	mature
Gompertz	46.41 <sup>a</sup>	56.06		30.84	58.23	
Logistic	51.02	58.63		38.69 <sup>c</sup>	60.27	
Brody	40.25	59.84		21.71 <sup>a</sup>	62.15	
Hill	36.66	57.87		19.36 <sup>ab</sup>	61.16	
Spline model	27.64	NA		14.57 <sup>b</sup>	NA	
Polynomial	44.63 <sup>a</sup>	57.21		36.81 <sup>c</sup>	60.90	

Means within a column with the same superscript were not significantly different (P < 0.05); means without superscripts were significantly different

**Conclusions** When modelling liveweight to maturity in female ewes, growth models need to cope with the effects of pregnancy and lactation on growth. Model parameters need to be flexible or designed to cope with these effects.

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## Adaptation of Meat Standards Australia Quality System for Northern Irish Beef

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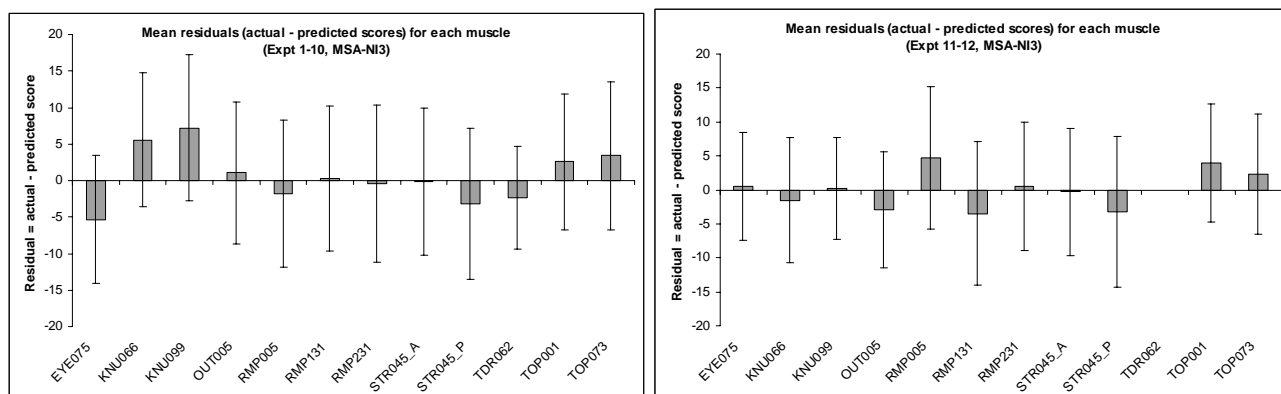
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**Introduction.** The “Meat Standards Australia” (MSA) quality assurance system for beef eating quality is based on consumer data and predicts the final eating quality for a particular muscle and cooking method from information recorded for each animal/carcass (Thompson, 2002, Polkinghorne *et al.*, 2008). Prediction traits included muscle, position within muscle, hanging method, % *Bos indicus* breed, use of hormonal growth promoters, marbling, maturity (by ossification score), carcass weight, rib fat depth, meat colour, ultimate pH, ageing time and cooking method (Thompson, 2002). In addition, participating meat plants minimize handling stress and ensure that the electrical stimulation/chilling regime allows an appropriate rate of pH decline against temperature. Some factors, especially % *Bos indicus* breed and use of growth promoters, do not apply in the EU, while the impact of maturity will be less due to different production practices. Likewise, the MSA system did not include bulls, beef of dairy origin or beef cooked “well-done” in its prediction model. This paper summarises the outcomes of a project to evaluate the performance of MSA, adapt it to NI beef and consumers and to test the validity of the adapted model.

**Materials and methods.** Experiments were conducted to evaluate the role of pre-and post-slaughter factors, such as gender, breed, hanging method, ageing, electrical stimulation/chilling, muscle and position within muscle on eating quality. Carcass information, namely breed, sex, hot standard carcass weight and EUROP grade were recorded as were MSA grading measurements (Thompson, 2002). A total of 192 animals and 36000 consumer tastings (6000 consumers) were used to develop a version of the model for NI. A further 10080 consumer tastings on beef from 48 animals were used to validate the adapted model (MSA-NI). Consumers scored portions for tenderness (TE), juiciness (JU), flavour liking (FL) and overall liking (OL) using a 100 mm line scale (Farmer *et al.*, 2009). A combined meat quality score (MQ4) was obtained using the equation,  $MQ4 = 0.4*TE + 0.1*JU + 0.2*FL + 0.3*OL$ .

**Results and Discussion** The standard MSA system accurately predicted the eating quality of beef for consumers from Northern Ireland. Nevertheless, some differences were found: NI consumers responded slightly differently to Australian consumers and some muscles and groups of animals were less accurately predicted. The adapted MSA model included adjustments to the boundaries between grades, adjustments to the predicted scores for certain muscles, removal of factors relating to *Bos indicus* and growth promoters and an adjustment for bulls. Figures 1 shows the ability of the adapted model (MSA-NI) to predict the eating quality of (a) beef used to develop the model and (b) that used for validation.



Key to muscles: EYE075 = *semitendinosus*, KNU066 = *rectus femoris*; KNU099 = *vastus lateralis*, OUT005 = *biceps femoris*, RMP 005, 131, 231 = *biceps femoris*, *gluteus medius*, *gluteus medius* (eye), STR045A, M, P = *longissimus dorsi*, anterior, middle and posterior, TDR062 = *psoas major*, TOP001, 073 = *adductor femoris*, *semimembranosus*.

**Figure 1** Mean difference ( $\pm$ se) between predicted and actual consumer MQ4 scores from MSA-NI for (a) the calibration data (36000 tastings) and (b) the validation data (10080 tastings). Bars indicate standard deviations.

**Conclusions** An adapted MSA-NI grading model predicted the eating quality of the NI beef with good accuracy and precision.

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## Finishing strategies for Belgian Blue and Limousin cross steers

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**Introduction** Belgian Blue and Limousin sires are used for cross breeding with Holstein-Friesian dairy cows and the resultant male calves are used for beef production. In beef winter finishing enterprises, a preliminary period of variable feeding may precede the finishing period to postpone slaughter date until beef prices are higher in late spring. The optimum feeding level in such a preliminary period has not been established. The aim of this study was to determine the effects of three feeding levels during a preliminary finishing period on feed intake, growth and carcass traits of Belgian Blue x Holstein-Friesian (BB) and Limousin x Holstein-Friesian (LM) steers.

**Materials and methods** Forty eight steers (24 BB and 24 LM) were blocked on live weight (BB 368, LM 391, s.d. 11.3, kg) within breed and assigned to 3 feeding levels for a preliminary feeding period of 112 days followed by finishing on concentrates *ad libitum* to a target slaughter weight of 610 kg. The 3 feeding levels in the preliminary period were (i) grass silage only (S0), (ii) S0 + 2.57 kg concentrate dry matter (DM) per head daily (S3), (iii) S0 + 5.13 kg concentrate DM per head daily. The silage analysis was 208 g/kg DM, 146 g/kg crude protein in DM, 701 g/kg *in vitro* DM digestibility, pH 3.9. The concentrate formulation was 875 g/kg rolled barley, 65 g/kg soya bean meal, 45 g/kg molasses and 15 g/kg mineral/vitamin premix. Accommodation was a slatted shed fitted with Calan boxes for individual feed recording. The animals were slaughtered in a commercial abattoir where cold carcass weight (0.98 hot weight), carcass grades for conformation and fatness, and weights of perirenal plus retroperitoneal fat were recorded. The data were statistically analysed as a 2 (breeds) x 3 (feeding levels) factorial, using general linear model least squares procedures, with terms for block, breed, feeding level and breed x feeding level.

**Results** As a consequence of their heavier initial live weight, LM were still heavier ( $P < 0.001$ ) than BB after 112 days, but at slaughter there was no difference in live weight between the breeds. BB tended to have higher daily live weight gains than LM during finishing but the differences were not significant. BB had a higher ( $P < 0.001$ ) kill-out and a lower ( $P < 0.001$ ) carcass fat class than LM. At the end of the preliminary period, S3 was 53 kg heavier ( $P < 0.05$ ) than S0, and S6 was 26 kg heavier ( $P < 0.05$ ) than S3. After 97 days on *ad libitum* concentrates (day 209), S3 had overtaken S6 in live weight, and the difference between S0 and S3 was reduced to 34 kg ( $P < 0.05$ ). Both S3 and S6 reached slaughter weight at the same time while S0 required a further 35 days to slaughter weight. During the preliminary period, daily live weight gain responses to the first and second 2.57 kg/day concentrate DM increments were 483 and 220 g, respectively. The corresponding differences in the opposite direction afterwards to 209 days were 189 and 300 g. Other than carcass fat class which was higher for S0 there were no differences in carcass traits. For similar slaughter and carcass weights, S6 required more ( $P < 0.001$ ) concentrates and net energy than both S0 and S3, while S0 required more ( $P < 0.001$ ) silage and net energy than S3.

**Conclusions** BB had superior finishing traits to LM with a higher kill-out proportion, better carcass conformation and a lower carcass fat class. S3 achieved similar slaughter and carcass weights to S6 with 357 kg less concentrate DM (but 154 kg more silage DM) and 290 UFV less net energy. S0 and S3 had similar concentrate intakes but S0 required 163 kg more silage DM and 148 UFV more net energy. Where a preliminary feeding period precedes a finishing period on *ad libitum* concentrates, the target growth rate during that period should be around 0.8 kg/day. Higher growth rates will be compensated during finishing while lower growth rates require an extended finishing period.

**Table 1** Effects of breed and finishing strategy on steer performance

	Breed (B)		Finishing Strategy (F)			s.e. <sup>1</sup>	Significance		
	BB	LM	S0	S3	S6		B	F	BxF
LW at 112 days (kg)	463	481	428 <sup>a</sup>	481 <sup>a</sup>	507 <sup>c</sup>	3.5	***	***	NS
LW at slaughter (kg)	609	618	615	614	611	6.7	NS	NS	NS
ADG 0 – 112 days (g)	848	805	431 <sup>a</sup>	914 <sup>b</sup>	1134 <sup>c</sup>	26.0	NS	***	* <sup>4</sup>
ADG 112 – 209 days <sup>2</sup> (g)	1365	1310	1563 <sup>a</sup>	1374 <sup>b</sup>	1074 <sup>c</sup>	57.3	NS	***	NS
ADG 0 days-slaughter (g)	1076	1009	945 <sup>a</sup>	1101 <sup>b</sup>	1081 <sup>b</sup>	29.5	NS	**	NS
Days to slaughter	226	226	249	214	214	-	-	-	-
Carcass weight (kg)	338	339	333	334	334	4.0	NS	NS	NS
Kill-out (g/kg)	556	533	542	545	547	2.8	***	NS	NS
Fat class	3.1	3.8	3.6 <sup>a</sup>	3.4 <sup>b</sup>	3.4 <sup>b</sup>	0.05	**	*	*** <sup>5</sup>
Total silage intake (kg) <sup>3</sup>	820	830	985 <sup>a</sup>	822 <sup>b</sup>	668 <sup>c</sup>	16.6	NS	***	NS
Total concentrate intake (kg) <sup>3</sup>	1469	1476	1369 <sup>a</sup>	1345 <sup>b</sup>	1702 <sup>b</sup>	9.0	NS	***	NS
Total net energy intake (UFV) <sup>4</sup>	2267	2282	2276 <sup>a</sup>	2128 <sup>b</sup>	2418 <sup>c</sup>	21.8	NS	***	NS

<sup>1</sup>For Breed; <sup>2</sup>Last common weight before slaughter; <sup>3</sup>DM; <sup>4</sup>Unite Fourragere Viande; <sup>5</sup>Values for S0, S3 and S6 = 401, 901 and 1234 (BB), and 453, 927 and 1035 (LM); Values for S0, S3 and S6 = 3.0, 3.0 and 3.3 (BB), and 4.2, 3.7 and 3.6 (LM);

<sup>abc</sup>Values with a

common superscript do not differ significantly ( $P > 0.05$ ); LW = live weight; ADG = average daily gain.



## The relationship between beef quality and carcass quality attributes measured under commercial conditions

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**Introduction** Objective measures of beef carcass quality appear to resolve some of the issues surrounding the subjective aspect of beef carcass classification (Allen and Finnerty 2000). Carcass conformation is important to the producer and processor whereas meat colour and tenderness are important to the consumer. Yet eating quality is not part of the current grading system. In this study, the relationships between carcass yield traits in commercial cattle and objective measures of meat quality were analysed considering both VIA predicted and recorded weights of primals. The aim was to establish whether both carcass yield and meat quality goals can be achieved in a value-based marketing system that rewards producers on both meat yield and meat quality parameters.

**Materials and methods** Animals were selected based on breed and sex in an abattoir where a commercial VIA machine (E+V GmbH, Oranienburg, Germany) was operating on-line. On the Monday of each week, eight Charolais-cross, eight Limousin-cross and eight Dairy-cross animals were selected for the study. For each breed group, four steers and four heifers were selected per week except for the dairy cross group where young bulls were substituted for heifers. Over 10 weeks, six classes of animals and samples were obtained in 10 batches for the VIA predicted traits, while actual recorded primal weights were recorded on the first six batches only. Age at slaughter, together with VIA based grades (based on the 15 point scales), weights and yield estimations for weight from the right hand half carcass were recorded. Quartering was at the 9<sup>th</sup>/10<sup>th</sup> rib, where a section of the longissimus muscle was recovered for pH (Testo 205) and colour analysis on site allowing a 45min blooming period (Minolta CR-410, D65 illumination, 2° standard observer with a 50 mm aperture). Carcasses were subject to electrical stimulation (90V for 30 sec 10 minutes PM). Complete sirloins were removed intact from the hind quarter (Pistola) at 48hrs PM and commercially processed into the marketable striploin and tenderloin with each measured trait being weighed (Table 1). Longissimus steaks were cooked to an internal temperature of 71°C and slice shear force (SSF) was measured three days PM as outlined by Shackelford *et al.*, (1999). A logarithmic transformation (base 10) was applied to SSF measurements for the analysis. Partial correlations between meat quality and VIA-predicted yields and recorded primal weights were obtained using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) including batch, and combined breed-sex classes as fixed effects with age as a co-variable.

**Results** Basic statistics and partial correlations are given in table one. A small but significant negative correlation observed between SSF and VIA predicted striploin weight (-0.18), VIA conformation (-0.21) and measured tenderloin weight (-0.18) were favourable because a lower SSF value indicates greater tenderness ( $P < 0.05$ ). Young bulls were significantly less tender than steers ( $P < 0.001$ ) or heifers ( $P < 0.05$ ), but no significant differences in tenderness were observed among breeds. Small but significant negative correlations were estimated between pH (48 hr) & VIA pistola weight (-0.18) and measured striploin weights. But there were no significant differences in pH between sexes or breeds. Positive correlations were shown between Lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) and measured sirloin weights. No differences in colour traits were observed between the sexes, but Charolais sirloin steak was lighter and more yellow ( $P < 0.001$ ) than Limousin and redder ( $a^*$ ) than Dairy ( $P < 0.05$ ). A stronger and more significant positive correlation was obtained between  $L^*$  and VIA fat class (0.28,  $P < 0.01$ ) compared to  $L^*$  and measured fat trim (0.19  $P < 0.05$ ).

**Table 1** Basic statistics for predicted and measured traits also showing partial correlations between pH, colour and tenderness measures and carcass quality traits  $P < 0.05^*$   $P < 0.01^{**}$   $P < 0.001^{***}$

VIA predicted:	n	mean	CV%	pH (48h)	$L^*$	$a^*$	$b^*$	SSF
Forequarter weight (kg)	196	63.24	17.4	-0.150	0.144	0.284 <sup>*</sup>	0.308 <sup>**</sup>	-0.105
Striploin weight (kg)	196	7.22	20.0	-0.139	0.149	0.344 <sup>*</sup>	0.323 <sup>***</sup>	-0.181 <sup>*</sup>
Pistola weight (kg)	196	104.52	17.9	-0.177 <sup>*</sup>	0.146	0.298 <sup>*</sup>	0.318 <sup>***</sup>	-0.109
Conformation (15pt)	196	6.78	31.1	0.014	0.086	0.232 <sup>*</sup>	0.152	-0.211 <sup>*</sup>
Fat class (15pt)	196	10.31	22.0	-0.172	0.283 <sup>**</sup>	0.187 <sup>*</sup>	0.280 <sup>**</sup>	-0.044
Measured trait (kg):	n	mean	CV%	pH (48h)	$L^*$	$a^*$	$b^*$	SSF
Complete weight	140	15.29	15.5	-0.198 <sup>*</sup>	0.202 <sup>*</sup>	0.343 <sup>*</sup>	0.377 <sup>***</sup>	-0.091
Boned striploin weight	140	8.32	18.6	-0.198 <sup>*</sup>	0.233 <sup>**</sup>	0.357 <sup>*</sup>	0.407 <sup>***</sup>	-0.055
Trimmed striploin weight	141	7.22	18.8	-0.212 <sup>*</sup>	0.197 <sup>*</sup>	0.351 <sup>*</sup>	0.375 <sup>***</sup>	-0.057
Tenderloin weight	141	3.42	16.6	-0.121	0.045	0.220 <sup>*</sup>	0.167	-0.184 <sup>*</sup>
Fat trim weight	141	1.10	36.4	-0.020	0.192 <sup>*</sup>	0.137	0.238 <sup>**</sup>	-0.011

**Conclusions** Significant correlations between carcass yield and tenderness were favourable, indicating no antagonism between these traits. Significant associations were found between carcass characteristics and colour, suggesting that the redness and yellowness of the meat colour increases with carcass weight. Breed influenced meat colour but sex had no detectable effect.

**Acknowledgements** Quality Meat Scotland, C. Alma Baker Award, Scottish Government, E+V GmbH. The contribution from abattoir staff is gratefully appreciated. Thanks also to Lesley Deans, Lutz Bunker and Jimmy Hyslop for assistance.

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## External preference mapping of beef: Relationship of fatty acids and flavour precursors with consumer's preferences

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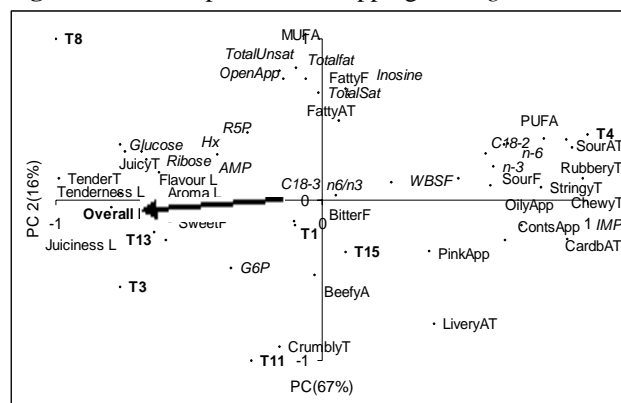
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**Introduction** The beef eating quality attribute, tenderness, has been traditionally considered more important than flavour (Miller, 2001). However, recent sensory research has found a higher correlation between flavour liking and consumers overall acceptability than tenderness and overall liking (Oliver *et al*, 2006). These beef eating quality attributes can be influenced by pre and post slaughter factors. Amongst the pre-slaughter factors, diet can change the fatty acid composition of the meat, with an impact on the nutritional characteristics of the meat and also on flavour (Wood *et al*, 2004). Recent studies have reported that diet can also influence in the content of the water soluble flavour precursors on beef and therefore could affect the formation of the flavour volatiles (Koutsidis *et al*, 2008). The aim of this experiment is to identify, through external preference mapping, the beef eating quality attributes driving the preference of consumers and relate these attributes to instrumental analyses of tenderness and flavour precursors.

**Materials and methods** Twenty eight cattle from 14 treatments were selected with the intention of achieving a wide range of eating quality attributes. The pre-slaughter factors which varied were age, breed, sex and diet and the post-slaughter factors were ageing time, electrical stimulation and hanging method. The animals were slaughtered on four different days at a commercial abattoir. The carcasses were stored at 10°C for 10 hours and then the air temperature dropped to 2°C for the next 38 hrs. The carcasses were boned out 48 hours after slaughter and the *longissimus dorsi* was removed, vacuum packed and aged for the time assigned to each group. The muscle was cut into samples for profiling panels, consumer panels, Warner Bratzler shear force (WBSF), fatty acids profile, reducing sugars (glucose, ribose, glucose-6-phosphate and ribose-5-phosphate) and nucleotides (AMP, IMP, inosine and hypoxanthine). During profiling panels, trained panellists assessed *l. dorsi* grilled steaks from the 14 treatments. The data obtained was analyzed by ANOVA and principal components analysis and the 7 most different treatments were selected for consumer panels. These treatments were presented to 120 consumers to determine scores for the liking of aroma, flavour, tenderness, juiciness and overall liking in a line scale between dislike extremely to like extremely (0 and 100). External preference mapping, using a vector model was conducted using sensory profiling data, consumer preference data and instrumental data. Direct correlations were also conducted between the attribute descriptor and instrumental analysis.

T1, T3, T4, T8, T11, T13, T15 = treatments; L=liking of attribute

**Figure 1** External preference mapping of *longissimus dorsi* grilled beef steaks for overall liking PC1/PC2 (83% Variation)



**Results** Overall liking of the consumers in the external preference map showed association with the descriptors, “sweet flavour”, “juiciness” and “tender texture”; in the opposite direction were found “rubbery”, “stringy”, “chewy texture,” “sour” flavour and aftertaste and “cardboard aftertaste” (Figure 1). Overall liking is also related to components such as ribose, hypoxanthine, AMP, glucose and less closely to ribose-5-phosphate and glucose-6-phosphate. IMP is in opposite direction to the consumers’ preferences. When direct correlations were conducted between the attributes and the flavour precursors, “juicy texture” was positively correlated ( $P<0.05$ ) with ribose ( $r=0.97$ ) and negatively with IMP ( $r=-0.85$ ). The polyunsaturated fatty acids (PUFA), omega 3 and 6 fatty acids (n-3 and n-6) and linoleic

acid (C18:2) are situated opposite to consumer liking. These fatty acids appear related to the descriptors of “sour” flavour and aftertaste, “cardboard aftertaste” and “oily” appearance. PUFAs were positively correlated ( $P<0.05$ ) with “sour flavour” ( $r =0.78$ ) and “sour aftertaste” ( $r =0.77$ ) and negatively correlated with “sweet flavour” ( $r =-0.59$ ). Total fat is correlated ( $P<0.05$ ) with the attributes, “fatty flavour” ( $r=0.72$ ) and “fatty aftertaste” ( $r =0.80$ ). These attributes seem not to be associated with overall liking and, as expected, is related to the texture descriptors of “rubbery”, “chewy” and “stringy”.

**Conclusions** The technique of external preference mapping demonstrates relationships between the overall liking of consumers, specific flavour and texture attributes and instrumental measurements of flavour and texture.

**Acknowledgments** The authors gratefully acknowledge funding from Dunbia

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## Effect of lipid-rich plant extract on the fatty acids composition and meat quality of Belgian-Blue cross bred steers

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**Introduction** We have previously demonstrated the ability of a lipid-rich plant extract to enhance levels of polyunsaturated fatty acid (PUFA) in beef (Kim *et al.*, 2008). The plant extract (PX) is developed from the liquid fraction extracted from fresh lucerne (*Medicago sativa* L.), and then heat-treated and dried. The PX has a high degree of rumen protection. This study investigated the effect of incremental inclusion of PX in steers fed on grass silage relative to concentrate on the fatty acid composition of beef and meat quality. Effects of additional vitamin E within the concentrate were also investigated.

**Materials and methods** Following summer grazing on perennial ryegrass/white clover swards, forty Belgian-Blue × Holstein steers (~ 400 kg liveweight) were allocated to one of five dietary treatments: 1) grass silage *ad libitum* (GS), 2) grass silage *ad libitum* plus 75 g PX/dry matter intake (DMI; GS-LPX), 3) grass silage *ad libitum* plus 150 g PX/DMI (GS-HPX), 4) restricted barley straw and control concentrate (40:60 on a DM basis; S-CC), and 5) restricted barley straw and concentrate with PX (25% in concentrate on a DM basis) (40:60 on a DM basis; S-PXC) and additional vitamin E (~ 300 mg/kg). The PX was manufactured by Désialis–France Luzerne, France. Liveweight was monitored every 28 days and the liveweight gain was used to modify feed intake of the S-CC and S-PXC animals to achieve a similar growth rate to those fed on *ad libitum* forage. Animals were slaughtered when they achieved fat class 3 and samples of *longissimus thoracis et lumborum* were taken at 48 h post-mortem for fatty acid analysis, vitamin E analysis, 10-d aged samples for shelf life studies in modified atmosphere packs. An analysis of variance was conducted with diet as the main factor using GenStat (11th edition) statistical software.

**Results** Liveweight gain, conformation score and fatness score were similar across diets averaging 1.1 kg/d, 76.7 and 56.5, respectively. Small differences in carcass weight were noted (Table 1). Total fatty acids and amounts of the major saturated fatty acids were not different (Table 1). Feeding grass silage relative to concentrate increased deposition of n-3 relative to n-6 PUFA. Incremental PX on grass silage resulted in additional deposition of 18:3n-3 (and 18:2n-6) and longer chain derivatives EPA and DHA resulting in improvements in P:S and n-6:n-3 ratio. Additional vitamin E in the diet of S-PXC increased its content in muscle impacting on lipid stability (TBARS).

**Table 1** Animal performance, fatty acid composition (mg/100 g muscle) of *longissimus thoracis et lumborum*, and TBARS and vitamin E content of muscle in Belgian-Blue cross bred steers given experimental diets

	GS	GS-LPX	GS-HPX	S-CC	S-PXC	s.e.d.	P
Right-side cold carcass (kg)	169 <sup>ab</sup>	174 <sup>b</sup>	170 <sup>ab</sup>	177 <sup>b</sup>	165 <sup>a</sup>	3.8	0.032
Total fatty acids	2551	2510	2433	2532	1999	379.3	NS
16:0	665	623	596	654	488	107.6	NS
18:0	325	332	339	344	274	57.2	NS
18:1n-9	880	850	794	825	581	134.8	NS
CLA ( <i>cis</i> -9, <i>trans</i> -11)	12.9	14.4	14.1	14.7	13.4	2.46	NS
18:2n-6	56.7 <sup>a</sup>	70.2 <sup>ab</sup>	72.7 <sup>b</sup>	121.3 <sup>c</sup>	134.5 <sup>c</sup>	7.37	<0.001
18:3n-3	26.9 <sup>a</sup>	38.3 <sup>b</sup>	41.3 <sup>b</sup>	13.8 <sup>a</sup>	35.7 <sup>b</sup>	3.05	<0.001
20:5n-3 (EPA)	14.3 <sup>bc</sup>	16.1 <sup>cd</sup>	18.3 <sup>d</sup>	9.8 <sup>a</sup>	12.3 <sup>b</sup>	0.79	<0.001
22:6n-3 (DHA)	2.42 <sup>bc</sup>	2.28 <sup>b</sup>	2.77 <sup>c</sup>	1.58 <sup>a</sup>	1.63 <sup>a</sup>	0.176	<0.001
P:S ratio	0.08 <sup>a</sup>	0.11 <sup>ab</sup>	0.13 <sup>b</sup>	0.13 <sup>b</sup>	0.23 <sup>c</sup>	0.018	<0.001
n-6:n-3 ratio	1.83 <sup>b</sup>	1.69 <sup>ab</sup>	1.62 <sup>a</sup>	6.28 <sup>c</sup>	3.36 <sup>c</sup>	0.049*	<0.001
Vitamin E (mg/kg muscle)	3.86 <sup>ab</sup>	4.41 <sup>b</sup>	4.16 <sup>b</sup>	3.17 <sup>a</sup>	7.75 <sup>c</sup>	0.373	<0.001
TBARS, d10 (mg/kg muscle)	0.59 <sup>ab</sup>	0.79 <sup>b</sup>	1.41 <sup>c</sup>	1.39 <sup>c</sup>	0.28 <sup>a</sup>	0.236	<0.001

TBARS=Thiobarbituric acid reactive substances; \*on log scale; Means within a row with different superscripts differ (P<0.05).

**Conclusions** Feeding incremental PX to grass silage-fed animals resulted in enhancement of 18:3n-3 and increased the longer chain derivatives EPA and DHA, resulting in improved P:S and n-6:n-3 ratios. Within the concentrate treatments PX also increased 18:3n-3 and EPA. The highest P:S ratios were noted on S-PXC treatment reflecting the high levels of 18:2n-6 and 18:3n-3 deposited. Under these circumstances the additional vitamin E fed helped to control oxidative stability as reflected in the lower TBARS.

**Acknowledgements** This work was supported by DEFRA, EBLEX, HCC, LMC and QMS, UK (“Probeef”) and European Commission ProSafeBeef (FOOD-CT-2006-36241). The PX was kindly provided by Désialis–France Luzerne.

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## The potential of a plant extract to provide fluorescent chlorophyll derivatives to act as markers of faecal contamination on carcasses

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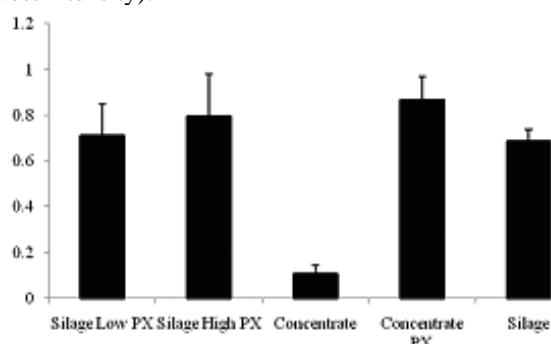
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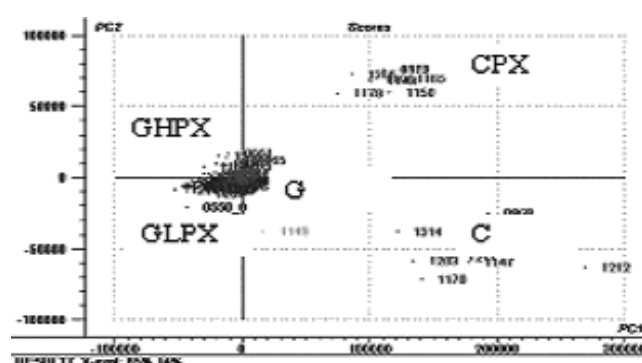
**Introduction** Cleanliness in the abattoir is of the utmost importance and strategies are carried out on farm to ensure that the animals arrive at the abattoir with limited faecal matter clinging to the hide. Currently carcasses are checked by 'eye' and trimmed to remove contaminated areas. Small areas of faecal contamination may not be visible to the eye and may harbour millions of pathogenic bacteria. Spectroscopic imaging is a rapidly evolving research area, with the potential to provide real-time solutions for the detection of faecal contamination on carcasses (Ashby *et al.* 2007). Chlorophyll is ubiquitous in green plants and thus grazing diets. During digestion in the gut, chlorophyll is only partially degraded to coloured and fluorescent intermediates: the phaeophytin, chlorophyllide, phaeophorbide and pyropheophorbide derivatives of chlorophylls a and b (Lee *et al.* 2009). This study assessed the potential of a chlorophyll containing feed (PX - an extract from lucerne; *Medicago sativa*) to provide fluorescent markers in the faeces which could then be used for on-line detection in the abattoir.

**Materials and methods** Forty-four Belgium-Blue steers were maintained on grass silage until the end of April 2008 and then at pasture until housing in October 2008. Animals were then allocated to 1 of 5 treatments: grass silage (G); grass silage + 75g/kg silage DM intake of PX (lucerne extract, Desialis, France; GLPX); grass silage + 150 g/kg silage DM intake PX (GHPX); straw + standard concentrate (C); straw + PX-concentrate (22% PX) (CPX). Straw and grass silage were offered *ad libitum* and concentrate was fed at a rate of 8 kg DM/d. Animals were kept on treatment for 16 weeks with feed samples taken daily and bulked per week. Before slaughter faecal samples were collected. Chlorophyll catabolites were determined by HPLC in the feed and faeces and analysed using a general ANOVA. Fluorescence emission spectra were measured directly on the faeces. The fluorescence emission spectra were measured with excitation at 382 and 430 nm, using an optical bench system. The spectra were collected by an imaging spectrograph (Acton SP-150, Acton Research Corporation, Acton, MA) connected to a sensitive charge coupled device (CCD-camera) (Roper Scientific NTE/CCD-1340/400-EMB, Roper Scientific, Trenton, NJ). Cut-off filters at 400 nm (for the 382 nm excitation) (Melles Griot 03FCG049) and 475 nm (for the 430 nm excitation) (Melles Griot 03FCG068) were positioned in front of the spectrograph slit to suppress excitation light reflected from the samples. Exposure time was 10 and 5 sec for excitation at 382 and 430 nm, respectively. The temperature of the samples was 4 °C. All the samples were measured twice and an average was used in the analysis. The resulting spectra were analyzed using PCA (Principal Component Analysis) and catabolite concentration using general ANOVA.

**Results** Intake of chlorophyll and its catabolites was different ( $P < 0.001$ ) across the 5 treatments: 12.9, 15.3, 0.56, 24.1 and 10.4 g/d for GLPX, GHPX, C, CPX and G, respectively. This corresponds with the levels of chlorophyll and its catabolites in faeces (Figure 1), where CPX was higher than C and G ( $P < 0.05$ ) but there was no difference between silage based treatments. These results are confirmed in the PCA cluster analysis for the fluorescent spectra emitted from the faeces which showed no difference between GHPX, GLPX and G but with clear separation from CPX (highest intensity) and C (lowest intensity).



**Figure 1** Concentration (mg/g DM) of chlorophyll catabolites in faeces



**Figure 2** PCA cluster analysis of faecal fluorescent spectra

**Conclusions** Inclusion of PX within a concentrate significantly increased the level of chlorophyll catabolites in faeces but had little effect increasing the levels when animals were offered silage. This suggests potential for its use to increase fluorescent intensity in concentrate finishing systems making it easier to detect small traces of faecal contamination on carcasses in the abattoir and thereby improve product safety.

**Acknowledgements** The authors gratefully acknowledge funding from an EU sixth framework programme ProSafeBeef.

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## Influence of season and finishing diet on the fatty acid composition of beef longissimus dorsi muscle

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**Introduction** The fatty acid (FA) composition of ruminant meat is influenced by many aspects of the production system including the animal finishing diet. Beef produced from grass-fed animals contains greater concentrations of the beneficial long-chain (LC) n-3 polyunsaturated fatty acids (PUFA) than beef produced from concentrate-fed animals (French *et al*, 2000). Since the animal diet is likely to vary over the farming year (McAfee *et al*, 2009), it is possible that the season of animal slaughter will also influence quantities of LCn-3 PUFA found in meat and subsequently available for human consumption. The aim of this study was to determine the FA composition of beef produced in Northern Ireland, examining the effects of both season of slaughter and reported finishing diet.

**Methods** Samples of beef longissimus dorsi (LD) muscle (n= 234) were collected fortnightly from a commercial abattoir over a 12 month period. Producers were identified and contacted to obtain information on whether the finishing diet provided in the month prior to slaughter was grass, concentrates, grass supplemented with concentrates or silage supplemented with concentrates. Total lipid was extracted from lean tissue according to the Folch method (Folch *et al*, 1957) and FA methyl esters were analysed using gas chromatography. Principle components analysis (PCA) was performed on the proportional FA data (% w/w) to investigate the influence of season and reported finishing system on FA profiles. To analyse for the effect of season and diet, a factorial ANOVA (SPSS v. 11.5) was used including season and diet as well as their interaction as fixed effects. Bonferroni post hoc test was used to adjust for multiple comparisons. Seasons defined as Spring: March – May; Summer: June-August; Autumn: September – November; Winter: December – February.

**Results** The intramuscular fat content of the LD muscle was not significantly affected by season. Beef cattle finished in autumn had significantly higher concentrations of alpha-linolenic acid (C18:3n-3), eicosapentaenoic acid (C20:5n-3), docosapentaenoic acid (C22:5n-3) and total conjugated linoleic acid (CLA) ( $P<0.01$ ) than cattle finished in other seasons. There were significant interactions between season and diet for C22:5n-3 ( $P<0.01$ ), LCn-3 PUFA ( $P<0.01$ ) and total n-3 PUFA ( $P<0.05$ ) in beef samples from animals reportedly finished on grass in autumn. This interaction showed that in autumn animals that were reportedly finished on grass had higher proportions of these FA compared to those finished on concentrates alone or concentrates plus forage.

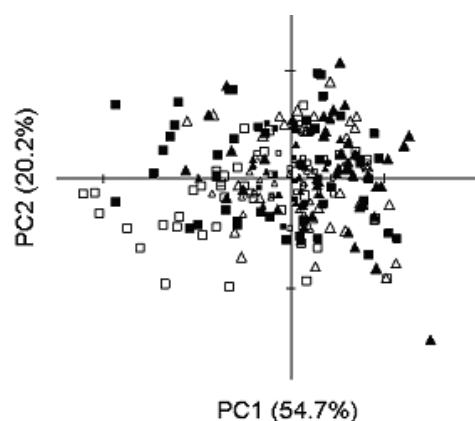
Two components in the PCA analysis explained 75% of total FA variation (PC1 55%, PC2 20%). The PCA scores plot (Fig 1) showed that samples of LD muscle from beef produced in autumn (□) were grouped mainly in the lower left quadrant. The loadings plot showed that autumn produced beef was associated with higher levels of stearic acid C18:0), linoleic acid (C18:2n-6), C18:3n-3, arachidonic acid (C20:4n-6), C20:5n-3 and C22:5n-3. There is no clear separation between the other seasons on the PCA scores plot (Figure 1).

**Conclusions** Results of this study provide evidence that there is seasonal variation in the concentration of a number of FA in beef produced under a range of commercial production systems. The higher concentrations of LCn-3 PUFA and total CLA in autumn-produced beef may have potential benefits for consumer health. Further research is needed however, to determine the time course of these changes in order to optimise conversion of the C18:3n-3 from grass to LCn-3 PUFA in the beef muscle.

**Acknowledgements** The authors gratefully acknowledge funding from LMC, AgriSearch and DEL Co-operative Award in Science and Technology (CAST).

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**Figure 1** PCA scores plot for FA analysis.  $\Delta$  Spring;  $\blacktriangle$  Summer;  $\square$  Autumn;  $\blacksquare$  Winter

## Genetic relationships between muscle density, measured by X-ray computed tomography (CT), and lamb growth and carcass traits

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**Introduction** With successful selection for leanness and muscularity in different sheep breeds, care must be taken to ensure that carcass composition is not changed in a way that is detrimental to meat quality. Recent research in different sheep breeds has found that average muscle density (MD) measured *in-vivo* in cross-sectional CT scans has strong negative genetic and phenotypic correlations with intramuscular fat (IMF), as well as taste panel scores for flavour, juiciness and palatability (Karamichou *et al.*, 2006, Navajas, 2008). Therefore, CT provides the means to quantify IMF (and potentially other meat quality traits), at the same time as carcass fat and muscle, in live lambs, which could be exploited in selection programmes. Genetic parameters are required for MD, including estimates of correlations with other growth and carcass traits, before its inclusion in breeding programmes can be assessed.

**Materials and methods** Performance and pedigree data were available from Scottish Blackface lambs from two hill farms of contrasting severity, giving a total of approximately 16800 lamb records collected over 15 years. At weaning, live weight (WWT) was recorded, as well as ultrasonically-measured (US) fat depth (UFD) and muscle depth (UMD). A sample of lambs from each farm was CT scanned at weaning in four years, giving a total of 844 lambs scanned. Total weights of carcass fat (CFAT), muscle (MUSC), internal fat (IFAT) and bone (BONE) were predicted from CT scans (Lambe *et al.*, 2006). In the hind leg and loin regions of the body, muscle volume (HLMV and LRMV, respectively) and muscularity indices (HLMi and LRMI, respectively) were calculated (Navajas *et al.*, 2007). MD was calculated in three cross-sectional reference scans, taken at the leg, loin, and chest, and averaged (MD in each scan weighted by proportion of muscle area in that scan across the data set – i.e.  $ISCMD \times 0.5$ ,  $LV5MD \times 0.27$ ,  $TV8MD \times 0.23 = AVMD$ ). Surplus lambs were slaughtered when they reached commercial slaughter criteria (minimum live weight ~37.5kg and condition score 3). Carcass records (~7300 in total) included hot carcass weight (CWT) and MLC classification scores for fatness (MLCF; scored on a 7 point scale and transformed to estimated subcutaneous fat percentage) and conformation (MLCC; scored on a 5 point scale). Using ASREML (Gilmour *et al.*, 2001), the heritability of AVMD was estimated using a univariate analysis, whilst genetic ( $r_g$ ) and phenotypic ( $r_p$ ) correlations between AVMD and other lamb traits were estimated using bivariate analyses. For each trait, a model was fitted that included random effects of animal and maternal permanent environment and fixed effects of age, farm, year, sex, litter size at 1 week, dam age, grazing area, and interactions of farm with year and sex.

**Results** AVMD was moderately heritable ( $h^2 = 0.30$ , s.e. 0.09). Phenotypic correlations (Table 1) with weights and carcass traits measured *in-vivo* were low to moderate and negative, suggesting that larger lambs with more fat and muscle and greater muscularity had less dense muscle (linked to better meat quality). However, only genetic correlations with UFD and CFAT were significantly different from zero, suggesting a negative genetic association of MD with fatness, but no significant association with muscling or muscularity. Neither  $r_g$  (range: -0.03 to -0.08) nor  $r_p$  (range: 0.01 to 0.04) with carcass traits (CWT, MLCC, MLCF) differed significantly from zero.

**Conclusions** The results suggest that inclusion of MD as a predictor of IMF, or other meat quality traits, in sheep breeding programmes would not be antagonistic with breeding goals aimed at increasing muscling, muscularity or growth. Unfavourable genetic associations with fat measurements support the case for including predictors of both carcass fat and IMF in breeding programmes, to optimise carcass and meat quality simultaneously. Similar results were reported by Navajas (2008) in the Texel breed, although further larger studies in terminal sire breeds would be relevant.

**Acknowledgements** Financial support for this research was received from the Scottish Government and Defra. The authors are grateful to staff at SAC Kirkton and Castlelaw farms and the CT unit for data collection.

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**Table 1** Genetic ( $r_g$ ) and phenotypic ( $r_p$ ) correlations (and standard errors in parenthesis) between AVMD and other lamb growth, US and CT traits.

	Trait	$r_g$	$r_p$
Live weight	WWT	-0.06 (0.17)	<b>-0.27</b> (0.04)
US traits	UMD	0.11 (0.16)	<b>-0.11</b> (0.04)
	UFD	<b>-0.38</b> (0.14)	<b>-0.34</b> (0.03)
CT traits	MUSC	-0.02 (0.23)	<b>-0.22</b> (0.04)
	CFAT	<b>-0.57</b> (0.17)	<b>-0.47</b> (0.03)
	IFAT	0.07 (0.40)	<b>-0.24</b> (0.03)
	BONE	0.03 (0.24)	<b>-0.14</b> (0.04)
	HLMV	-0.01 (0.24)	<b>-0.21</b> (0.04)
	LRMV	-0.11 (0.30)	<b>-0.20</b> (0.04)
	HLMi	0.12 (0.19)	<b>-0.16</b> (0.04)
	LRMI	-0.09 (0.24)	<b>-0.16</b> (0.04)

Values shown in bold are significantly different from zero

## Effect of chicory grazing on killing out percentage and meat eating quality in lambs

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**Introduction** A growing body of evidence shows that lambs grazing pure stands chicory grow faster, and thus finish earlier in the season, and have lower levels of parasitism (Athanasiadou *et al.*, 2007; Kidane *et al.*, 2009). However, it is not known if chicory affects meat eating quality, although carcasses may be leaner when market weights are reached, compared to a more slowly finishing on grass/clover mixtures. Dietary influences on meat quality should be considered as these are very relevant for consumer acceptance. Here, we assessed the effect of chicory grazing on killing out percentage and meat eating quality in lambs.

**Materials and methods** Lambs grazed pure stands of chicory (CH) or grass/clover (GC) from turn-out at ~32 days of age for ~20 weeks. Body weight (BW) taken just before slaughter and carcass weights (CW) defined killing out percentage as (CW/BW)\*100%. Carcasses were hip-suspended at 2°C for 24 h before posterior 20 cm of left loins were retrieved, matured at 2°C for another 9 days in vacuum bags and frozen pending sensory quality assessment at University of Bristol. Loins were thawed overnight, de-boned on the day of assessment, cut in 8-10 2-cm thick samples and cooked until internal temperature reached 75°C. Samples were placed in an incubator (60 °C) prior to sampling by 10 qualified assessors, who were asked to rate 8 point category scales for tenderness, juiciness, lamb flavour intensity, abnormal lamb flavour intensity and two hedonic scales for flavour liking and overall liking. In addition, a thirteen descriptor flavour profile, using unstructured 100 mm intensity scales, was also used (0: nil intensity; 100: extreme intensity). Lambs were allocated to different sub-panels by sex. Reported results were derived from an ANOVA using REML.

**Results** CH and GC lambs weighed 39.1 and 36.4 kg (s.e.d. 1.35 kg; P=0.051) and killing out percentages were 39.9 and 37.2% (s.e.d. 1.00; P=0.01), respectively. Table 1 shows sensory results using data pooled across panels. Female CH lambs had juicier loins than GC lambs with reduced grassy flavour. However, in some sub-panels, effects on juiciness were stronger (5.2 vs 4.7; s.e.d. 0.36; P<0.001) and CH loins were scored more tender than GC loins (5.6 vs 5.1; s.e.d. 0.37; P<0.01) with a higher livery flavour (11.8 vs 5.4; s.e.d. 7.0; P=0.050). In the pooled data, no effects were observed for castrated lambs, although some sub-panels scored CH loins as higher acidic (8.3 vs 3.6; s.e.d. 4.1; P<0.05), rancidness (5.0 vs 1.0; s.e.d. 3.2; P<0.05), more tender (6.2 vs 5.0; s.e.d. 0.83; P<0.05) and with greater lamb flavour (4.5 vs 3.3; s.e.d. 0.79; P<0.01) than GC loins. Other descriptors (fatty/greasy, kidney, bitter, sweet, ammonia, fishy, soapy and dairy) and hedonic flavour and overall liking did not differ between CH and GC lambs, although in one sub-panel overall liking of CH loins was scored higher than GC loins (4.6 vs 3.5; s.e.d. 0.82; P<0.05).

**Table 1** Effect of forage type on sensory quality of loins from female and castrated lambs

	Female lambs				Castrated lambs			
	Chicory	Control	s.e.d.	P-value	Chicory	Control	s.e.d.	P-value
<i>8 point scale used</i>								
Texture	5.5	5.2	0.25	NS	5.2	5.5	0.20	NS
Juiciness	5.2	4.9	0.14	0.025	5.0	4.9	0.14	NS
Lamb flavour	4.2	4.4	0.30	NS	4.1	4.2	0.19	NS
Abnormal flavour	2.4	2.0	0.28	NS	2.5	2.4	0.15	NS
<i>Hedonic</i>								
Flavour liking	5.1	5.2	0.29	NS	4.8	4.8	0.19	NS
Overall liking	5.1	5.1	0.27	NS	4.7	4.7	0.20	NS
<i>100 mm line scale used</i>								
Livery	11.2	7.2	3.01	NS	9.9	10.5	1.88	NS
Rancid	0.7	0.5	0.55	NS	2.8	1.1	1.63	NS
Acidic	6.6	4.0	2.03	NS	6.7	4.2	1.65	NS
Grassy	6.2	10.6	2.37	0.077	9.9	9.1	1.64	NS

**Conclusion** These results suggest that grazing on chicory produces heavier carcasses with better killing out percentage, and increased loin juiciness, although the latter in female lambs only. Beneficial sensory scores for eating quality were given to chicory-reared lamb in several sub-sets but were cancelled out when data was pooled, suggesting the need for relatively large numbers of observations to avoid drawing invalid conclusions. Although it can not be excluded that sex-specific effects observed in this study would not be present had lambs been finished to commercial standards, overall the data suggest that using chicory as an alternative crop for finishing lambs is expected to yield higher carcass weights without detrimental effects on meat eating quality.

**Acknowledgements** This work is funded through Quality Meat Scotland, Meat Promotion Wales, English Beef and Lamb Executive, and Livestock and Meat Commission. SAC receives support from Scottish Government.

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## Effects of silage type and proportion in the diet on the growth and carcass characteristics of finishing lambs

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**Introduction** Within sheep systems in the UK and Ireland it is common for hill lambs to be housed late in the season and finished on high grain diets due to inadequate supplies of grass. However, due to a number of factors, concentrate feed costs have increased significantly in recent years so there is a need to investigate lower cost alternatives. Grass silage-based feeding systems for lambs have been studied extensively and are capable of sustaining good growth rates (>200 g/d) when supplemented with concentrates (Carson *et al.*, 2001). Forage maize has increased in popularity over the past 10 years and offers some opportunities to reduce forage costs on mixed beef/sheep farms, with the potential for high dry-matter yields (comparable to a 3-cut silage system) of high quality material from a single harvest (Keady *et al.*, 2008). However there is limited information on supplementation strategies for lambs offered maize silage. The aims of this study were to investigate the performance and carcass characteristics of lambs finished on grass silage or maize silage at two contrasting levels of concentrates.

**Materials and methods** Sixty crossbred lambs (48 females, 12 males) of mixed breeds, with a mean age of  $217 \pm 9.3$  days and a mean live weight of  $35 \pm 0.5$  kg, were allocated to four groups ( $n = 15$ ) balanced for sex, age and sire breed. Lambs were housed in groups of six and offered *ad libitum* grass silage (G) or maize silage (M) plus concentrates. Concentrates were offered to achieve (on a dry-matter basis) a HIGH (0.80, H) or LOW (0.50, L) proportion of forage in the diet, giving a total of 4 treatments: GH, GL, MH and ML. Daily concentrate allocations were estimated from the previous day's silage DM intake and were offered in two equal sized meals at 0930 and 1600 h. The grass silage was predicted by Near Infrared Reflectance Spectroscopy to supply 291 g DM/kg, 655 g/kg digestible organic matter/kg DM, 10.5 MJ ME/kg DM and 140 g crude-protein/kg DM. Maize silage was predicted to supply 346 g DM/kg, 11.3 MJ ME/kg DM, 87 g CP/kg DM and 293 g starch/kg DM. Concentrates fed to lambs offered grass silage and maize silage were formulated to supply 167 and 214 g CP/kg DM respectively. Silage was offered fresh daily at 0930 h while the concentrates were offered in two equal size meals at 1000 h and 1630 h. Intake of silage and supplement were recorded daily. Lambs were weighed fortnightly until they reached their target slaughter weight of 44 kg. Cold carcass weight, dressing proportion and grade were recorded at the point of slaughter. Carcass conformation was scored on a 5-point scale using the EUROP classification system (E=5, P=1) while carcass fatness was scored on a 6-point scale using the Livestock and Meat Commission (NI) classification system (1=1, 2=2, 3=3, 4L=4, 4H=4.5 and 5=5). Data were analysed in a 2 X 2 factorial arrangement (Forage type X Proportion) using Residual Maximum Likelihood (REML) analysis with covariates included for carcass weight, sire breed and sex.

**Results** Intake of maize silage was 17% higher than grass silage for lambs on the HIGH forage diet, and 14% higher on the LOW forage diet ( $P < 0.05$ ). Overall, total DM intake was increased by 15% when lambs were offered maize silage rather than grass silage ( $P < 0.001$ ). Consequently, daily live weight gain was 34 g/d higher for maize silage-fed lambs ( $P < 0.001$ ). Reducing the forage proportion from 0.80 to 0.50 decreased the intake of grass silage and maize silage by 17% and 14% respectively, but increased total DM intake by 32% ( $P < 0.001$ ) and average daily live weight gain by 53 g/d. Finishing diet had no effect on carcass fatness; however with grass silage-fed lambs only, carcass conformation increased ( $P < 0.05$ ) and dressing proportion tended to increase ( $P = 0.12$ ) as the proportion of forage in the diet increased.

**Table 1** Effects of silage type and proportion in the diet on the performance of finishing lambs

Silage type Silage proportion in the diet	Grass		Maize		s.e.d	Silage	Proportion	Silage X Proportion
	HIGH	LOW	HIGH	LOW				
Silage DM intake (kg/d)	0.66 <sup>b</sup>	0.56 <sup>a</sup>	0.77 <sup>c</sup>	0.64 <sup>b</sup>	0.010	***	***	*
Concentrate DM intake (kg/d)	0.16 <sup>a</sup>	0.53 <sup>c</sup>	0.19 <sup>b</sup>	0.60 <sup>d</sup>	0.007	***	***	***
Total DM intake (kg/d)	0.82 <sup>a</sup>	1.09 <sup>c</sup>	0.96 <sup>b</sup>	1.24 <sup>d</sup>	0.017	***	***	NS
Daily LWG (g/d)	79 <sup>a</sup>	149 <sup>b</sup>	129 <sup>b</sup>	166 <sup>b</sup>	20.2	***	**	NS
Conformation score	3.19 <sup>b</sup>	2.85 <sup>a</sup>	3.02 <sup>ab</sup>	3.12 <sup>ab</sup>	0.156	NS	NS	*
Fat score	2.94	2.96	3.00	2.97	0.234	NS	NS	NS
Dressing proportion	0.465 <sup>b</sup>	0.445 <sup>a</sup>	0.445 <sup>a</sup>	0.445 <sup>a</sup>	0.0088	NS	NS	P=0.12

HIGH, 80% silage on a dry-matter basis; LOW, 50% silage on a dry-matter basis; DM, dry-matter; LWG, live weight gain; Means sharing the same superscript are not statistically significant ( $P > 0.05$ )

**Conclusion** The results of this study demonstrate that high quality maize silage is an ideal forage for finishing lambs indoors, achieving higher intake characteristics and increasing daily live weight gain by up to 50 g/d compared to lambs offered medium quality grass silage. However the lamb performance benefits decrease significantly when diets contain high levels of concentrates.

**Acknowledgement:** Funding from the Department of Agriculture and Rural Development is gratefully acknowledged.

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Keady TWJ, Kilpatrick DJ, Mayne CS and Gordon, FJ. 2008. *Livestock Science* 119, 1-11



## Distinguishing between different beef production systems using $\alpha$ -tocopherol, $\beta$ -carotene and lutein measurements in beef

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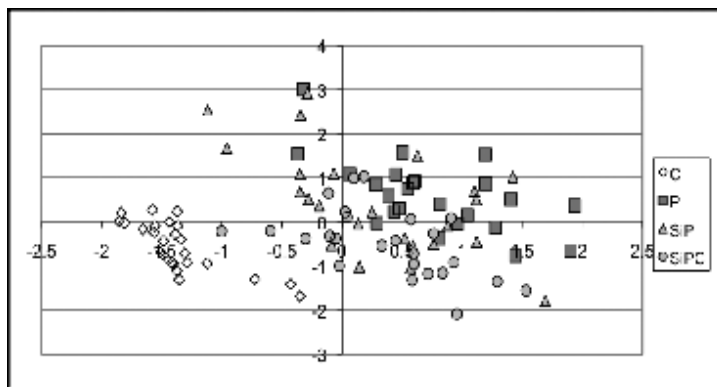
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**Introduction** Food authentication is an issue of increasing interest to consumers and food producers alike amid concerns about the origin of food and, in the case of animal-derived foods such as meat, concerns about its mode of production. Grass-based beef production is of particular interest to consumers because of its perceived healthiness and natural, animal-friendly production. The aim of the research was to determine if  $\alpha$ -tocopherol,  $\beta$ -carotene and lutein measurements in beef could be used to distinguish between different beef production systems.

**Material and methods** Charolais  $\times$  Limousin crossbred heifers were assigned to one of four treatments (25 heifers per treatment): grazed pasture from November 2006 to October 2007 (P); pasture silage offered *ad libitum* indoors from November 2006 to April 2007, then grazed pasture from April to October 2007 (SiP); pasture silage offered *ad libitum* indoors from November 2006 to April 2007, then grazed pasture plus 0.5 of the diet dry matter (DM) as supplementary concentrates from April to October 2007 (SiPC); concentrates and straw indoors from November 2006 to October 2007 (C). Mean ( $\pm$  s.d.) initial live weight was  $275 \pm 27.0$  kg and the mean initial age was  $252 \pm 28$  days. The composition of the concentrate was  $430 \text{ g kg}^{-1}$  rolled barley,  $430 \text{ g kg}^{-1}$  molassed beet pulp,  $80 \text{ g kg}^{-1}$  soybean meal,  $35 \text{ g kg}^{-1}$  molasses,  $20 \text{ g kg}^{-1}$  mineral/vitamin premix and  $5 \text{ g kg}^{-1}$  lime. The concentrate allowance for the C group was adjusted periodically to maintain a similar rate of growth to that the P group. At the end of the experiment the mean ( $\pm$  s.d.) carcass weight was  $275 \pm 21.6$  kg. Muscle (*longissimus dorsi*) and subcutaneous adipose tissue samples were taken for analysis following post-mortem storage of carcasses for 48h at  $4^\circ\text{C}$ . Muscle  $\alpha$ -tocopherol was extracted using a modified method of Buttriss and Diplock (1984) and determined by HPLC using an Agilent 1200 series (Agilent Technologies Inc.) equipped with a variable loop injector and a Synergi Hydro – RP 80A (250 mm  $\times$  4.6 mm),  $4 \mu\text{m}$  particle size column. The mobile phase was methanol-water (99:1, v/v) at a flow rate of  $2 \text{ ml min}^{-1}$  with detection by a diode array detector (Agilent Technologies, 1200 series) set at 292 nm.  $\beta$ -Carotene and lutein in adipose tissue were extracted following the method of Strachan *et al.* (1993) and determined by HPLC with a Phenomenex Jupiter C18 analytical column 300A (250 mm  $\times$  4.6 mm),  $5 \mu\text{m}$  particle size column.  $\beta$ -Carotene and lutein were eluted isocratically with methanol-water (99:1, v/v) at a flow rate of  $2 \text{ ml min}^{-1}$ , and detected at 436 nm and 450 nm, respectively. Statistical analysis of the data was performed using the SPSS software package. Analysis of variance (ANOVA) followed by a post hoc Newman – Keuls multiple comparison test was used to determine if significant differences existed between the P, SiP, SiPC and C groups. Principal component analysis (PCA) was performed and used as a pattern recognition method.

**Results** Mean ( $\pm$  s.d.)  $\alpha$ -tocopherol concentrations of muscle from the P ( $2.63 \pm 0.58 \mu\text{g g}^{-1}$ ) and SiP ( $2.43 \pm 0.86 \mu\text{g g}^{-1}$ ) groups were higher ( $P < 0.05$ ) than those of the SiPC group ( $1.77 \pm 0.54 \mu\text{g g}^{-1}$ ) which, in turn, was higher ( $P < 0.05$ ) than that of the C group ( $1.14 \pm 0.40 \mu\text{g g}^{-1}$ ). The lutein concentration in adipose tissue differed significantly between groups ( $P < 0.05$ ) with values (mean  $\pm$  s.d.) of  $0.13 \pm 0.02 \mu\text{g g}^{-1}$ ,  $0.10 \pm 0.03 \mu\text{g g}^{-1}$ ,  $0.08 \pm 0.03 \mu\text{g g}^{-1}$  and  $0.04 \pm 0.01 \mu\text{g g}^{-1}$  for the P, SiP, SiPC and C groups, respectively. Mean ( $\pm$  s.d.)  $\beta$ -carotene concentrations of adipose tissue from the P ( $0.54 \pm 0.15 \mu\text{g g}^{-1}$ ), SiP ( $0.49 \pm 0.11 \mu\text{g g}^{-1}$ ) and SiPC ( $0.49 \pm 0.11 \mu\text{g g}^{-1}$ ) groups were significantly higher ( $P < 0.05$ ) than those of C group ( $0.09 \pm 0.04 \mu\text{g g}^{-1}$ ). The PCA plot (Figure 1) revealed that it is possible to distinguish between beef from the C group and the other groups.



**Figure 1** PCA score plot of principal component 2 versus principal component 1 for all samples following  $\alpha$ -tocopherol,  $\beta$ -carotene and lutein analysis.

**Conclusions** Following analysis of  $\alpha$ -tocopherol,  $\beta$ -carotene and lutein in beef it is possible to distinguish beef from animals fed a diet based on concentrates and diets containing pasture or pasture/pasture silage/concentrate combinations but not between beef from animals fed the various pasture/pasture silage/concentrate combinations.

**Acknowledgements** The authors gratefully acknowledge funding from the Irish Department of Agriculture, Fisheries and Food under the Food Institutional Research Measure.

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# Instrumental meat quality and fatty acid composition of lean muscle from beef steers offered grass silage alone or in combination with legume/cereal based wholecrop silage at two concentrate levels

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**Introduction** With increasing consumer awareness of meat quality and the relationship between dietary fat and the incidence of cardiovascular related disease, research has been undertaken to manipulate fatty acid (FA) profiles in beef. Scollan *et al.* (2006) found that legume based diets resulted in higher polyunsaturated fatty acid (PUFA) levels in intramuscular fat in beef steers than those offered grass silage. The aim of this experiment was to assess the effect of offering lupins/triticale, lupins/wheat, peas/oat wholecrop silage and grass silage to continental beef cattle on instrumental meat quality and FA composition.

**Materials and methods** Ninety continental cross steers ( $555 \pm 41$  kg) were allocated to one of 10 dietary treatments in a 5 forage x 2 concentrate level experiment. The five forage diets offered were (1) perennial ryegrass based grass silage (PGS), (2) fescue/perennial ryegrass based grass silage (FGS), (3) lupins/triticale based silage combined with PGS 50:50 on a dry matter (DM) basis, (4) lupins/wheat based silage combined with PGS 50:50 on a DM basis, and (5) peas/oat wholecrop silage combined with PGS 50:50 on a DM basis. Each forage diet was offered *ad libitum* and supplemented with either 4 or 7 kg concentrates/head/day. A representation of steers from each forage and concentrate treatment were slaughtered over each of the 4 dates, ranging from 109 to 137 days on trial. Instrumental meat quality assessments (cooking loss, Warner Braztler shear force, meat colour) were carried out 7 days post mortem. FA analyses were undertaken on the *longissimus dorsi* obtained from the fore-rib joint using the direct methyl ester preparation method of O'Fallon *et al.* (2007) and capillary column gas chromatography. FA concentrations were expressed as g FA/100 g total FA. Data were analysed as a 5 forage x 2 concentrate level factorial experiment with genotype, farm of origin and start weight included as covariates using GenStat REML.

**Results** The effect of these treatments on production and carcass characteristics was presented previously by Kennedy and Dawson (2009). Forage offered to continental finishing steers had no effect on FA composition of muscle (Table 1). Increasing concentrate level significantly ( $P < 0.05$ ) decreased concentration of  $\alpha$ -linolenic acid (C18:3n-3) but had no effect on total n-3 PUFA, n-6 PUFA, PUFA:SFA ratio and conjugated linoleic acid (CLA) level in muscle. Forage had no effect on instrumental meat quality. Increasing concentrate level offered from 4 to 7 kg/head/day, increased  $a^*$  and  $b^*$  but had no effect on  $L^*$  of lean meat colour, cooking loss or Warner Braztler shear force (WBSF). An increase in  $b^*$  from lean meat offered a higher concentrate ratio to grass silage is in contrast to previous research (Cooke *et al.* (2004). There were no statistically significant interactions between forage diet and concentrate level.

**Table 1** Effect of forage type and concentrate level on instrumental meat quality and fatty acid composition of muscle (g FA/100 g total FA) from continental steers

	Forage (F)					Concentrate (kg/day) (C)		Significance			
	PGS <sup>1</sup>	FGS <sup>2</sup>	Lupins/triticale <sup>3</sup>	Lupins/wheat <sup>4</sup>	Peas/Oat <sup>5</sup>	sed	4	7	sed	F	C
<i>Fatty acid composition</i>											
n-3 PUFA	0.79	0.96	0.71	0.85	0.85	0.119	0.89	0.77	0.073	NS	NS
n-6 PUFA	2.53	3.13	3.19	3.53	3.20	0.473	3.07	3.16	0.292	NS	NS
C18: 3n-3	0.60	0.74	0.61	0.64	0.60	0.079	0.70	0.58	0.049	NS	*
PUFA:SFA	0.07	0.08	0.08	0.09	0.08	0.012	0.08	0.08	0.008	NS	NS
Total CLA	0.41	0.38	0.34	0.36	0.45	0.065	0.37	0.41	0.040	NS	NS
<i>Instrumental meat quality</i>											
Cooking loss (g/kg)	27.4	26.3	27.6	27.7	27.6	0.62	27.5	27.1	0.39	NS	NS
WBSF (kg/cm <sup>2</sup> ) <sup>γ</sup>	4.88	4.34	4.55	4.79	4.61	0.217	4.51	4.76	0.136	NS	NS
$L^*$ (lightness)	40.5	40.7	39.9	41.7	40.3	1.32	41.2	40.0	0.83	NS	NS
$a^*$ (redness)	25.6	25.6	25.0	24.3	26.0	1.14	24.5	26.1	1.61	NS	*
$b^*$ (yellowness)	17.1	16.9	15.8	16.3	17.6	0.89	16.2	17.3	0.56	NS	*

Treatments 1 to 5 as described in methods, \*  $P < 0.05$ , NS not statistically significant ( $P > 0.05$ )

**Conclusion** Finishing beef cattle on legume/cereal wholecrop silage had no effect on muscle fatty acid composition or instrumental meat quality relative to grass silage based diets. An increased level of concentrate supplementation decreased C18:3n-3 proportion and increased redness ( $a^*$ ) and yellowness ( $b^*$ ) of the meat.

**Acknowledgements** Funding from EBLEX, HYBU CIG CYMRU, QMS, AgriSearch and DARDNI is gratefully acknowledged.

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## Impact of genetic selection on feed efficiency on carcass traits in Irish beef cattle

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**Introduction** Feed is the largest variable cost on farms and there is much interest in selecting for improved feed efficiency (FE). However, prior to recommending selection on any trait, the expected response to selection on other economically important traits needs to be quantified. The objective of this study was to quantify the genetic relationship between feed efficiency and carcass fat (CF), carcass conformation (CC) and carcass weight (CW) in Irish beef cattle.

**Materials and methods** Data for this analysis originated from two separate sources. The first data set consisted of feed intake (FI) and bodyweight (BW) records collected at the Irish bull performance test station. Different measures of FE were subsequently calculated. For example, residual feed intake (RFI) was assumed to represent the residuals from a multiple regression model regressing FI on ADG and BW<sup>0.75</sup> with batch included as a contemporary group (CG) effect. These data were described in full by Crowley *et al.* (2008). The second dataset consisted of information on CC, CF and CW on 2,566,969 animals slaughtered in 30 abattoirs in Ireland during the years 2005 to 2008. In the present study, the EUROP classification grades were transformed to a 15-point linear scale as outlined by Hickey *et al.* (2007), with a carcass fat score of 1 implying low fat coverage and a carcass conformation score of 1 implying poor conformation. Dam lactation number was grouped as 1, 2, 3 to 4 and 5+. Animals with no sire information (n=1,242,138) were discarded. Only males slaughtered between 300 and 1,200 days of age and females slaughtered between 300 and 875 days of age were retained. Only animals with Aberdeen Angus (AA), Belgian Blue (BB), Charolais (CH), Friesian (FR), Hereford (HE), Holstein (HO), Limousin (LM) and Simmental (SI) breed fractions were retained leaving 822,763 records. Additionally, animals where less than 75% of their breed fraction was known were discarded. Carcass weights less than 150 kg and greater than 550 kg (n=11,150) were also omitted. Contemporary group was defined as herd by slaughter date and only CGs with 5 or more records were retained. A total of 386,729 individuals from 36,914 CGs remained. One quarter of CGs were randomly chosen to reduce the dataset size for variance component estimation. Genetic parameters were estimated using a linear mixed animal model in ASREML (Gilmour *et al.*, 2007). Fixed effects included in the model for the carcass traits were CG, gender, non-linear association with age at slaughter, parity of dam, breed proportion, heterosis and recombination loss. An interaction between gender and age at slaughter was also fitted. Fixed effects included in the model for traits measured on the performance tested bulls were CG of test group, parity of dam, breed, age at the end and a quadratic effect of age at the end of test.

**Results and Discussion** Absolute genetic correlations between the performance test measures, excluding FE traits, and the three carcass traits ranged from 0.004 to 0.33 (Table 1). Although standard errors were large, ranging from 0.10 to 0.16, they were considerably smaller than other studies with similar objectives (Nkrumah *et al.*, 2007). Most of the genetic correlations with performance traits estimated in this study were not more than two standard errors from zero with the exception of the correlation between CF and BW; selection for increased BW is expected to have a negative effect on CF. With regard to the feed efficiency traits, CC and CW were both negatively correlated with FCR suggesting selection for improved (i.e., lower) FCR will improve both CC and CW. Furthermore, CF and RFI were correlated indicating that selection for improved (i.e., lower) RFI will yield leaner carcasses. The lack of a genetic correlation between RFI and CW is somewhat expected given that RFI is phenotypically independent of BW.

**Table 1** Genetic correlations of carcass traits with feed intake and weight measurements and measures of feed efficiency

	FI ▼	BW	ADG ▲	FCR ▼	RFI ▼
Carcass Conformation	-0.12 (0.12)	-0.10 (0.11)	0.15 (0.14)	-0.33 (0.14)	-0.19 (0.13)
Carcass Fat	0.01 (0.14)	-0.33 (0.11)	-0.004 (0.16)	-0.02 (0.17)	0.32 (0.14)
Carcass Weight	0.01 (0.11)	0.08 (0.10)	0.19 (0.13)	-0.29 (0.13)	-0.17 (0.12)

▲=Indicates where a (more) positive value for this trait is desirable; ▼= Indicates where a lesser value for this trait is desirable

**Conclusion** Selection for improved feed efficiency will not have any unfavourable repercussions for carcass traits. If anything selection for improved feed efficiency (i.e., lower FCR and lower RFI) will result in larger, leaner carcasses with better conformation. However the standard errors of the genetic correlations were large similar to previous studies, attributable mainly to the relatively small datasets for the FE variables. Therefore, there may be merit in pooling data from international sources to increase the dataset size and potentially generate more precise estimates of genetic parameters.

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## Increasing feed efficiency using genetic improvement and intensive pasture based systems of milk production

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**Introduction** The increased milk production required to meet future global demands will be realised by expansion of dairy herds as well as increased efficiency of milk production both through improvements in management and in genetic merit for feed efficiency. The objective of this study was to investigate the influence of genetic improvement using the Irish total merit index, the economic breeding index (EBI) on feed efficiency within likely future pasture-based production systems under a post EU milk quota scenario.

**Materials and methods** The study was carried out at Moorepark dairy research centre in the Republic of Ireland over a 2 year period (2007 and 2008). Three genetic groups of Holstein-Friesian (HF) dairy cattle were established from within the Moorepark herd: LowNA, national average EBI North American HF (EBI = €49); HighNA, high EBI North American HF (EBI = €75); HighNZ, high EBI New Zealand HF (EBI = €88). Animals were randomly allocated to one of two intensive pasture-based feed systems (FS): 1) the Moorepark pasture (MP) system; 2.64 cows per ha and 344 kg concentrates per cow and 2) a high output per hectare (HC) system, 2.85 cows per ha and 1,056 kg concentrates per cow. Milk yield was recorded daily and milk composition was recorded weekly throughout the study. Animal bodyweight (BW) was recorded weekly with animal body condition score (BCS) measured every three weeks. Individual cow dry matter intake (DMI) was estimated on six occasions each year using the n-alkane technique.

Feed conversion efficiency (FCE) was defined as the kg of milk solids (MS) per cow per day divided by the total DMI of that cow on that day

Residual feed intake (RFI) was defined as:  $RFI_t = DMI_t - (year_y + fat\ yield_t + protein\ yield_t + lactose\ yield_t + BW_t^{0.75} + \Delta BW_t + BCS_t)$ ,

Residual solids production (RSP) was defined as:  $RSP_t = MS\ yield_t - (year_y + TDMI_t + BW_t^{0.75} + \Delta BW_t + BCS_t)$

where t = day post calving; and y = year (1 or 2);  $\Delta BW$  = instantaneous change in BW at day t estimated from the first derivative of a lactation profile and fitted in the model as a piecewise regression to account for the different energy requirements for BW gain compared to energy gained from tissue mobilisation.

The effect of genotype and FS on MS production, DMI and feed efficiency were determined using mixed models (Proc Mixed, Statistical Institute, 2006) with cow included as a random effect. Models included the effects of genotype, FS, year, parity, and their interactions.

**Results and discussion** The effects of genotype and FS on MS production, DMI and efficiency are described in Table 1. The HighNA and HighNZ genotypes produced greater MS yield compared to LowNA animals. The HighNZ animals were lighter but had greater BCS compared to their HighNA and LowNA contemporaries. Animals on the HC FS had greater total DMI across lactation compared to animals on the MP FS. Animals on the HC FS produced greater volumes of MS per day compared to animals on the MP FS.

The ranking of genotypes for feed efficiency was not consistent across the different definitions used. Both HighNA and HighNZ genotypes had superior FCE. However FCE is a poor definition of efficiency in breeding programmes as it is correlated with its component traits and responses to selection can be difficult to predict. The main reason for the inferior RFI of the HighNZ was because they were lighter despite producing more MS than the LowNA with similar intake. The HighNZ animals however had superior RSP. Furthermore, RFI is not correlated with MS production and thus a low (i.e., superior) RFI animal could be low yielding; RSP on the other hand was correlated with MS production implying that superior RSP animals, on averaged, yielded more MS.

**Table 1** The effect of genotype and feed system on production, intake, and feed efficiency of spring calving dairy cows.

	Genotype			Feed System		SE	P-value		
	LowNA	HighNA	HighNZ	MP	HC		G <sup>1</sup>	FS <sup>2</sup>	G X FS
Milk solids (kg/d)	1.43 <sup>a</sup>	1.48 <sup>b</sup>	1.50 <sup>b</sup>	1.39	1.55	0.025	0.02	< 0.001	0.93
Total DMI (kg/d)	16.6	16.6	16.5	15.9	17.2	0.22	0.76	< 0.001	0.50
BW (kg)	531 <sup>a</sup>	541 <sup>a</sup>	506 <sup>b</sup>	525	527	6.8	< 0.001	0.79	0.27
BCS (units)	2.75 <sup>a</sup>	2.82 <sup>ab</sup>	2.91 <sup>b</sup>	2.79	2.86	0.030	< 0.001	0.02	0.73
FCE (g/kg)	87 <sup>a</sup>	90 <sup>b</sup>	91 <sup>b</sup>	87	91	1.2	< 0.001	0.004	0.32
RFI (kg DM/d)	0.14 <sup>a</sup>	-0.23 <sup>b</sup>	0.21 <sup>a</sup>	-0.10	0.53	0.124	< 0.001	0.012	0.35
RSP (kg/d)	-0.04 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	-0.03	0.03	0.017	0.001	0.004	0.27

<sup>a-b</sup> Means within a row with different superscripts differ ( $P < 0.05$ ).

**Conclusions** The results suggest that genetic selection using EBI can significantly increase MS production through improved feed efficiency. The results show that while a number of definitions of feed efficiency exist, conventional definitions such as FCE and RFI may be inappropriate. Residual solids production is an alternative definition of feed efficiency that identifies increased MS production for a given feed intake. However, further genetic analysis needs to be undertaken to determine its association with other traits.

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## Food intake and feeding behaviour of Holstein-Friesian and Jersey x Holstein-Friesian crossbred dairy cows

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**Introduction** The Holstein-Friesian (HF) is the dominant dairy cow breed on Northern Ireland dairy farms, a reflection of the high efficiency of the breed for milk production. However, achieving high levels of food intake within grassland based milk production systems can be challenging, especially when compared with concentrate based production systems. While increased food intakes can be achieved through management strategies, there is anecdotal evidence suggesting some cow genotypes are more suited to forage based systems than others. The current experiment was designed to compare food intake, feeding behaviour and grazing behaviour of HF and Jersey x Holstein-Friesian (JxHF) crossbred dairy cows when offered conserved forage based diets and when grazing.

**Materials and methods** This experiment involved twenty-eight (14 HF and 14 JxHF) primiparous dairy cows (mean calving date, 20 February). At the start of the experiment cows were a mean of 96 days calved, while the HF and JxHF cows had a mean liveweight of 512 and 421 kg, respectively, and a mean daily milk yield of 23.6 and 22.5 kg/day, respectively. During two 10-day indoor periods, cows were maintained in two visually isolated genotype groups and had access to feed via a Calan Gate feeding system. Cows were offered a mixed ration consisting of forage and concentrates (66:34 DM basis) with the forage component of the diet comprising grass silage and maize silage (60:40 DM basis). Refused food was removed at 09:00 and replaced with fresh food each day. In addition, each cow was offered 1.0 kg concentrate/day in the parlour, 0.5 kg during each milking. Food intakes and feeding behaviour of individual cows (feeding time and number of meals) were automatically recorded during the final five days of each period using the Calan gate system. Cows then commenced grazing, with the two genotypes grazing in separate groups, with a minimum distance of 30 m always maintained between groups. A flexible rotational grazing system was adopted, with fresh herbage allocated daily after pm milking. Average pre- and post-grazing sward heights (measured using a rising plate meter) were 9.1 cm (s.d. 1.2) and 5.2 cm (s.d. 1.5), respectively. During the grazing period cows were offered 2.0 kg concentrate/cow/day in the parlour. Cows completed three grazing periods (28, 35 and 28 days in duration). During the final 12 days of each period herbage intake was estimated using the n-alkane technique while grazing behaviour was recorded using grazing behaviour recorders (2 x 24 hour periods/cow). Throughout the experiment milk yield was recorded daily while milk fat and protein concentrations were determined weekly. Data were analysed using repeated measures REML analysis.

**Results** Genotype had no significant effect on milk fat plus protein yield ( $P>0.05$ ), while the HF cows were approximately 75 kg heavier than the JxHF cows ( $P<0.001$ ). When housed indoors, HF cows had a significantly higher dry matter intake than JxHF cows ( $P<0.05$ ), although genotype had no effect on either total feeding time or on the number of meals/day. During the grazing period genotype had no effect on daily dry matter intake, while dry matter intake per kg liveweight<sup>0.75</sup> ( $P<0.05$ ) and time spent grazing ( $P<0.01$ ) were higher in JxHF cows. HF cows had more grazing bouts each day ( $P<0.01$ ).

**Table 1** Effect of cow genotype on mean performance during the experiment and on food intake and feeding behaviour

	Genotype		s.e.d	Sig.
	HF	JxHF		
Cow performance ( study mean)				
Fat + protein yield (kg/day)	1.47	1.42	0.037	NS
Liveweight (kg)	499	424	15.2	***
Indoor period				
Daily dry matter intake (kg/cow)	18.5	17.1	0.67	*
Daily dry matter intake (kg/kg liveweight <sup>0.75</sup> )	0.17	0.18	0.004	NS
Total feeding time (min/day)	248	236	18.0	NS
Number of meals per day	16.1	16.0	1.04	NS
Grazing period				
Daily dry matter intake (kg/cow)	17.0	16.3	0.83	NS
Daily dry matter intake (kg/kg liveweight <sup>0.75</sup> )	0.16	0.17	0.005	*
Total grazing time (min/day)	531	582	18.8	**
Grazing bouts per day	9.3	7.7	0.45	**

**Conclusions** Despite being significantly lighter, JxHF cows produced a similar fat + protein yield as HF cows. Although intakes were higher with the HF cows on the indoor system, none of the behavioural parameters measured were affected by genotype. However, when expressed on a metabolic liveweight basis the higher DM intakes of the JxHF in a grazing system highlighted their greater intake potential compared to HF cows. In addition, JxHF cows appeared to have a higher grazing drive evidenced by their increased grazing time and fewer grazing bouts.

**Acknowledgements** This study was co-funded by DARD and AgriSearch.

## Explaining variation in energy balance using high density SNP information

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**Introduction** Severe negative energy balance (EB) has been shown to have harmful effects on health and fertility. Estimates of heritability and genetic correlations suggest that EB is not only a consequence of a poor match between nutrition and production, but is also genetically induced (Veerkamp *et al.*; 2003, Friggens *et al.*, 2007). The importance of EB to animal breeders comes from the high costs of involuntary culling caused by poor health or failing to establish a successful pregnancy caused by impaired ovarian function or delayed resumption of oestrous cycles. Currently, high density SNP chips are available to investigate the genetics of EB and the use of genomic selection could increase the ability to select for complex traits such as EB. The aim of this study was to demonstrate that high density SNP information can be used to estimate genomic breeding values (GEBV) for genetically induced EB.

**Material and methods** A total of 588 Holstein-Friesian heifers with known pedigree born between 1990 and 1997 were genotyped for the Illumina 50K SNP panel (54,001 SNPs in total). Quality control and pedigree checking was performed and 43,011 SNPs and 548 animals were retained. During the first 15 wk of lactation live weight, feed intake, and milk yield were measured on 527 of these heifers. All heifers were fed *ad libitum*. Fat, protein, and lactose yields were measured at a fixed day of the week. Feed intake was recorded daily using automated feed intake units. EB (MJ/d) was calculated as the difference between energy intake and calculated energy requirements for milk, fat, and protein yields and maintenance costs as a function of live weight. The EB values were averaged across 14 weeks (2-15) to provide the EB phenotype that was used. A 10-fold cross validation approach was carried out, such that the data was randomly partitioned into 10 subsets. Each subset was retained once as the validation dataset and the remaining 9 sets were used to predict the GEBV of those animals in the validation set. The model described in Calus *et al.*, (2008) was used to predict the GEBV. The GEBV were calculated as the sum of the estimated SNP effects and the polygenic effect. The same data subsets and approach were used with a simple polygenic model excluding the SNP information for comparison. The GEBV were assessed using accuracy  $r_{yg}^2$  of the predicted GEBV ( $\hat{g}$ ) when compared with the phenotypes ( $y$ ) and thus the  $r_{yg}^2$ . The accuracy of selection ( $r_{gg}^2$ ) when comparing the true breeding values ( $g$ ) and GEBV has been reported to be a function of the heritability, the number of phenotypic records and the number of effective QTL (Daetwyler *et al.*, 2008 ; Goddard, 2009). This function was adapted for use with the accuracy when comparing phenotypes and GEBV. It was then applied to predict the number of effective QTL for EB and the number of records needed to reach different levels of accuracy of selection.

**Results** The model including the SNP information yielded an overall accuracy of 0.294 and thus an  $r_{yg}^2$  of 0.086 ( $r_{gg}^2=0.265$ ) when comparing the phenotypes and GEBV in the combined validation sets. The maximum  $r_{yg}^2$  that this model could have gained was equal to the heritability which was calculated separately as 0.325. For the model excluding the SNP information with only the polygenic effect an overall accuracy of 0.211 and  $r_{yg}^2$  of 0.044 was found. The effective number of QTL for EB was predicted to be 472 and a total of 5818 records with phenotype and genotype information was predicted as needed for an  $r_{gg}^2$  of 0.80.

**Conclusions.** The use of SNP information confirm the genetic background of EB. Using the SNP information an increase in the accuracy of selection for EB was achieved over the simple polygenic model. Thus, EB could be selected for using genomic selection. The size of the data set directly impacts the expected accuracy and thus an increase in the total number of phenotypic records used would be required to improve the accuracy of selection.

**Acknowledgements** The authors acknowledge funding from SABRETRAIN, Productschap Zuivel, Senter Novem and RobustMilk.

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## Single nucleotide polymorphisms in the bovine Neuropeptide Y5 Receptor gene and their predicted role in physico-chemical characteristics of the receptor protein

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**Introduction** In Ireland, enteric fermentation by ruminants contributes ~14% of the total greenhouse gas emissions. For a 'sustainable beef production system' there is an increasing demand to select livestock species with high feed efficiency and consequently a reduction in enteric methane emissions (Hegarty *et al.*, 2007). Neuropeptide Y (NPY) is a neurotransmitter that regulates appetite and energy homeostasis in animals and humans. The physiological functions of neuropeptide are mediated through a number of membrane bound G-protein coupled receptor (GPCR) molecules. Among the various types of GPCRs, neuropeptide Y5 receptor (NPY5R) is an important molecule which plays vital roles in the feed intake behaviour of animals (Kalra *et al.*, 2007). Single nucleotide polymorphisms (SNP) in the bovine *NPY5R* gene are likely to influence feed intake behaviour in cattle and may act as a potential genetic marker for selection of animals with high feed energy utilization efficiency. The aim of this research was to identify SNPs in the bovine *NPY5R* gene and predict the alteration of functionality of the mutated receptor.

**Materials and methods** Genomic DNA was extracted from blood samples (n=73) of beef cattle: Aberdeen Angus (9); Aubrac (1); Belgian Blue (1); Blonde d'Aquitaine (1); Charolais (11); Hereford (6); Limousin (15); Parthenais (3); Salers (3); Shorthorn (1); Simmental (14); *Bos Indicus* (8). The samples were sourced from the performance trials conducted by the Irish Cattle Breeding Federation, Tully (Co. Kildare) and Teagasc Grange Beef Research Centre (Co. Meath) and *Bos indicus* from India. Two sets of PCR primers were designed to amplify a total length of 2.1 kb of the bovine *NPY5R* gene (GeneID: 781872). Sequencing of the PCR products were performed in both forward and reverse directions. SNPs were identified by multiple sequence alignment, using Molecular Evolutionary Genetics Analysis (MEGA) software. Physico-Chemical properties of the mutated receptor protein were determined using ProtParam software (Gasteiger *et al.*, 2005).

**Results** Based on the alignment of the 2.1 kb sequence, a total of 18 SNPs were identified (Table 1). Of these SNPs, 4 were non-synonymous and 10 were synonymous. Of the total 17 SNPs, 4 were present in the regulatory region (5' UTRs) and 13 in the exonic region which corresponds to the seven transmembrane domain of the receptor molecule. Interestingly, one SNP (G/A) causes an amino acid substitution (M67I) in the first intracellular loop of the receptor molecule. While another two SNPs (C/T, C/T) cause amino acid substitutions at positions 312 and 313, one SNP (C/T) introduces a stop codon that occurs in the third intracellular loop of the 7 transmembrane domain of the Y5 receptor molecule. This stop codon is likely to cause a premature termination of the polypeptide leading to a truncated Y5 receptor protein. The predicted changes in the physico-chemical properties of the Y5 receptor protein (Table 2) suggest important physiological consequences due to the presence of this SNP.

**Table 1** Alleles and functions of the SNPs identified.

SNPs	Function	SNPs	Function
T/C	5' UTR	A/C	Synonymous
G/T	5' UTR	C/T	Synonymous
T/G	5' UTR	C/T	Leu→Phe
C/T	5' UTR	C/T	Pro→Leu
C/T	Synonymous	C/T	Arg→Stop codon
G/A	Met→Ile	C/T	Synonymous
C/T	Synonymous	T/C	Synonymous
G/A	Synonymous	A/G	Synonymous
C/T	Synonymous		

**Table 2** Properties of wild and truncated Y5 receptor protein

Properties	Wild type Y5 receptor	Truncated Y5 receptor
Number of amino acids	446	364
Molecular weight	50775	41282
Theoretical pI	9.19	9.31
Extinction coefficient (M <sup>-1</sup> cm <sup>-1</sup> )	56225	46005
Formula	C <sub>2303</sub> H <sub>3628</sub> N <sub>610</sub> O <sub>620</sub> S <sub>31</sub>	C <sub>1864</sub> H <sub>2950</sub> N <sub>498</sub> O <sub>515</sub> S <sub>22</sub>

**Conclusion** There is high degree of genetic variation (1SNP/123 base) present in the bovine *NPY5R* gene. The SNPs identified in the regulatory and exonic regions of the bovine *NPY5R* gene, specifically those causing amino acid change and premature termination of the Y5 receptor protein and leading to alteration in the physico-chemical properties are likely to play vital physiological roles in the neuropeptide Y mediated energy homeostasis in cattle. Hence, genetic associations of the SNPs identified, with the feed intake traits of the animals is currently being investigated.

**Acknowledgements** This research was supported by a Teagasc Walsh Fellowship.

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## Effect of breed and sire expected progeny difference for carcass weight on the expression of growth related genes in muscle of steers

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**Introduction** Growth rate of bovine skeletal muscle is a trait of economic importance to beef production (Sudre *et al.* 2005). Selective breeding to increase muscle growth rate in cattle would enhance beef production in Ireland. Currently, the Irish Cattle Breeding Federation (ICBF) compiles breeding values for growth in cattle, expressed as expected progeny differences for carcass weight (EPD<sub>cwt</sub>) but this is based on an amalgam of muscle, fat and bone growth. Elucidation of the molecular regulation of muscle growth rate may lead to the identification of molecular markers for growth traits which could subsequently be incorporated into breeding programs. The objective of this study was to examine the effect of breed and sire EPD<sub>cwt</sub> on the expression of growth related genes in muscle of beef cattle.

**Materials and methods** This study utilised muscle samples harvested at slaughter from the study of Campion *et al.* (2009). The animals used were cross-bred Aberdeen Angus (AA; n=16) and Belgian Blue (BB; n=16) steers, born to Holstein Friesian dams and sired by AI bulls with either high (H) or low (L) expected progeny difference for carcass weight (EPD<sub>cwt</sub>). The 32 animals represent the progeny of 16 different sires (AA; n = 7 and BB; n =9). At the end of their 2<sup>nd</sup> grazing season the animals were assigned to one of two mean slaughter weights (SW) viz. 560kg (Light) or 620kg (Heavy), having been blocked within breed and sire EPD<sub>cwt</sub> and balanced for live weight, sire and age. The finishing diet consisted of a total mixed ration with a grass-silage:concentrate ratio of 30:70 on a dry matter basis. Following slaughter, samples of *Longissimus dorsi* muscle were harvested from the animals; snap frozen in liquid nitrogen and stored at -80°C. RNA quantity and quality was determined prior to first strand cDNA synthesis. GAPDH was selected as the most suitable reference gene using GenORM software. Quantitative real time reverse transcription RT-PCR reactions were carried out to measure the relative expression of a number of muscle growth related genes. The software package GenEx 4.2.2 (MultiD Analyses AB) was used for normalisation to the reference gene (GAPDH). Non-normally distributed data were transformed as appropriate by raising to the power of  $\lambda$  (TransReg procedure, SAS, 2001). Data were analysed using mixed models ANOVA (PROC MIXED, SAS 2001). Breed, genetic merit (GM) and slaughter weight were included as fixed effects in the statistical model, together with all appropriate two- and three-way interactions. Sire was included as a random effect.

**Results** No three-way interaction was detected. There were GM x SW interaction for IGFBP5 ( $P < 0.05$ ), IGF1 receptor ( $P < 0.01$ ) and IGF2 receptor ( $P < 0.05$ ), B x SW interactions ( $P < 0.05$ ) for IGFBP3, growth hormone receptor (GHR) and myostatin and a B x GM interaction for IGF2. There was no clear effect of the main factors of B or GM on the expression of any gene examined though IGFBP2 was higher at heavier SW.

**Table 1** Effect of breed (B), sire EPD<sub>cwt</sub> (GM) and slaughter weight (SW) on the relative expression of 10 genes examined ( $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ )

Gene	B		H	GM		SW		SEM	B	GM	Statistical Significance			
	AA	BB		L	L	Heavy	Light				SW	BxGM	BxSW	GMxSW
IGFBP2	11.3	13.2	15.4	9.09	16.25	8.30	3.70	NS	NS	*	NS	NS	NS	NS
IGFBP3	41.9	5.09	30.3	16.7	25.59	21.4	3.55	***	*	NS	NS	*	NS	NS
IGFBP4	6.06	8.06	7.50	6.62	6.93	7.19	1.90	NS	NS	NS	NS	NS	NS	NS
IGFBP5	14.8	19.0	19.4	14.5	9.53	24.4	8.23	NS	NS	NS	NS	NS	NS	*
IGFBP6	3.8	3.54	4.50	2.79	3.97	3.33	0.56	NS	NS	NS	NS	NS	NS	NS
IGF1-R	15.5	3.94	8.27	11.1	8.00	11.4	1.18	***	NS	NS	NS	NS	NS	**
IGF2	16.1	21.1	15.6	21.6	14.32	22.9	2.32	NS	NS	*	*	NS	NS	NS
IGF2-R	6.38	11.2	8.97	8.59	9.30	8.26	1.09	**	NS	NS	NS	NS	NS	**
GHR	10.7	4.74	6.00	9.44	4.64	10.8	2.61	*	NS	*	NS	*	NS	NS
Myostatin	10.6	5.50	7.09	9.04	4.99	11.1	4.12	*	NS	NS	NS	NS	*	NS

**Conclusion** The data indicate that the local expression of some genes involved in muscle growth and proliferation can be influenced by the interaction of both genetic and management factors. These interactions are also consistent with the animal performance data of Campion *et al.* (2009) and merit further investigation. Overall, this information is important to the understanding of the biological control of muscle growth and to the interpretation and planning of gene expression based studies.

**Acknowledgements** The authors acknowledge funding from the Teagasc Walsh Fellowship Programme.

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## Effect of variety, nitrogen fertilization and regrowth interval on fatty acid levels of perennial ryegrass post establishment

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**Introduction** Grassland based ruminant production systems dominate Irish agriculture and perennial ryegrass (PRG) dominates reseeding programs. Perennial ryegrass is a rich source of poly unsaturated fatty acids (PUFA) namely linoleic (C18:2) and linolenic (C18:3) acid in the ruminant diet. Recent work (Cristilli *et al.*, 2009) shows that along with season the variety has a strong effect on fatty acid profile of PRG and offers an avenue to increase the supply of fatty acids to grazing ruminants. Additionally nitrogen fertilization (Boufaied *et al.*, 2003), and cutting interval (Elgersma *et al.*, 2003), have been linked with variation of PUFA concentrations in grass herbage. The objective of this work was to evaluate the effect of variety, nitrogen application level (N) and regrowth interval (R) on the fatty acid profile of PRG in Autumn.

**Material and methods** A 12 x 2 x 2 factorial design study was carried out to examine the effect of variety, N level and R interval on fatty acid concentration and yield with four replicated fully randomized blocks and twelve PRG (*Lolium perenne* L.) varieties. Plots (1.5m x 4.5m) were harvested to a post cutting height of 5cm using a Haldrup, Logstor, Denmark. Varieties were sown on 26<sup>th</sup> May 2008. Two N rates: 25 (low, N1) and 70 (high, N2) kg N/ha were applied over the experimental period from July to October 2008, directly after the preceding cut. Regrowth interval were three (short, R1) or five (long, R2) weeks. A one step methylation method (Sukhija and Palmquist, 1988) was used for FA methylation. The FA profile was determined by gas chromatography using a Varian GC (Chromopak) CP-3800 equipped with a FID detector and a CP-7420 column (Varian-Chromopak). Data were analysed using the mixed procedure of SAS with terms included for variety, N level and R interval and the associated interactions.

**Results** The effect of selected varieties, N level and R interval on fatty acid content is shown in Table 1. Regrowth interval affected ( $P < 0.001$ ) total FA and C18:3 concentrations, with R1 showing higher levels. Interactions between R and cut ( $P < 0.001$ ) were found and manifested through R1 with an increase of FA levels from an early to a late cutting time, and through R2 with an increase of total FA and a decrease of C18:2 and C18:3. No interactions were found between N level and R interval in FA levels. Magician presented the highest levels of individual FA while Denver had the lowest levels. Overall, there were no significant effects of variety and N level on total FA, while C18:2 and C18:3 were increased by N2. Regarding FA yield, R2, N2 and a late cut positively affected total and individual FA yield. Magician and Gilford presented the highest and the lowest FA yield respectively, with the total FA yield amounting to 90.8 and 69.2 Kg FA/ha. Interactions between R interval and N level ( $P < 0.001$ ) showed highest FA yields in R2N2 and lowest in R1N1.

**Conclusions** Fatty acid content of PRG varies with variety and management factors post establishment. A reduction in R interval lead to an increase in total fatty acid content. Monitoring and manipulation of these factors, together with genetic selection could lead to high lipid varieties and potentially to an increase in the content of essential PUFA in ruminant products.

**Table 1** Effect of variety, nitrogen and regrowth on fatty acid content (g kg<sup>-1</sup> DM)

	C18:2	C18:3	Total
Variety			
Denver	9.5 <sup>b</sup>	29.4 <sup>b</sup>	62.7
Magician	11.4 <sup>a</sup>	33.3 <sup>a</sup>	68.1
SEM <sup>†</sup>	0.37	0.73	1.79
Significance <sup>‡</sup>	*	***	NS
Nitrogen			
N1	10.0 <sup>b</sup>	30.5 <sup>b</sup>	65.5
N2	10.6 <sup>a</sup>	32.2 <sup>a</sup>	67.3
SEM <sup>†</sup>	0.15	0.30	0.73
Significance <sup>‡</sup>	**	***	NS
Regrowth			
R1	10.1	36.87 <sup>a</sup>	70.7 <sup>a</sup>
R2	10.4	25.82 <sup>b</sup>	62.1 <sup>b</sup>
SEM <sup>†</sup>	0.15	0.30	0.73
Significance <sup>‡</sup>	NS	***	***
Interactions			
R1*cut1	8.4 <sup>c</sup>	23.2 <sup>c</sup>	51.9 <sup>d</sup>
R1*cut2	11.8 <sup>a</sup>	50.5 <sup>a</sup>	89.5 <sup>a</sup>
R2*cut1	11.5 <sup>a</sup>	27.4 <sup>b</sup>	60.5 <sup>c</sup>
R2*cut2	9.3 <sup>b</sup>	24.3 <sup>c</sup>	63.7 <sup>b</sup>
SEM <sup>†</sup>	0.21	0.41	1.03
Significance <sup>‡</sup>	***	***	***

<sup>†</sup>SE of mean; <sup>‡</sup> \*\*\* =  $P < 0.001$ ; \*\* =  $P < 0.01$ ; \* =  $P < 0.05$ ; NS = Not significant

**Acknowledgement** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food.

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## The effect of grass species on nitrogen response in grass clover swards

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**Introduction** Changes to EU regulations and increased fertiliser costs have resulted in reduced nitrogen (N) use in grassland systems. This has implications for the choice of grass variety sown by growers. Currently, Irish grasslands are populated with varieties bred to produce high yields under optimum N conditions. Such swards are largely dominated by Perennial Ryegrass, however, as N levels are reduced it is uncertain whether this will remain the most productive species. This study compares the performance of Perennial Ryegrass to that of four alternative species grown at different N levels.

**Material and methods** From 2006 to 2009, six forage grasses, Cocksfoot (Donata), Meadow Fescue (Pradel), Tall Fescue (Barolex), Timothy (Dolina), diploid Perennial Ryegrass (Portstewart) and tetraploid Perennial Ryegrass (Navan), were grown along with a companion white clover (Chieftain) at Crossnacreevy Plant Testing Station, Belfast. Plants were grown at three N levels: high (420 kg ha<sup>-1</sup> N), medium (210 kg ha<sup>-1</sup> N) and low (105 kg ha<sup>-1</sup> N). Trial plots measured 1.5m x 7m and were replicated three times within each treatment in a randomised block design. Each treatment was managed to maximise dry matter yield, with a target of 10 cuts per season using a nominal regrowth period of 21 d with periods of up to 35 d towards the end of the season. Plot fresh weight was determined by harvesting the entire plot to a stubble height of 5 cm using a plot harvester (Haldrup, Logstor, Denmark). A random subsample of c. 250 g was taken from each plot for dry weight determination after oven drying at 80 °C for 16 h. Botanical composition was also determined for two cuts each year by taking a subsample from each plot. Average dry matter yields over the three years were compared using two-way ANOVA (Genstat 8; VSN International Ltd., Hemel Hempstead, UK).

**Results** In year one, yields were low and there was a clear pattern between treatments with lower N levels resulting in lower yields and higher clover contents. Yields improved in subsequent years, however, in low and medium N plots clover content also increased and by year three clover typically accounted for more than 50 % of the yield. Table 1 shows the total plot yield over the season (up to 30<sup>th</sup> October) averaged over the three years. There were significant differences between N levels, between species and for N level x species. Generally, tetraploid perennial ryegrass plots were the highest yielding at high N, but showed the largest decrease where N was limited. The diploid perennial ryegrass plots were more tolerant to the low and medium N conditions, with yields similar to or greater than Fescue and Cocksfoot plots. Timothy plots outperformed all of the others, especially under the low and medium N conditions. The botanical separations showed that much of the yield differences were due to differences in clover yield rather than yield of the sown grass species. In addition, Timothy and Cocksfoot plots were particularly prone to encroachment from other grasses.

**Table 1** Average yield (dry matter t ha<sup>-1</sup> y<sup>-1</sup>) of six grass clover swards at three Nitrogen levels (data in parenthesis shows the range of yields over three years)

	Nitrogen (kg ha <sup>-1</sup> )		
	105	210	420
P. Ryegrass (4n)	7.8 (5.1-10.2)	8.8 (6.3-11.8)	12.9 (10.9-16.7)
P. Ryegrass (2n)	8.2 (6.1-10.3)	9.4 (6.8-12.1)	12.2 (10.2-16.0)
Timothy	8.9 (7.8-10.2)	10.3 (8.9-12.3)	13.0 (11.6-15.3)
T. Fescue	8.6 (7.8-9.2)	8.9 (7.7-10.0)	12.5 (11.3-14.6)
M. Fescue	8.8 (7.9-9.7)	9.1 (7.8-10.7)	12.2 (11.0-14.6)
Cocksfoot	7.9 (4.8-10.0)	8.5 (5.5-11.2)	10.7 (8.0-14.3)

**Conclusion** Reducing N reduced plot yields and increased clover content for all of the species tested, this in general agreement with other studies (Frame *et al.* 1998). In this case, clover came to dominate all of the low N swards regardless of the choice of sown species. The results suggest that swards based on diploid perennial ryegrass may be more tolerant to low N than those based on tetraploid perennial ryegrass. There may also be potential to improve yields at low N by including alternative species, such as Timothy, but the interaction with clover and other grasses needs to be considered.

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## Organic management strategies and its effect on clover-based grassland production

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**Introduction** In Ireland, there is a price premium if 50% of annual milk produced on organic farms is supplied during the winter (September to March). Organic systems of production operate at low stocking rates compared with conventional systems, offering the potential to extend the grazing season throughout the autumn, winter and early spring.

**Materials and methods** In 2008/2009, an experiment was conducted at Solohead Research Facility; latitude 52° 51' N, longitude 08° 21' W. The aim of the experiment was to substantially lower winter feed costs for organic winter milk production systems, through the supply of 0.50 of the diet from grass-clover swards during the autumn and winter. The systems of production compared had: (i) a mean calving date of 17 February, stocking density of 2.15 cows/ha, receiving 90 kg/ha of annual fertilizer N input (Control); (ii) a mean calving date of 17 February, stocking density of 1.6 cows/ha, receiving no fertilizer N input (S-NFN) and (iii) a mean calving date of 16 April, stocking density of 1.6 cows/ha between calving and 1 September and a stocking density of 1.2 cows/ha between 1 September and 18 February, receiving no fertilizer N input (W-NFN). Cows were turned out to pasture after calving and the number of days at pasture was recorded as 1 day when cows were outside both day and night and 0.5 when cows were outside only by day. Exceptionally high rainfall was recorded during the summer and autumn 2008 (28% above normal; 641 mm compared to 10-year average, 501 mm). Cows were randomised into four main groups on the basis of lactation number (1, 2, 3 & ≥4) within 1 week of parturition and then sub-divided into sub-groups of three on the basis of calving date. From within each sub-group one cow was randomly assigned to each herd. Herds were randomly assigned to each system. There were 18 cows per system. Animal production data (milk production, milk composition, live-weight and body condition score (BCS)) were subjected to ANOVA using SAS, 2008. Pre-calving live-weight and BCS were used as covariates when analysing live-weights and BCS.

**Results** There were no ( $P > 0.05$ ) differences between systems in production of milk yields and milk composition or live-weight and BCS during or at the end of lactation (Table 1). The W-NFN system produced 0.48 of milk between 1 September and 18 February. High rainfall had lead to difficult grazing conditions consequently cows in the control system were housed by night from 7 October and fulltime from 23 October. This is approximately five weeks earlier than normal at Solohead for spring calving herds resulting in 220 days at pasture for the control group. Lower stocking densities on the NFN systems allowed cows to be kept outside for longer ( $P < 0.001$ ), although there was no ( $P > 0.05$ ) difference in days at pasture between the NFN systems (Table 1).

**Table 1** Production of milk, fat, protein and lactose, milk composition for 290 d lactation, the mean number of days that cows were at pasture, concentrates fed, mean cow live-weight during lactation, and body condition score (BCS; scale 1 to 5) during and at the end of lactation

	Control	S-NFN	W-NFN	s.e.m.	P value
Milk (kg/cow)	6371	6511	6605	182	NS
Fat (kg/cow)	274	282	273	18.8	NS
Protein (kg/cow)	230	236	228	5.9	NS
Lactose (kg/cow)	301	308	309	8.5	NS
Fat + Protein (kg/cow)	504	518	502	13.0	NS
Fat (%)	4.31	4.34	4.18	0.096	NS
Protein (%)	3.62	3.63	3.48	0.050	0.065
Lactose (%)	4.73	4.72	5.04	0.144	NS
Days at pasture (days/cow)	220	234	231	1.7	***
Concentrate fed (kg/cow)	590	590	847	21.3	***
Live-weight (kg/cow)	602	594	590	9.2	NS
Mean BCS during lactation	3.00	3.03	2.97	0.039	NS
BCS at the end of lactation	3.02	2.97	2.89	0.068	NS

**Conclusions** A long grazing season was possible with the late-calving W-NFN herd. A later mean calving date than 16 April is necessary to produce 0.50 of annual milk between September and March.

**Acknowledgements** This project is funded by the Department of Agriculture and Food Research Stimulus Fund (Project RSF07-511).

## A meta-analysis of the impact of stocking rate on the productivity of pasture-based milk production systems

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**Introduction** The abolition of milk quotas by 2014 will have a major influence on the Irish dairy industry with factors such as land, stock and labour availability constraining production in future years. Stocking rate (SR), expressed as cows per hectare (ha) is the major factor determining productivity from pasture and recent research by Macdonald *et al.* (2008) reported that an increase in SR results in an increase in milk production per ha but a decrease in milk production per cow. The objective of this study is to quantify the response in milk production per cow and per ha associated with a change in SR using meta-analysis techniques on experimental data from 1960 to 2008. A meta-analysis is a quantitative review using scientific methods, based on statistics, to summarise previous research in a particular topic (Sauvant *et al.* 2008).

**Materials and methods** A thorough literature review of SR experiments resulted in the final database containing 44 papers and 109 experiments for analysis. For inclusion in the database, an experiment had to contain a comparison of at least two SR under the same experimental conditions. Experimental data included experiment length, grazing cow-days per ha, the various SR and milk production and bodyweight (BW) results per cow and per ha. As the objective of the meta-analysis was to analyse the effect of an increase in SR from a low level, the lowest SR treatment within each experiment was considered the base SR, with the milk production at this SR considered the base milk production performance. Large variability in animal BW was observed over both time and between experiments within the database, consequently each SR was also described in terms of an additional 100kg BW per ha. For analytical purposes, two main subsets of data were created. In common experimental length (Type C) experiments, the experimental period was common to all SR treatments within the respective experiment with the effects observed due to differences in feed allowance over varying periods of time. In variable experimental length (Type V) experiments, the length of the experimental period varied between SR treatments (*i.e.* full lactation studies). A study effect (IdExp) representing the variance between studies not accounted for by the other variables in the model was included in each model as described by Sauvant *et al.* (2008). The independent variables actual and proportional change in milk production according to a change in SR were continuous in nature and analysed using linear mixed models using the statistical procedures software of SAS (Proc MIXED) with IdExp included as a random effect and an unstructured variance covariance structure (UN) among records.

**Results** Average SR and experimental length within the Type C database was 3.83 cows per ha and 168 days respectively. The average SR and experimental length within the Type V database was 2.8 cows per ha and 272 days respectively. An increase in SR of 1 cow per ha resulted in a decrease in daily milk yield per cow of 7.4 and 8.7% for Type C and V data respectively while milk yield per ha increased by 20.1 and 19.6%, respectively (Table 1). Within the Type V dataset, a 1 cow per ha increase in SR also resulted in a 15.1% reduction in lactation length (equivalent to 42 days). Predicted milk production performance was also calculated using equations based on SR and BW per ha. Low residual standard errors indicated a good precision of the predictive equations with the exception of proportional change in milk production per cow.

**Table 1** Change in milk production per cow and per hectare for an increase in stocking rate of 1 cow per hectare for common (Type C) and variable (Type V) experimental length data.

	Type C				Type V			
	No. data	Base	Change	% change	No. data	Base	Change	% change
Performance per cow								
Milk yield (kg)	99	18.1	-1.23	-7.42	22	15.8	-1.38	-8.66
Milksolids <sup>1</sup> yield (kg)	69	1.33	-0.08	-6.97	20	1.37	-0.13	-9.15
Performance per hectare								
Milk yield (kg)	99	8,868	1,657	20.1	22	9,878	1,568	19.6
Milksolids yield (kg)	69	689	113	18.5	20	957	101	11.3

<sup>1</sup> Milksolids = fat + protein

**Conclusion** The results illustrate that whilst milk production per cow is reduced as SR increases, a strong positive relationship exists between SR and milk production per ha. The low predictability of proportional change in milk production per cow according to the classical SR definition of cows per ha suggests that SR would be more appropriately defined in terms of the change in available feed offered per animal within each treatment.

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## The effect of sward perennial ryegrass content and defoliation method on seasonal and total dry matter production

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**Introduction** It is widely accepted that perennial ryegrass dominant swards are more productive. (Frame, 1991). Quantifying the production loss, as sward perennial ryegrass (PRG) content decreases is an important issue for grassland farmers. The evaluation of grass varieties in Ireland uses mechanical defoliation methods and visual ground score estimates of the proportion of PRG in a sward. The objective of this study was to quantify the effect of i) sward PRG content and ii) defoliation method on seasonal and total dry matter (DM) production, as well as the effectiveness of ground cover score estimates in predicting sward PRG content and DM production under actual animal grazing.

**Materials and methods** Ninety plots were established on free draining, acid brown earth soil with a sandy loam to loam texture in Autumn 2007. The study incorporated three varieties of perennial ryegrass (*Lolium perenne* L.; PRG) at five different seeding rates 5, 7.5, 12, 20 & 30kg/ha, under 2 defoliation managements; i) a simulated mechanical grazing management (SG) ii) actual animal grazing by dairy cows (AG). Each variety was replicated three times at each seeding rate under the two defoliation managements (3 varieties x 5 seeding rates x 2 defoliation managements, replicated 3 times). The five seeding rates were used to establish swards with approximately 15%, 25%, 40%, 65% and 100% PRG content. Plot size was 1.4m x 5m (7m<sup>2</sup>) SG, and 2.8 x 5m (14m<sup>2</sup>) AG. Plots were grazed nine times during their first full grazing season (February to October 2008). SG plots were harvested using a motor Agria mower (Etesia UK Ltd., Warick, UK). The fresh weight of a 1.2x5m strip (>4cm) was recorded and a sub-sample (100g) dried at 80°C for 16 h to determine dry matter. Herbage yield (kg DM/ha) was then calculated. The herbage yield for the AG plots was determined prior to each grazing by cutting a 0.25m<sup>2</sup> sample (>4cm) using a 0.5 x 0.5 m quadrat and Gardena grass shears (Gardena, GmbH, Ulm Germany), with dry matter determined as described above. Tiller density was assessed in Autumn '08. Three turves (10cm x 10 cm) were cut from each plot and dissected. From this, PRG tiller density and total tiller density/m<sup>2</sup> was calculated. All plots were ground cover scored (score 1- 5) based on the proportion of PRG present in the sward. SG plots received 350kg nitrogen (N)/ha over the grazing season, with AG plots receiving 250kg N/ha, the difference accounting for the nitrogen recycling which would occur on the AG plots. All dung paths were removed from the AG plots after grazing. Data was analysed using PROC GLM in SAS.

**Results** Sward PRG proportion had no significant effect on PRG or total tiller density. Defoliation method approached significance ( $P<0.07$ ) for PRG tillers/m<sup>2</sup> but had no effect on total tiller density. As sward PRG proportion increased, spring, summer and total dry matter yield increased significantly ( $P<0.001$ ). Defoliation method also had a significant effect ( $P<0.001$ ) on Spring, Summer and total dry matter yields, with AG having higher yields. There was a significant interaction ( $P<0.02$ ) between sward PRG proportion and defoliation method in Spring, with AG swards consistently having a higher yield across all PRG proportions. Sward PRG proportion had a significant effect on ground score, increasing for 15%, 25% and 40% swards, with no difference between 65% and 100% swards.

**Table 1** Effect of sward perennial ryegrass content and defoliation method on tiller density, DM production and ground score (Year 1)

Sward PRG proportion	15%	25%	40%	65%	100%	Sig.	SG	AG	Sig.	SED	Inter
PRG Tillers (m <sup>2</sup> )	4764	5258	5483	5058	5831	$P<0.569$	5660	4898	$P<0.07$	422.01	NS
Total Tillers (m <sup>2</sup> )	9322	8631	9383	7619	8314	$P<0.268$	9054	8253	$P<0.162$	572.3	NS
Spr. DM prod (kg/ha)	364	447	538	822	910	$P<0.001$	333	899	$P<0.001$	55.8	$P<0.02$
Sum. DM prod (kg/ha)	5832	6424	6457	6852	7414	$P<0.001$	6299	6893	$P<0.001$	169.2	NS
Aut. DM prod (kg/ha)	3958	3903	4212	4269	4036	$P<0.109$	4368	3783	$P<0.001$	101.7	NS
Total DM prod (kg/ha)	10154	10774	11206	11943	12360	$P<0.001$	11000	11575	$P<0.015$	232.2	NS
Ground Score	1.89	2.34	2.91	3.72	3.71	$P<0.001$	2.89	2.94	$P<0.652$	0.12	NS

SED=Standard error of the difference; PRG=Perennial ryegrass; Spr.=Spring ; Sum.=Summer; Aut=Autumn; NS=non significant

Inter=Interaction between sward perennial ryegrass content and defoliation method

**Conclusions** The results of this study show that increasing the PRG proportion of swards significantly increased seasonal and total DM production of grass swards. Results suggest that swards with less than 65% PRG content are close to the reseeding threshold, particularly if spring DM production is important for the enterprise. Mechanical defoliation of swards compared with AG recorded lower DM yields, with this difference attributed to spring and summer DM production, however overall it appears to be reasonably reflective of AG systems. Visual ground cover scores are a useful indicator of sward production potential. It can also be concluded that sward response to applied nitrogen increases as PRG content increases as all swards received the same level of N fertiliser.

**Acknowledgements** The Department of Agriculture and Food Stimulus Fund is acknowledged for its financial support.

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## Semi-natural, western hill vegetation under defined management systems, 1995 to 2008

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**Introduction** The status of upland habitats, especially western blanket bog and wet heath, is a matter of official concern (NPWS, 2008). The Teagasc Hill Sheep Farm, which ranges in altitude from 15 to 275 m O.D., is c. 20 km inland from the mid-western sea-board and contains c. 250 ha of unimproved hill vegetation that mainly represents a complex of blanket bog and wet heath habitats. The objective was to assess the influence of physical factors on the semi-natural vegetation under defined management systems over time.

**Material and methods** The unimproved area was grazed at 0.9 ewes/ha from 1995 to 1998 and 0.8 ewes/ha from 1999 to 2008 with a relatively consistent monthly variation throughout. A grid, 100 m x 100 m, was surveyed giving 226 intersection points which formed the basis for quantifying the physical attributes, physiography (6 categories), soil (4 categories) and altitude (3 categories), of the hill and vegetation. The latter was estimated by a modified point quadrat system giving 100 observations at ground level each time. Animal holding areas, which accounted for six to eight intersection points, were excluded. Vegetation height, 1999 to 2008, was calculated using the same quadrat to give 20 hits at each intersection point. The associations between physiography, soil and altitude and the frequency of the overall vegetation, its individual groups and vegetation height were evaluated by PROC GLM (SAS, 2002-3).

**Results** The mean frequency (%) of the vegetation occurrence, which consisted of grasses, sedges, bryophytes, heathers and 'other forbs', increased from 64.5 in 1995 to 82.8 in 2008. Grasses and sedges together accounted for 73% of the vegetation in 1995 and 66% in 2008. Physiography was significantly associated with the distribution of the overall vegetation frequency ( $P < 0.001$ ) and with that of sedges ( $P < 0.001$ ), bryophytes ( $P < 0.05$ ) and heathers ( $P < 0.05$ ), in 1995 while it was significantly associated only with 'other forbs' ( $P < 0.05$ ) in 2008. Soil was significantly associated also with the overall vegetation frequency ( $P < 0.05$ ) and with that of grasses ( $P < 0.01$ ), sedges ( $P < 0.001$ ) and 'other forbs' ( $P < 0.05$ ) in 1995 and maintained that association but with the replacement of 'other forbs' by heathers ( $P < 0.05$ ) in 2008. Altitude, which was limited in amplitude, was strongly associated only with the frequency of 'other forbs' ( $P = 0.06$ ) in 1995 but had no significant association with the distribution of the total vegetation or its groups in either 1995 or 2008. The changes in vegetation frequency at all category levels from 1995 to 2008 were mostly positive. Physiography was significantly associated with the change in sedges ( $P < 0.05$ ), soil with that in heathers ( $P < 0.01$ ) and 'other forbs' ( $P < 0.05$ ), while altitude was not significantly associated with any of the changes. Overall mean vegetation height remained relatively unchanged at c. 21 cm but the changes at category level were both negative and positive. Physiography was significantly associated ( $P < 0.05$ ) with vegetation height in 1999 and very significantly ( $P < 0.001$ ) in 2008 when both soil and altitude were also significantly associated ( $P < 0.05$ ) with vegetation height. All factors, physiography ( $P < 0.01$ ), soil ( $P < 0.001$ ) and altitude ( $P < 0.01$ ), were significantly associated with the change in vegetation height from 1999 to 2008. Some examples of the associations are contained in Table 1.

**Table 1** Least square means (s.e.) for frequency of sedges and vegetation height by soil category

Soil (n)	Frequency of sedges (%)		Vegetation height (cm)	
	1995	2008	1999	2008
Lithosol (72)	17.8 (1.9)	22.5 (2.1)	20.0 (1.5)	15.4 (1.6)
Gley/Podzol (22)	20.1 (2.4)	26.4 (2.7)	17.1 (1.9)	19.7 (2.0)
Peat <50 cm (27)	22.4 (2.2)	27.9 (2.5)	18.9 (1.8)	19.4 (1.9)
Peat ≥50 cm (89)	28.0 (1.6)	34.1 (1.8)	18.7 (1.3)	18.1 (1.3)

**Conclusion** The changes in the frequency of vegetation from 1995 to 2008 generally over-shadowed the initial association with the physical factors, except soil. The latter persisted with a significant association not only with the overall vegetation but also with grasses, sedges and heathers. Soil is thus an important factor in monitoring the effect of land use in this environment. The substantial increase in the general vegetation cover and decrease in the dominance of the two main groups, grasses and sedges, suggest that the management system on the hill is increasing biodiversity and is sustainable.

**Acknowledgements** The authors gratefully acknowledge the input and support of Dr. J.P. Hanrahan and funding from the N.D.P.

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## An evaluation of tyfon and chicory, as the sole forage or in combination with perennial ryegrass on the performance of finishing lambs

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**Introduction** High levels of lamb growth post weaning can be achieved from grazed grass as the sole diet (Keady and Hanrahan 2009a,b). However many commercial producers are unable to finish lambs without concentrate supplementation. Keady and Hanrahan (2007) concluded that, whilst it is not financially justifiable, concentrate supplementation increased lamb performance. In recent years there has been interest in the use of alternative forages, grazed *in situ*, and offered either as the sole forage or in combination with grazed grass to improve lamb growth post weaning. The aim of the current study was to evaluate the effect of tyfon and chicory offered either as pure stands or in combination with perennial ryegrass on lamb performance. The effect of old permanent pasture was evaluated to determine the benefits to lamb performance from reseeding with perennial ryegrass.

**Materials and methods** Three 1.36 ha paddocks, each were divided into three sections. On 29 May one third of each paddock was reseeded with either perennial ryegrass (PRG) at a seeding rate of 12 kg/ha, tyfon/PRG mixture at 1.5 and 10.5 kg/ha or chicory/PRG mixture at 1.5 and 10.5 kg/ha, respectively. A fourth paddock was divided into 4 equal sections and 2 random sections were seeded with either tyfon (2.5 kg/ha) or chicory (2.5 kg/ha). Prior to reseeding all paddocks received the following fertiliser (kg/ha): 51 kg N, 25 kg P and 111 kg K. Old permanent pasture (OPP) (1.3 ha) (*Poa species*, *Lolium perenne*, *Dactylis glomerata*, *Trifolium repens*, *Phleum pratensis* and weed species accounted for 39%, 27%, 11%, 8.5%, 7.5% and 7% of sward cover, respectively) which had been grazed by ewes and lambs for the previous 20 years was divided into 3 paddocks and received fertiliser N (51 kg/ha). On 7 July 269 lambs (Suffolk-x, mean live weight 29.5 kg) were allocated to the six treatments at random balanced for dam age, breed, and rearing type. The initial number of lambs per treatment was based on the DM yield at the start of the study. The lambs were rotationally grazed without concentrate supplementation to pre-determined sward heights. At 3-week intervals lambs were drafted for slaughter and carcass weight, and conformation and fat classifications were recorded. Additional lambs were used in a “put and take” system to enable grazing days for each treatment to be determined. All treatments received fertiliser N (31 kg/ha) after the first rotation and in early September. The data were analysed using PROC GLM of SAS.

**Results** The effects of forage type on lamb performance to slaughter are presented in Table 1. Lambs grazing chicory/PRG had a higher carcass weight ( $P<0.05$ ) and kill out ( $P=0.05$ ) but tended to have a lower daily live weight gain ( $P=0.06$ ) relative to lambs grazing PRG reseed. Relative to chicory/PRG, lambs grazing tyfon/PRG were lighter at slaughter ( $P<0.05$ ), had a lighter carcass weight ( $P<0.05$ ) and were slaughtered at a younger age ( $P<0.05$ ). Lambs grazing chicory had higher daily live weight gain ( $P<0.01$ ), slaughter weight ( $P<0.01$ ) and carcass weight ( $P<0.01$ ) and tended ( $P=0.07$ ) to be younger at slaughter relative to lambs grazing tyfon. Relative to PRG reseed, lambs grazing the OPP were younger at slaughter ( $P<0.05$ ). Ram lambs had significantly higher live weight gain ( $P<0.001$ ) than ewe lambs (251 v 182 g/d) and were younger at slaughter (68 v 91 days). Treatment had no effect ( $P>0.05$ ) on carcass conformation or fat classification. The PRG sward had the highest number of lamb grazing days whilst the pure stand of chicory resulted in the lowest stock carrying capacity.

**Table 1** The effects of forage type on lamb performance

	Forage							Contrasts						
	Perennial	Chicory	Tyfon	Chicory	Tyfon	Permanent	s.e.	sig						
	ryegrass	PRG	PRG	(C)	(T)	pasture			PRG	PRG	CP	C	PRG	
	(PRG)	(CP)	(TP)			(OPP)			v	v	v	v	v	
									TP	CP	TP	T	OPP	
Days to slaughter	86	90	80	78	75	78	3.5	*	NS	NS	*	P=0.07	*	
Slaughter wt (kg)	45.1	45.5	44.6	45.7	44.4	45.2	0.33	NS	NS	NS	*	**	NS	
Weight gain(g/d)	220	206	212	226	208	223	6.2	NS	NS	P=0.06	NS	**	NS	
Carcass wt (kg)	19.0	19.6	18.9	19.8	19.0	19.0	0.24	*	NS	*	*	**	NS	
Kill out (g/kg)	421	432	426	434	429	421	4.7	NS	NS	P=0.05	NS	NS	NS	
Lamb grazing days/ha	4833	4788	4553	2698	4462	-	-	-	-	-	-	-	-	

**Conclusions** Including alternative forages with perennial ryegrass during reseeding had no beneficial effects on lamb performance. Post weaning lamb performance on old permanent pasture was similar to that achieved from new reseeded perennial ryegrass pasture.

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## Nitrogen recovery in herbage from dung pats and urine patches applied at two different times during the growing season

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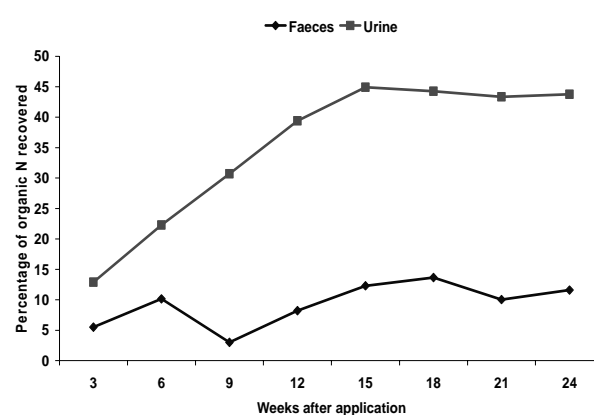
**Introduction** There is increasing pressure to increase nitrogen (N) use efficiency within agricultural systems, as loss of N to waterways and the atmosphere contributes to aquatic and terrestrial eutrophication, and to nitrous oxide emissions. Dairy cows typically excrete between 80 - 120 kg of manure N per animal year, and in intensively grazed pastures, urine N is the largest contributor to nitrate leaching (Di *et al.*, 2002). Understanding the efficiency with which grass swards take up N excreted by grazing dairy cows is a prerequisite to improving the efficiency of N use within grazing systems. The current study was designed to identify the proportion of N contained within 'urine patches' and 'dung pats' which is subsequently taken up by herbage during the growing season.

**Material and methods** Thirty-six circular grass plots (radius, 60 cm) were used in a 2 x 3 factorial design experiment (randomised block, n = 6). Treatments examined comprised two application dates; 1<sup>st</sup> May (M) and 13<sup>th</sup> August (A), and three organic N treatments; control (zero N: C), faeces N (2350 g faeces/plot: F), and urine N (2630 g urine/plot: U). On each application date (M and A), faeces or urine (as appropriate) were applied in the centre of the plot by simulated urination and defecation. Weights of faeces and urine applied were based on the average weights of 15 defecation/urination events recorded from grazing cows. In addition, all plots received inorganic fertiliser N on 1<sup>st</sup> May, and thereafter at 3 week intervals (50, 45, 35, 30, 25, 20 and 15 kg N per hectare, respectively). Total inorganic fertiliser N application was 220 kg/ha over the experiment. Plots with organic N applied in May and August were harvested eight times and three times respectively, following applications, thus simulating a rotational grazing system. Grass was cut to a height of 50 mm using Gardena shearers. Faeces and urine were analysed for total N. Herbage was analysed for oven dry matter (ODM) and total N. A repeated measures approach using the residual maximum likelihood procedure was used to analyze the data set.

**Table 1** Effect of faeces/urine application and application date on herbage growth and N recovery during the first 9 weeks after application

Date	May application			August application			SED	N treat.	Date	Int.
N treatment	C	F	U	C	F	U				
Herbage DM yield (g/m <sup>2</sup> )	87.7	88.2	123.8	32.5	37.4	45.8	7.56	***	***	*
N content of herbage (g/kg DM)	31.2	32.7	39.6	31.0	32.9	35.7	1.47	***	ns	ns
Herbage N yield (g/m <sup>2</sup> )	2.70	2.76	4.87	1.01	1.23	1.75	0.27	***	***	***
N recovered (%)	-	3.0	29.7	-	12.2	19.8	6.7	*	ns	ns

**Results** Nitrogen concentrations in urine and faeces, applied in May and August, were 9.16 and 4.81 g N per litre and 3.56 and 2.61 g N per kg fresh respectively. The application of urine increased ( $P < 0.001$ ) herbage N yield and DM yield in the first 9 weeks after application by 80.3% and 41.2%, respectively, when applied in May, and by 73.3% and 40.1%, respectively, when applied in August (Table 1). The application of faeces had no significant ( $P > 0.05$ ) effect on herbage N yield or DM yield. Application date had a significant ( $P < 0.001$ ) effect on herbage DM and N yields, with yields being lower in August. Date of application had no significant effect on the percentage of N recovered or herbage N content. Following urine application on 1<sup>st</sup> May, herbage N and DM yields were significantly greater than the control for 15 weeks after application. Twenty-four weeks after organic N application (May), 43.8% of the N applied via urine, and 11.6% of the N applied via faeces had been recovered in the herbage (Figure 1).



**Figure 1** Percentage of N applied in faeces and urine on 1<sup>st</sup> May subsequently recovered in herbage

**Conclusions** Herbage DM and N yields were significantly increased with the application of urine to grass plots compared to the application of faeces. Also, a much higher percentage of the N available in urine (43.8%) was recovered by herbage than N available in faeces (11.6%).

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## Relationships between IGF-I concentration and age at first calving of Holstein-Friesian heifers with their survival up to third calving

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**Introduction** Dairy cows survive on average only three lactations; this severely limits opportunities for on-farm selection of breeding cows in addition to presenting a welfare issue and causing economic loss. Furthermore, around 15% of liveborn heifers fail to reach first calving, and about 20% of those which do are culled or die during their first lactation. The somatotrophic axis (growth hormone, insulin-like growth factor-I [IGF-I], insulin and glucose) in the female dairy calf is of key importance for growth, development, and reproductive function. We have previously found a strong positive correlation between IGF-I concentration and growth in heifers between 1 and 6 months of age, and both were also associated with age at first calving (AFC). The aim of this study was to determine if IGF-I concentration, or AFC, are useful in predicting future survival up to third calving of Holstein-Friesian animals on dairy farms.

**Materials and methods** A total of 17 dairy farms across southern England milking Holstein-Friesian cows were recruited during 2003 and 2004. Recruited heifers ( $n=337$ ) were monitored from birth until they calved for a third time. For animals failing to reach third calving, the number of calvings they achieved before they died, or were culled or sold was recorded. Blood samples were collected at one ( $28 \pm 0.8$  d,  $n=334$ ), six ( $184 \pm 0.8$  d,  $n=317$ ) and fifteen ( $452 \pm 3$  d,  $n=305$ ) months of age, and at weeks -1, +1 and +8 around first and second calving for the measurement of plasma IGF-I using commercial ELISA kits. Age at first service (AFS) ( $15 \pm 0.1$  months,  $n=305$ ) and AFC ( $26 \pm 0.2$  months, range 21 to 37 months,  $n=286$ ) were recorded. Animals were subdivided on the basis of the number of calvings they achieved; 0 ( $n=51$ ), 1 ( $n=68$ ), 2 ( $n=55$ ), or 3 ( $n=163$ ). One-way analysis of variance (ANOVA) was initially used to compare IGF-I concentration, AFS and AFC according to the number of calvings. To further investigate relationships which showed a significant relationship with the number of calvings, analyses were performed using a linear mixed effects model with posthoc LSD tests, including farm as a random effect.

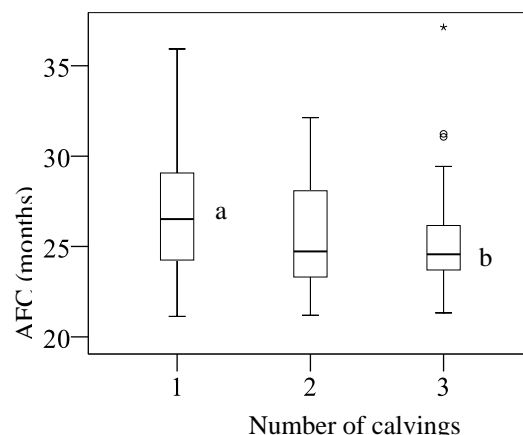
**Results** Of the initial 337 heifers recruited, 85% ( $n=286$ ) reached first calving, whilst only 48% ( $n=163$ ) survived to third calving. Infertility was the main reason for culling after first ( $n=20/68$ ) and second ( $n=20/55$ ) calving. Plasma IGF-I concentration increased during the rearing period up to 15 months, then declined at each subsequent calving before increasing again by 8 weeks PP; concentrations were lower around second than first calving. IGF-I concentrations according to the number of calvings achieved are presented in Table 1. Heifers only calving once had lower IGF-I levels at 15 months compared to those calving three times ( $P<0.05$ ). Animals calving only twice had lower concentrations immediately before and after second calving compared to animals reaching their third lactation ( $P<0.05$ ). Animals calving three times were younger at first service ( $15 \pm 0.1$  months) and at first calving ( $25 \pm 0.2$  months) compared to heifers only calving once ( $16 \pm 0.4$  months, and  $27 \pm 0.4$  months, respectively) (Figure 1).

**Table 1** Plasma IGF-I concentration (ng/ml) during the rearing period, and around first and second calving, according to number of calvings achieved.

	Number of calvings				P-value#
	0, n=51	1, n=68	2, n=55	3, n=163	
1 month	40±5	40±3	43±4	46±2	NS
6 months	92±9	83±5	87±5	90±3	NS
15 months	99±9	97±4 <sup>a</sup>	99±5	109±2 <sup>b</sup>	0.03
Pre-calving 1		73±4	66±4	71±2	NS
Week 1 PP		34±3	40±5	42±2	0.1
Week 8 PP		56±3	58±3	59±2	NS
Pre-calving 2			49±4 <sup>a</sup>	61±3 <sup>b</sup>	0.04
Week 1 PP			17±2 <sup>a</sup>	27±2 <sup>b</sup>	0.03
Week 8 PP			41±4	44±2	NS

#Linear mixed model with farm included as a random effect.

PP=postpartum



**Figure 1** AFC according to number of calvings; 1 ( $27 \pm 0.4$  months,  $n=68$ ), 2 ( $26 \pm 0.4$  months,  $n=55$ ), and 3 ( $25 \pm 0.2$  months,  $n=163$ ).  $a>b$ ,  $P<0.001$

**Conclusion** Only half the heifers recruited at birth survived until a third calving. Animals failing to complete a single lactation are clearly uneconomic to rear as replacement heifers. Heifers culled after first calving, mainly for infertility, had lower IGF-I concentrations at 15 months, and animals culled after second calving had lower concentrations immediately before and after calving. This suggests that differences in the somatotrophic axis may affect their fertility and hence longevity, and these differences may be amplified on entering second lactation. Animals reaching their third lactation were younger at first breeding and calving, highlighting the importance of early growth and development in terms of their subsequent performance.

**Acknowledgements** This project was funded by Defra and DairyCo.

## Factors associated with selling price of dairy calves at livestock marts

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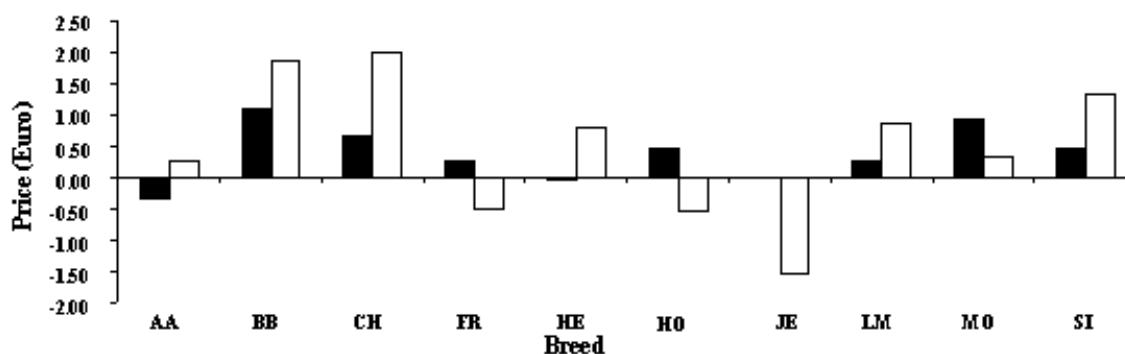
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**Introduction** In Ireland livestock marts remain an important marketing outlet for dairy calves, with dairy calves accounting for 26% of total dairy cattle mart movements (Department of Agriculture, Fisheries, and Food, 2008). Previous US studies have attempted to quantify the factors associated with calf price (Troxel and Barham, 2007), however little is known on the association between calf price and factors such as dam age, heterosis, recombination, calving difficulty, and whether the animal was a singleton or twin. The objective of this study was to determine the factors associated with selling price received for dairy calves in Irish livestock marts. Results from this study may be used to help farmers make improved management decisions, and provide information for bio-economic models for evaluating production systems or estimating economic values.

**Materials and methods** In the present study calves were defined as animals from dairy cows sold between 2 days of age and 12 weeks of age. A total of 306,640 calf price records from 71 livestock marts in Ireland between the years 2000 to 2008 inclusive, were available. In Ireland, calves from dairy herds are generally sold in livestock marts from a couple of days of age. Not all calves are sold in livestock marts and some are sold privately among individuals; no data on these sales were available. To accurately quantify associations, only animals sold individually at the livestock marts were retained. Animals were removed if their herd of origin or the mart of sale were unknown. Only calves sold for a price between €2 and €450 were retained. Animals retained had to have at least 66% of their breed fraction known. Following all edits 53,838 calves remained. Factors associated with calf price were determined using mixed linear models (ASReml; Gilmour *et al.*, 2009). Mart, date of sale nested within mart, and herd nested within year of sale were included as random effects. Fixed effects considered for inclusion in the models were: year of sale, month of sale, gender, age of animal at selling, parity of dam, calving ease, whether the animal was born as a singleton or twin, the proportion of the 10 most common dairy and beef breeds used in Ireland, heterosis, and recombination loss, as well as interactions.

**Results** The mean selling price for all calves in the dataset was €157 (standard deviation = €79). The majority of calves (57%) were sold between January and April and 91% of all calves were sold before 42 days of age, with an abrupt drop in sales at day 42. Crossbred, male, singleton calves from older cows received a price premium ( $P < 0.001$ ) and the price paid increased linearly with age. The association between age and price, however, differed by gender, with a greater increase in males (€1.15 per day of age) compared to females (€0.94 per day of age). The difference in calf price from different parity dams was small, with the exception of calves from first parity cows where they, on average, received €14.03 less ( $P < 0.001$ ) than calves from mature cows (*i.e.* parity  $\geq 5$ ). Each 1% increase in proportion Belgian Blue was worth an extra €1.89 in males compared to €1.06 in females (Figure 1).



**Figure 1** Change in calf price per one percent increase in breed proportion for female (■) and male (□) calves across breeds.

AA= Aberdeen Angus, BB= Belgian Blue, CH= Charolais, FR= Friesian, HE= Hereford, HO= Holstein, JE= Jersey, LM= Limousin, SI= Simmental.

**Conclusion.** Factors such as breed, birth type, calving difficulty, heterosis, age at selling, gender, and age of dam were shown to have varying associations with the price obtained for an animal. Purchasers were willing to pay premium prices for older, crossbred, singleton male calves born from older cows. Although prices varied across years, there were similarities in season trends; namely the age of the animal and the seasonality of selling. Knowledge of these factors and the seasonal trends in sales can help farmers to maximise prices attainable for their animals and thus the profitability of their production system.

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## The effect of winter diet on the weight gain and body condition score of weanling dairy replacement heifers

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**Introduction** Heifers should be managed to achieve 55% to 60% of mature bodyweight (BW) at mating start date (Patterson *et al.*, 1992). Ensuring the optimum development of replacement heifers is critical and needs to be accomplished at low cost without sacrificing performance. The feed costs of kale are over 30% less than grass silage. Yet, feed and labour costs associated with kale increase when the recommendation of offering grass silage with kale is adhered to, thus a 100% kale diet would be attractive. The objective of this study was to investigate four contrasting winter feeding regimes on heifer BW gain and body condition score (BCS).

**Materials and methods** One hundred and twenty spring born weanling replacement dairy heifer calves were balanced on the basis of breed (Holstein Friesian; 87% of herd, Montbelliarde × Holstein; 13% of herd), age ( $282 \pm 16.3$  days), bodyweight (BW;  $214 \pm 22.8$  kg) and body condition score (BCS;  $2.94 \pm 0.309$ ) in a randomised block design. They were then randomly assigned to one of four winter feeding treatments from 17 November 2008 to 10 February 2009 (85 days). The four feeding treatments were: i) indoors offered *ad libitum* high quality grass silage (HQS); ii) indoors offered *ad libitum* poor quality grass silage (PQS); iii) outdoors offered 70% kale and 30% grass silage (70K); iv) outdoors offered 100% kale (100K). The high quality grass silage offered was >70% DMD (dry matter digestibility) while the poor quality grass silage was <65% DMD. The forage kale was grazed *in situ*. The 70K animals were offered grass silage bales >65% DMD. Prior to the commencement of the experiment all animals received one Tracesure® I bolus to provide iodine, selenium and cobalt supplementation. The 100K treatment animals were offered straw for the first week of the study to adjust them to the 100% kale diet – no further fibre source was offered after the first week. All animals were offered fresh feed daily; the indoor animals grass silage refusals were removed daily, the outdoor animals were offered a fresh allocation of kale each morning by moving a temporary electric fence. All treatments were grouped individually. During the experimental period all animals were weighed weekly and condition scored every three weeks. All animals were turned out to pasture on 10 February and offered *ad libitum* grazed grass. All animals were then weighed weekly to the start of the breeding season (15 April) and monthly thereafter, while BCS was recorded monthly from turnout. All data were analysed using PROC MIXED in SAS. Animal was used as the experimental unit. Pre-experimental values were used as a co-variate in the model. The data are reported in three periods, PI: the 85 day experimental period, PII: the period of time from turnout to the start of the breeding season and PIII: from after the commencement of the breeding season to 15 September 2009.

**Results** The DMD of the HQS was 72 ( $\pm 3.0$ ) % and the dry matter (DM) was 22.2 ( $\pm 1.32$ ) %. The DMD and DM of the PQS were 60 ( $\pm 1.6$ ) % and 26.1 ( $\pm 2.36$ ) %, respectively. The estimated Unité Fourragère Lait (UFL) value of the kale offered was 1.02 UFL/kg DM and was 0.78 UFL/kg DM for the HQS and 0.75 UFL/kg DM for the PQS. During PI the BW gain of the 70K treatment was significantly greater ( $P < 0.001$ ) than all other three treatments (0.26 kg/day). During PII BW gain for the 100K treatment was greater ( $+0.29$ ;  $P < 0.001$ ) than all other treatments (0.85 kg/day). There was no difference in BW gain during PIII (0.93 kg/day). During PI the BCS of the HQS, PQS and 100K animals did not differ significantly (2.85) however the BCS of the HQS and PQS animals was less ( $P < 0.001$ ) than the 70K animals. There was no difference in BCS between the 70K and 100K treatments (2.93). All animals had similar BCS in PII (3.08) and PIII (3.24). The lower weight gain of the 100K treatment relative to the 70K treatment is partly attributable to gut fill, especially given that there was no difference in BCS at the end of PI and the high BW gain in PII. There was no difference in total BW accretion between the 70K and 100K (237 kg) but it was higher ( $P < 0.001$ ) than the HQS and PQS (220 kg).

**Table 1** Effects of winter feeding treatment on bodyweight (BW) and body condition score (BCS)

	HQS	PQS	70K	100K	SED	Sig
BW gain/day PI (kg/day)	0.28 <sup>a</sup>	0.27 <sup>a</sup>	0.52 <sup>b</sup>	0.24 <sup>a</sup>	0.133	0.001
BW gain/day PII (kg/day)	0.84 <sup>a</sup>	0.89 <sup>a</sup>	0.82 <sup>a</sup>	1.14 <sup>b</sup>	0.153	0.001
BW gain/day PIII (kg/day)	0.94	0.89	0.93	0.94	0.150	0.598
Average BCS PI	2.84 <sup>a</sup>	2.83 <sup>a</sup>	2.97 <sup>b</sup>	2.89 <sup>ab</sup>	0.1464	0.001
Average BCS PII	3.12	3.06	3.09	3.05	0.1973	0.610
Average BCS PIII	3.23	3.18	3.26	3.28	0.1476	0.076

S<sup>abc</sup> values in the same row not sharing a common superscript are significantly different

### Conclusion

The results from this study indicate that higher BW gains can be achieved during the winter period by offering a kale based diet which resulted in higher BW at mating start date. Furthermore, 100K animals had high BW gain in PII suggesting that it is possible to offer 100% kale, with no additional fibre source, over the winter period.

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## A clinical and pathological investigation of perinatal mortality in dairy calves: preliminary results from a recent prospective study

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**Introduction** Perinatal mortality (calf death within 48 hours of birth following a gestation period of at least 260 days) has increased recently internationally and in Irish dairy herds (Mee *et al.*, 2008). While traditionally the predominant aetiology was dystocia (Mee, 2009) the current causes of such loss are unclear. Results from necropsy examinations of calves at regional veterinary laboratories provide causes of death but may be biased towards causes of death not immediately obvious to the farmer or their veterinarian. Such passive surveillance data may also lack detailed epidemiological background information on the circumstances of the calf death. In contrast, a prospective active surveillance study design can gather both detailed anamnestic and pathological data on all calf mortality, irrespective of the perceived cause of death and hence give a more comprehensive picture of the causes of total herd perinatal mortality.

**Materials and methods** Between January and May 2009, 40 necropsy examinations were carried out on calves which died within 48 h of calving on seven spring-calving dairy farms (1-15 calves/farm). In addition, blood samples were collected from a random selection of ten pregnant dams per herd. Epidemiological data (single/twin, parity, sex, breed, body condition score, gestation length, calving date and time and degree of assistance, calf clinical signs and time of death) were collected on carcass submission questionnaire forms and necropsies (body and organ weights, gross pathology, histopathology, microbiology, serology and micronutrient sampling) were carried out at Moorepark Dairy Production Research Centre. Additional laboratory analyses were conducted by the veterinary laboratories of the Department of Agriculture and Food in Cork (microbiological culture, fetal serology for *Leptospira hardjo* and *Neospora caninum*-Immunocomb test kits), Limerick (maternal blood selenium) and Backweston (liver copper, cobalt and selenium and kidney selenium analysis and BVDv tissue PCR, and BVDv serum antigen and antibody assays), the Veterinary Sciences Division of AFBI in Stormont, Northern Ireland (maternal blood plasma inorganic iodine, copper and magnesium) and the Scottish Agricultural Colleges (SAC), Penicuik, Scotland (neonatal hepatic and serum vitamin A and E analyses).

**Results** Of the 40 carcasses submitted, 38 (95%) were singletons, 29 (73%) were from pluriparae, 28 (70%) were from Holstein-Friesian sires and 23 (58%) were male. Sixteen calves (40%) were unobserved/unassisted at calving and 15 (38%) had difficult (score  $\geq 3$ ; 1-5 scale) calvings. The mean (sd) death weight of the calves was 34.6 (8.2) kg. The majority of calves were dead at birth/stillborn (78%) with 5, 10 and 8% dying 1min-1h, >1h-24h and >24-48h after calving, respectively. In over half the calves their lungs were atelectatic (53%) with the remained partially atelectatic (33%) or inflated (15%). Results were available for 32 calves where serum was examined for BVD antigen, of which none had antigen and 4 had antibodies (3 of which were >1h old), respectively. Of 26 calves with results for *L. hardjo* serum antibodies, 3 had titres  $\geq 1:50$  (all 3 were >1h old). Of 31 calves with results for *N. caninum* antibodies, 2 had inconclusive or positive titres (1 of which was >1h old). Of 33 calves with abomasal contents culture results none yielded significant isolates. Mean (sd) thyroid gland weight was 14.1g (5.95) with 2.5% of glands >30g and a mean (sd) thyroid/body weight ratio of 0.04 (0.014). The mean (sd) liver copper (n=36), selenium (36) and cobalt (11) concentrations were 1.8 (0.74) mmol/kg, 6.7 (2.19)  $\mu$ mol/kg and 0.2 (0.07)  $\mu$ mol/kg, WM, respectively. The mean (sd) kidney selenium (n=35) concentration was 9.1 (1.69)  $\mu$ mol/kg. Using the analyzing laboratories reference ranges (liver Co 0.7-5  $\mu$ mol/L, liver Cu 0.06-2.5 mmol/L, liver Se 0.75-3  $\mu$ mol/L and kidney Se 5-20  $\mu$ mol/L, WM), all of liver Co values were below range, 14% of liver Cu values were above range, all of kidney Se values were within range and all of liver Se values were above range. All of the herds had normal dam blood selenium, copper and magnesium status but only one had a normal blood iodine status; the others were high. Based on the clinical histories and necropsy and laboratory findings, causes of perinatal mortality were classified into foetal disorders [17, (43%); lethal congenital defects (5), umbilical haemorrhage (3), dead in utero (3), anoxia/eutocia (2), prematurity (2), goiter (1) and anaemia (1)], calving problems [14, (35%); malpresentation (7), dystocia (3), uterine torsion (2), milk fever (1), twinning (1)], placental problems [5, (13%), premature placental separation] and unexplained perinatal mortality [4, (10%)].

**Conclusions** From this preliminary study it is evident that the majority of perinatal mortality cases were stillborn, singleton males from pluriparae sired by Holstein-Friesian bulls with partially or completely atelectatic lungs without significant infections or micronutrient imbalances; calf mortality was primarily associated with foetal disorders and calving problems. The value of combining whole herd active surveillance with anamnesis and necropsy examination supported by laboratory testing is evident from the inclusion of both clinical and pathological diagnoses in the causes of death detected.

**Acknowledgements** The author acknowledges the technical assistance of Tom Condon and Jonathon Kenneally and the collaboration of colleagues in the Veterinary Laboratory Service of DAFF, AFBI and SAC.

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## Effect of weaning system on the performance of dairy calves fed using computerised milk and concentrate dispensers

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**Introduction** Feeding milk or milk replacer can be a labour intensive practice, therefore both feed and labour costs can be minimized by weaning at an earlier age (Gleeson *et al.*, 2007). Basing the decision to wean calves on concentrate intake, Greenwood *et al.* (1997) reduced weaning age by almost 14 d without impacting on calf performance. Group housing of calves has increased in popularity, but until the recent development of computerized feeders, managing calves in groups did not enable weaning based on individual calf concentrate intake. Consequently, the objective of the present study was to investigate the effect of weaning method on performance and feed intake of Holstein-Friesian calves reared from 5 d of age using computer-controlled feeders.

**Material and methods** Group-housed Holstein Friesian calves ( $n = 77$ ), were randomly assigned to milk feeding systems with either (1) weaning at 8 weeks (Standard) or (2) weaning based on concentrate intake (Auto). Calves commenced the study at 4 days of age and were fed using computerized milk and concentrate dispensers. For Treatment 1 calves, milk replacer was offered at a rate of 500 g/d from 4 to 51 days of age and then reduced in equal steps from day 51 to weaning at day 56. For Treatment 2 calves, milk replacer was offered at a rate of 500 g/d until concentrate intake averaged 500 g/d for 4 consecutive days at which stage the computer-controlled programme automatically reduced milk replacer intake. When calves on the auto treatment consumed an average of 1.5 kg/d of concentrate over a 4-d period, milk replacer feeding was finished. Calves that did not achieve a concentrate intake of 500 g/d and initiate the auto weaning facility before day 51, were weaned following the standard treatment. Calf performance, health and feed intakes were recorded and both feed and economic efficiency determined. Weekly milk and concentrate intakes were analyzed by repeated measures analysis of variance using the Genstat REML procedure. This fitted a model with week as the time factor and fixed effects for sex, birth weight and weaning system plus the week by fixed terms interactions. Live weight and body size data were analyzed both separately and together for male and female calves, with males removed from the study at week 10. Female calf live weight and body size from week 10 onward were analyzed by ANOVA with week 1 value used as a covariate.

**Results** Total milk and concentrate intake until weaning was 23.1 and 21.9 kg DM/calf respectively, for calves on the standard treatment and 18.6 and 17.0 kg DM/calf respectively for calves on the auto treatment (Table 1). Weaning age was reduced by 8 days on the auto treatment, resulting in calves that were lower in live weight (-7.6 kg,  $P < 0.001$ ) at weaning. However at 40 wk of age, weaning treatment had no effect on heifer live weight.

**Table 1** Performance of calves weaned at a fixed age or based on concentrate intake

Performance parameters	Weaning system		SED	Sig.
	Auto	Standard		
Milk replacer intake until weaned (kg)	18.6	23.1	0.53	***
Concentrate intake until weaned (kg DM)	17.0	21.9	1.53	**
Weaning age (day)	47.2	55.0	1.17	***
Live weight at weaning (kg)	59.3	66.9	1.31	***
Feed cost until weaning (£)	30.07	37.56	0.93	***
Gain : kg DM	0.59	0.64	0.028	0.088
Gain : £ feed	0.68	0.75	0.030	*
Live weight at 40 weeks of age (kg)	226	234	7.7	NS

**Conclusions** When using computerized feeders, weaning calves based on the level of concentrate intake as opposed to calf age reduced the age and live weight at weaning, although differences in live weight disappeared post-weaning. Efficiency of liveweight gain until weaning was lower with calves weaned based on concentrate intake however overall feeding costs were reduced compared with calves weaned at a fixed age.

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## Factors affecting ovulation potential in Holstein-Friesian dairy cows

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**Introduction** Reproductive performance in high yielding Holstein-Friesian (HF) dairy cows has severely declined over the last 40 years. The cause is multifactorial, however, there is substantial evidence suggesting that the degree of negative energy balance (NEB) is highly influential (Beam and Butler, 1999). During early lactation, high yielding dairy cows are unable to consume sufficient food to support the high level of milk production and therefore experience severe and prolonged NEB, causing excessive mobilisation of body energy reserves. Severe NEB has been shown to extend the interval from calving to the commencement of luteal activity and the interval to EB nadir is correlated with the number of days to the first post-partum ovulation (Staples *et al.*, 1998). The aim of the current study was to assess the effects of NEB on growth of the dominant follicle (DF), on the pulsatility and surge of LH preceding ovulation and on the growth of the corpus luteum thereafter.

**Materials and methods** Post-calving; ten multiparous HF dairy cows (mean parity, 4.2) were offered, *ad libitum*, a total mixed ration comprised of 55% concentrate and 45% forage (80% grass silage + 20% maize silage; DM basis). The diet contained 12.5 MJ metabolisable energy and 186 g crude protein per kg DM. Animals were synchronised to ovulate at days 42, 70 and 98 postpartum using controlled intra-vaginal drug release (CIDR) of progesterone. A rectal ultrasound scan was performed on alternate days after CIDR insertion to assess follicular growth; then at 6 hr intervals for 72 hrs after CIDR removal to detect ovulation; and also on alternate days from 72 hrs after CIDR removal to day 7 post-insemination, to assess growth of the corpus luteum. Prior to CIDR removal, an indwelling catheter was placed in the jugular vein. Following CIDR removal, blood was collected at 10 minute intervals for 8 hrs to determine LH pulsatility and at 3 hr intervals thereafter, for a total of 72 hrs, to detect the LH surge preceding ovulation. Milk progesterone was assessed daily from CIDR insertion to day 7 post-insemination. Animals were inseminated 56 hrs after CIDR removal and upon detection of ovulation. Data were analysed using REML and linear regressions via Genstat.

**Results** During the experimental period, cows had an average milk yield of 41.4 kg/d (S.E.D. 2.3), average dry matter intake of 21.7 kg/d (S.E.D. 0.4) and average EB of -33.4 MJ/d (S.E.D. 13.2). There was no difference in milk yield between cows that ovulated and those that did not but cows that ovulated had a significantly higher DMI (20.2 vs. 18.5 kg DM/d; SED, 0.76;  $P < 0.05$ ) during weeks 1 to 5 postpartum. Animals that ovulated had higher peak LH concentration, a higher DF growth rate and a larger DF at ovulation than those that failed to ovulate (Table 1).

**Table 1** Effects of energy, blood and follicular parameters on ovulation.

	Ovulated	Not ovulated	SED	Sig
LH peak concentration (ng/ml)	9.28	4.70	1.87	*
Interval from CIDR removal to LH peak (hrs)	37.0	44.0	5.49	NS
DF growth rate from CIDR insertion to CIDR removal (mm/day)	0.9	0.4	0.16	**
DF growth rate from CIDR removal (mm/day)	2.9	1.4	0.25	***
DF diameter at CIDR removal (mm)	23.7	18.2	0.91	***
DF area (mm <sup>2</sup> )*	241	133	27.35	***
DF max diameter (mm)*	27.9	20.3	1.71	***
Milk progesterone concentration (ng/ml) on d7 post-insemination	14.4	6.60	2.68	**

\* DF area/ maximum diameter at ovulation or 69 hrs post CIDR removal in ovulated and non-ovulated animals respectively

There were no significant effects of blood metabolites during weeks 1 to 5 postpartum on ovulation; however, significant ( $P < 0.05$ ) relationships were observed between plasma NEFA concentrations and the interval from CIDR removal to LH peak (positive relationship) and between plasma glucose concentrations and the interval from CIDR removal to the LH peak (negative relationship).

**Conclusions** High yielding Holstein-Friesian dairy cows that did not ovulate showed reduced follicular development, delayed LH peak and reduced peak LH concentration compared to animals that ovulated. Increases in blood metabolite concentrations associated with the mobilisation of body reserves negatively affected DF growth and the interval to peak LH, both of which have previously been associated with reduced reproductive performance.

**Acknowledgements** This study was sponsored by DARDNI and AgriSearch.

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## The effects of genetic merit for fertility traits on ovarian dynamics during the oestrous cycle in lactating dairy cows

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**Introduction** The incorporation of fertility traits into selection indexes has allowed identification of animals with similar genetic merit for milk production traits, but divergent genetic merit for fertility traits. Using a high and low fertility genetic model established in this manner, Cummins *et al.* (2009) demonstrated similar phenotypic milk production, but divergent phenotypic fertility performance. The aim of this study was to characterise the ovarian dynamics of a complete oestrous cycle in cows with divergent genetic merit for fertility traits but similar genetic merit for milk production traits.

**Material and methods** The Estimated Breeding Values (EBV) of the high (n = 19) and low (n = 12) fertility cows used in this study are summarised in Table 1. Cows were synchronised with a CIDR based protocol (GnRH + CIDR in on day 0, PGF<sub>2α</sub> on day 7 and CIDR out on day 8). Starting on the day of synchronised oestrus, transrectal ovarian ultrasound examinations were carried out daily, and continued until ovulation at the end of the ensuing cycle. The number, size and location of all ovarian structures were recorded daily. Statistical analysis was carried out using SAS. The number of follicular waves (NumWaves) was analysed using PROC NPAR1WAY and the Kruskal-Wallis test. Mixed model methodology was used to analyse continuous data using the model  $Y = \mu + \text{genotype} + \text{lactation} + \text{NumWaves} + e$ . Repeated measures was used to compare corpus luteum (CL) volume from day 0 to 16, and the number of small follicles (<5mm) during the first follicle wave. The model used was  $Y = \mu + \text{genotype} + \text{lactation} + \text{day of cycle} + \text{day of cycle} * \text{genotype} + e$ . Cow nested within genotype was included as a random effect in all mixed models.

**Table 1** The EBV (and SD) for milk production and fertility in cows with high and low EBV for fertility traits

Trait	High Fertility	Low Fertility
Milk BV (kg)	232 (69)	213 (64)
Calving interval BV (days)	-3.3 (0.7)	2.5 (1.0)
Longevity BV	1.6 (0.4)	-0.1 (0.5)
EBI production sub-index (€)	46 (16)	43 (12)
EBI fertility sub-index (€)	54 (8)	-30 (14)

**Results** Oestrous cycle measurements are summarised in Table 2. The high fertility sub-index group tended ( $P < 0.07$ ) to have fewer follicular waves (2.2 vs. 2.7), and on average a shorter oestrous cycle than the low fertility sub index group. Both groups commenced first wave follicular growth on similar days (1.4 days), but the low fertility group took longer ( $P < 0.05$ ) for the first wave dominant follicle (DF) to achieve its peak diameter; this peak diameter tended to be larger (16.1 vs. 14.7 mm) in the low compared to the high fertility group. There was no difference in the number of follicles <5 mm in diameter during the first follicle wave. The largest diameter of the ovulatory DF tended to be greater in the high than the low fertility group. Average CL volume was greater in the high than the low fertility group during the first 16 days of the oestrous cycle.

**Table 2** Oestrous cycle characteristics (and s.e.) of high and low fertility sub-index groups

	High Fertility	Low Fertility	P
Day of 1 <sup>st</sup> wave DF peak (Days)	7.7 (0.6)	9.9 (0.7)	0.019
Day of ovulation (Days)	21.9 (0.7)	24.1 (1.0)	0.054
Ovulatory DF max diameter (mm)	17.6 (0.4)	16.4 (0.5)	0.059
No. of follicles <5mm in 1 <sup>st</sup> wave	18.5 (1.4)	16.8 (1.7)	n.s.
CL volume (mm <sup>3</sup> )	6757 (280)	5737 (353)	0.026

**Conclusion** Some differences in ovarian follicular and CL dynamics were observed, demonstrating that genetic merit for fertility traits may be manifest in measurable changes in ovarian function. Further work is necessary to characterize the reproductive hormone profiles associated with the recorded differences in ovarian follicular and CL dynamics.

**Acknowledgements** Funding from the National Development Plan and the Dairy Levy Trust is gratefully acknowledged.

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## The relationship between plasma progesterone concentration during the early luteal phase and embryo survival in dairy heifers

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**Introduction** In cattle, early embryo death is a major cause of cow reproductive wastage. Low post ovulatory systemic progesterone (P4) or a delay in the normal increase in P4 concentrations during the early luteal phase have been associated with reduced embryo survival rate in dairy cows. However, the relationship(s) between systemic concentrations of P4 during the early luteal phase and the repeatability of these from cycle to cycle is as yet unequivocal. The objective of this study was to establish i) the relationships between plasma concentrations of P4 during the early luteal phase and subsequent embryo survival rate and ii) the repeatability of P4 concentrations during the early luteal phase from cycle to cycle in dairy heifers.

**Materials and methods** A total of 118 Holstein-Friesian heifers were used in the study. Oestrus was synchronised using two injections of a synthetic prostaglandin F<sub>2α</sub> analogue administered intramuscularly 11 days apart. At the oestrus following the second PGF<sub>2α</sub> injection, heifers were artificially inseminated (AI) by one of two operators using thawed frozen semen from one high fertility bull. Embryo survival was determined by ultrasonography on day 30-35 after AI. A positive pregnancy diagnosis was based on the presence of an apparently viable foetus with a visible heartbeat and clear amniotic fluid. Following pregnancy diagnosis all heifers were injected with PGF<sub>2α</sub> to induce embryo loss and return to oestrous cycles. Six weeks after the induced embryo loss, the entire experimental protocol was repeated on all heifers. All inseminated heifers were blood sampled twice daily (am and pm) on days 4, 5, 6 and 7 following each insemination. Concentrations of P4 were determined by radioimmunoassay (Coat-a-Count) and a mean concentration of P4 was calculated for each day. Logistic regression was used to evaluate the relationship between plasma concentrations of P4 on days 4, 5, 6 & 7 post insemination and embryo survival rate. Quadratic components included in the final model where significant. Regression analysis was used to evaluate the relationships between P4 on different days. Repeatability estimates ( $r_e$ ) for P4 were derived from an analysis of variance as the intraclass correlation among records on the same heifer on the same day for the two different rounds of insemination.

**Results** Embryo survival rate of heifers was 67 and 79% ( $P=0.06$ ) following inseminations 1 and 2, respectively. There was a positive linear ( $P<0.01$ ) and quadratic ( $P<0.01$ ) association between concentrations of P4 on days 4, 5, 6 and 7 post insemination and changes ( $\Delta$ ) in P4 on days 4 to 5 and 7 and embryo survival rate (Table 1). Higher embryo survival rates were initially associated with increasing concentrations of P4 while embryo survival rates declined at the highest concentrations of P4.

**Table 1** The relationship between early luteal phase concentrations of P4 and embryo survival (presented as Odds Ratios (OD)) in dairy heifers with upper and lower confidence intervals (CI)

	Adj R <sup>2</sup>	Progesterone fitted as Linear Component				Progesterone fitted as Quadratic Component			
		OD	Lower CI	Upper CI	Significance	OD	Lower CI	Upper CI	Significance
Day 4	0.085	11.986	2.559	56.138	$P<0.002$	0.554	0.372	0.827	$P<0.004$
Day 5	0.01	5.098	1.550	16.765	$P<0.01$	0.789	0.641	0.972	$P<0.03$
Day 6	0.11	3.732	1.695	8.219	$P<0.001$	0.874	0.791	0.965	$P<0.01$
Day 7	0.17	14.803	3.267	67.068	$P<0.001$	0.770	0.661	0.897	$P<0.001$
$\Delta 4-7$	0.15	9.810	2.502	38.459	$P<0.001$	0.723	0.592	0.883	$P<0.001$
$\Delta 5-7$	0.08	3.142	1.280	7.71	$P<0.01$	0.784	0.638	0.964	$P<0.02$
$\Delta 6-7$	0.003	0.049	0.762	1.445	$P>0.801$	-	-	-	-

Repeatability estimates for plasma concentrations of P4 on days 4 to 7 varied from 0.05 to 0.20. The relationships between concentrations of P4 on days 4, 5, 6 to concentration on day 7 are presented in Table 2.

**Table 2** The relationship between concentrations of P4 on days 4, 5 and 6 to that on day 7

Dependent Variable	Relationship to Day 7 Concentration	R <sup>2</sup>	Significance
Progesterone on Day 7	$3.01+1.94(P_4 \text{ on Day 4}) -0.066(P_4 \text{ on Day 4})^2$	0.22	$P<0.001$
Progesterone on Day 7	$1.97+1.14(P_4 \text{ on Day 5}) -0.04(P_4 \text{ on Day 5})^2$	0.39	$P<0.001$
Progesterone on Day 7	$1.24+1.066(P_4 \text{ on Day 6}) -0.04(P_4 \text{ on Day 6})^2$	0.57	$P<0.001$

**Conclusions** There was both a linear and quadratic relationship between concentrations of P4 on days 4 to 7 and changes in P4 between these days and embryo survival rate. Increasing concentrations of P4 were associated with increasing embryo survival rates while embryo survival declined at higher concentrations of P4. Early luteal phase concentrations of P4 had a low repeatability from cycle to cycle. Early luteal (days 4-5) concentrations of P4 were a reasonable predictor of concentrations on day 7 and could be used to predict animals at risk of embryo loss.

**Acknowledgment** Funded by Science Foundation Ireland (07/SRC/B1156).



## Uterine endometrial gene expression in heifers of high or low fertility on day 14 of the estrous cycle

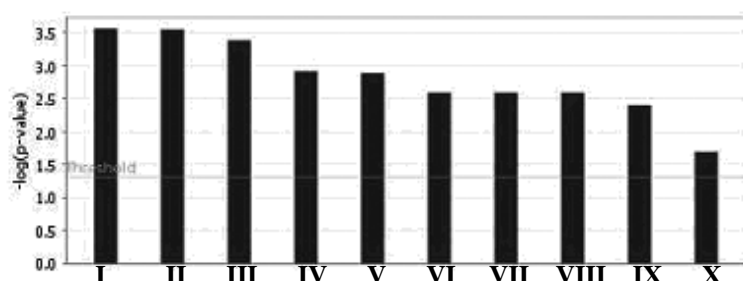
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**Introduction** Early embryo loss, occurring within the first two weeks of pregnancy, constitutes 70-80% of reproductive wastage (Diskin *et al.*, 2002). Repeatable differences between heifers in their ability to become pregnant have been established and uterine rather than ovarian factors are generally thought to account for these differences (McMillan *et al.*, 2001). However, the biochemical mechanisms governing uterine mediated early embryo development, or indeed the specific timeframe within the first two weeks of pregnancy during which most loss occurs, are not yet known. We previously reported that a number of genes with identified roles in embryonic development were differentially expressed in uterine endometrial tissue on day 7 of the estrous cycle, between heifers ranked either high or low for fertility (Killeen *et al.*, 2009). The aim of the current study was to further elucidate the underlying molecular mechanisms contributing to early embryo loss by examining differential uterine endometrial gene expression of high or low fertility heifers on day 14 of the estrous cycle.

**Material and methods** Reproductively normal crossbred beef heifers (n=120) were inseminated and pregnancy diagnosis was carried out 28 days later, after which animals were programmed to return to estrus. Animals were re-inseminated followed by pregnancy diagnosis on a further four occasions. On the basis of pregnancy rate to 5 successive inseminations, animals were divided into two groups: i) those that established pregnancy on all 5 occasions (high fertility group) and ii) animals achieving pregnancy on only one occasion (low fertility group). Estrous cycles were then synchronised following administration of a prostaglandin F<sub>2α</sub> analogue (Estrumate®). Animals from both high (n=7) and low groups (n=7) were slaughtered on day 14 of the estrous cycle and uterine endometrial tissue (ipsilateral to corpus luteum) was harvested and stored at -80°C in RNAlater solution. RNA was extracted using TRIzol® reagent, purified, DNase treated, and quantified and quality control checked using the 2100 Agilent Bioanalyser. RNA samples were then biotinylated and hybridised to the Affymetrix 23,000 bovine master Gene Chip according to manufacturers' protocol. Normalisation and statistical analysis were carried out using R and the PUMA method in Bioconductor (Pearson *et al.*, 2009). Functional analyses were generated through the use of Ingenuity Pathway Analysis (IPA, CA, USA).

**Results** Differentially expressed genes (DEGs) totalled 430 ( $P < 0.05$ ), 156 of these were up-regulated and 274 down-regulated. Genes mapped were implicated in a host of biological pathways including lipid metabolism, LDLR, PTGS2, PCCB, ACOT4 & ALOX5; cellular growth and proliferation, PPARG, ABTB1, HCRT, BRAF & MC4R; vitamin and mineral metabolism, SLC27A4, G6PC, ARG2 & CYP4F3; nutritional diseases, MC4R & LDLR; inflammatory response, APRT, PTGS2 & C6 and embryonic development, PPARG, BRAF & ITGA6. The top canonical pathways generated found to contribute to low conception rate, included mitochondrial function, citrate cycle, oxidative phosphorylation and linoleic acid metabolism. The many mapped canonical pathways, while simultaneously contributing to an extensive list of generated biological processes (Figure 1), illustrates the likely multifactorial nature of endometrial function between high and low fertility animals. In agreement with these findings, a previous study by our group (Killeen *et al.*, 2009) using endometrial tissue collected on day 7 of the estrous cycle show similar transcript profiles between high and low fertility heifers. For example, DEGs expressed similarly in both studies include: PCCB, SLC25A24, DAP and COL4A4 ( $P < 0.05$ ). Of interest, PCCB; the gene encoding propionyl-CoA carboxylase beta polypeptide, is contained within the blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) locus. There are two types of this congenital defect: BPES I & II. Female infertility differentiates the former from the latter (Piemontese *et al.*, 1997). Thus, future work will determine if variants of this gene could potentially serve as a marker for fertility in cattle.



**Figure 1** Key gene networks expressed in bovine endometrial tissue.

- I Organismal Functions
- II Lipid Metabolism
- III Vitamin & Mineral Metabolism
- IV Cell Growth & Proliferation
- V Nutritional Disease
- VI Cell Morphology
- VII Haematological System
- VIII Inflammatory Response
- IX Tissue Morphology
- X Embryonic Development

**Conclusion** Expression of genes involved in key biological pathways including embryonic development are differentially regulated in the uterine endometrium of heifers of high compared with low fertility on day 14 of the estrous cycle.

**Acknowledgements** The authors greatly acknowledge funding from Teagasc.

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## Incidence of fertilization failure and embryo loss in Holstein Friesian heifers and postpartum dairy cows

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**Introduction** The cost of replacing cows that are culled due to reproductive failure is approximately £18,000 per 100 cow herd per year. It is estimated that fertilisation rates following artificial insemination are >90% (Diskin *et al.*, 2006) and yet calving rate to a single insemination is in the order of 31% (Law *et al.*, 2009). The majority of this reproductive wastage occurs between insemination and maternal recognition of pregnancy, approximately 16 days after ovulation, with relatively little late embryonic/foetal mortality. The objective of this study was to quantify reproductive wastage prior to day 7 post insemination and to evaluate energetic, metabolic and hormonal effects in early lactation (day 0-42) on embryonic loss.

**Material and methods** Sixty-three autumn-calving Holstein Friesian cows (mean parity 3.1) and 32 nulliparous Holstein Friesian heifers (mean age 15 months) were used (Study 1). Lactating cows were offered a total mixed ration (TMR) comprising 60% concentrates and 40% forages (60% grass silage and 40% maize silage) on a DM basis. The complete diet contained 185 g CP/kg DM and 12.4 MJ ME/kg DM. An average daily energy balance for each individual cow was calculated for each week of lactation according to Thomas (2004). Heifers were offered grass silage *ad libitum* and 3 kg concentrate per head per day. A subsequent study (Study 2) was conducted on 16 spring calving Holstein Friesian cows (mean parity 2.8) and 18 nulliparous Holstein Friesian heifers (mean age 14 months) to validate the technical aspects of single embryo recovery. Lactating cows were offered a TMR comprising 50% concentrate and 50% forage (65% grass silage and 35% maize silage) on a DM basis. Heifers were offered grass silage *ad libitum* and 3 kg concentrate per head per day. Individual cow intakes were not recorded in Study 2. Oestrous cycles were synchronised to ovulate at days 42, 70 and 98 post calving in Study 1 and 61 d post-calving in Study 2, using a controlled intra-vaginal drug release (CIDR) of progesterone. Animals were bred by AI approximately 56 h after CIDR removal. The uteri of all animals were non-surgically flushed 7 days post-insemination to recover and classify embryos; the proportion of unfertilised oocytes, degenerate embryos and viable embryos (morula/blastocyst stage) was recorded. Data were analysed using logistic regression models via Genstat.

**Results** Only animals that were detected in oestrus and had normal luteal phase progesterone concentrations were included in the data set. In Study 1, cows had an average milk yield of 38.0 kg/d (100 d), an average DM intake of 21.5 kg/d (100 d), and an average six week energy balance of -28.0 MJ/d. In Study 2, cows had an average milk yield of 34.8 kg (61 d). The recovery rate did not differ ( $P>0.05$ ) between heifers and cows but the proportion of oocytes fertilised (as a proportion of recovered structures) was significantly lower in lactating cows than heifers (32.5 vs. 81.3%, respectively;  $P<0.001$ ).

**Table 1** Recovery and fertilisation rates from single oocyte flushes of Holstein Friesian cows and heifers (Studies 1 & 2)

	Period	Flushes (n)	Nothing recovered (n)	Unfertilised Oocyte (n)	Blastocyst Morula (n)	/	Recovery rate (%)	Fertilization rate (%)
Heifers	Study 1	35	25	3	7		28.6	70.0
	Study 2	18	12	0	6		33.3	100.0
	Total	53	39	3	13		30.2	81.3
Cows	Study 1	91	55	24	12*		39.5	33.3
	Study 2	16	12	3	1		25.0	25.0
	Total	107	67	27	13		37.4	32.5

\* Two structures were degenerate; one at the 2 cell stage and the other at the 8-16 cell stage.

Cows with fertilised oocytes (Study 1) had a more positive average daily energy balance (EB) in week 1 post calving than cows yielding an unfertilised oocyte (-10.5 vs. -46.8 MJ/d;  $P<0.01$ ; SED 11.49). Similarly, cows with fertilised oocytes had a more positive cumulative EB in week 1 (-75.7 vs. -326.6 MJ;  $P<0.01$ ; SED 80.0) and 2 (-252.9 vs. -614.4 MJ;  $P=0.058$ ; SED 183.6) post calving than cows with unfertilised oocytes. Plasma urea concentrations were lower in cows with fertilised oocytes in week 1 post calving compared to those with unfertilised oocytes (4.30 vs. 5.66 mM;  $P<0.01$ ; SED 0.48).

**Conclusions** The results clearly demonstrate that single embryo recovery is difficult with 66.3% of animals flushed yielding no oocyte/embryo. Fertilisation failure occurred in 53.5% of animals (67.5% of cows, 18.8% of heifers) which is greater than 10-20% estimated by Diskin *et al.* (2006). Furthermore, energy status in early lactation significantly affected fertilisation rate. Reproductive wastage during the 7 day period post insemination was lower than anticipated, with 15.4% of fertilised oocytes from lactating cows being degenerate.

**Acknowledgements** This project was jointly funded by DARDNI and SFI

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## Mathematical model of the bovine oestrous cycle

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**Introduction** Systems biology aims at understanding how the various components of a biological system function together, rather than investigating individual parts. One approach is the translation of a conceptual biological model into a set of mathematical equations that represent dynamic relations between system components. The purpose of building such mathematical models is to interpret and predict dynamics of complex biological systems, and to identify new research questions. One example of a dynamic biological system is the bovine oestrous cycle. Regulation of the cycle is controlled by numerous factors, interacting through feedback loops. Therefore, it is hard to obtain insight in the dynamics of the system by describing isolated parts. Mathematical modelling of the involved mechanisms is expected to improve insight in biological processes underlying female reproduction, and could thereby help to find causes of declined fertility in dairy cows (Boer *et al.*, 2009). Such a model is recently developed for the human menstrual cycle (Reinecke and Deuflhard, 2007). The objective of this work was to develop a mechanistic model that simulates the dynamics of the bovine oestrous cycle at individual cow level.

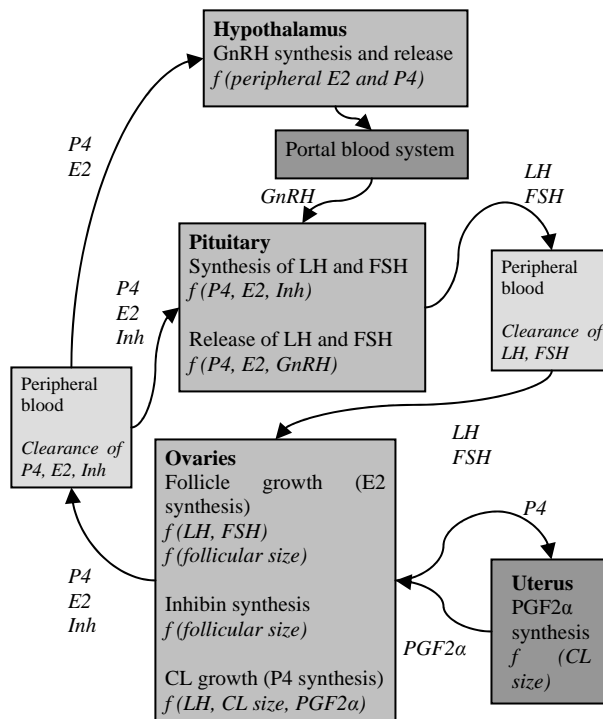
**Material and methods** The model was constructed by first defining a number of key components of the system and their interactions, which were represented in a flow chart. Subsequently, a set of differential equations were derived to describe the relations mathematically. Time delays were incorporated when appropriate, to capture the time needed for factors to influence each other. Hill functions were used for modelling of inhibitory and stimulatory effects of hormones.

**Results** The current model comprises a mathematical representation of follicle development and accompanying fluctuations in hormone concentrations in a cycle with three follicular waves. The main organs involved in the regulation of the oestrous cycle are the ovaries, hypothalamus and pituitary. These organs interact via hormones in the blood (Figure 1). Differential equations describe the control of the hormones oestradiol (E2), progesterone (P4), gonadotropin releasing hormone (GnRH), luteinizing hormone (LH), follicle stimulating hormone (FSH), inhibin (Inh) and prostaglandin (PGF2 $\alpha$ ) on the growth of follicles and CL. Simulations show that the model is able to describe the system consistent with empirical data for cows (Figure 2).

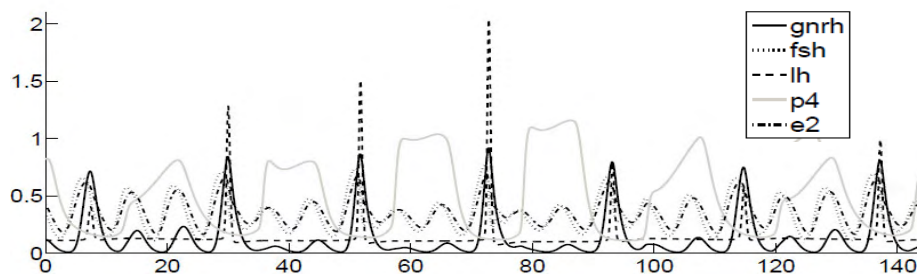
**Conclusions** This model could help in understanding the dynamic regulation of the bovine oestrous cycle. It is expected that this model will serve as a basis for more elaborate models and simulations, with the ability to study effects of external manipulations and genomic differences. Possible extensions of the model could be in the field of energy metabolism, stress, and factors affecting the expression of oestrous behaviour. The model can be used to determine the level of control exerted by various system components on the functioning of the system. Further, hypothesised causes of declined fertility in dairy cows could be tested by changing model parameters.

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**Figure 1** Schematic representation of the dynamic model of the bovine oestrous cycle. Each process is represented by a set of differential equations, indicated by  $f(\dots)$ .



**Figure 2** Preliminary simulation result, showing hormone fluctuations in consecutive cycles.

## Effect of calving ease on the subsequent performance of cow and calf in UK Holstein-Friesian cattle

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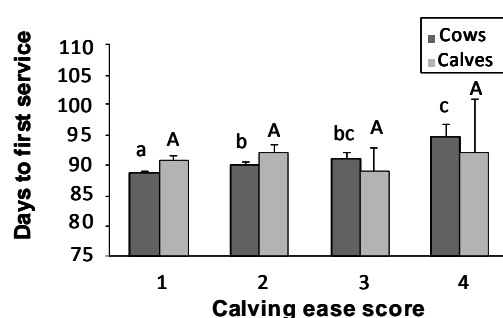
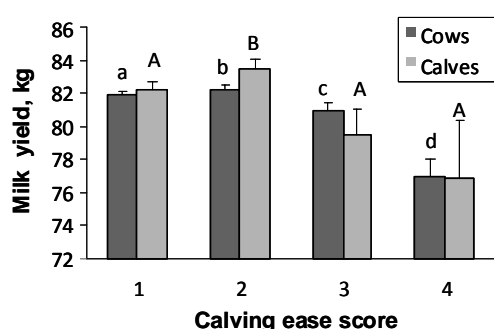
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**Introduction** Being one of the most economically significant non-production traits, calving ease has a large impact on the dairy industry (Dekkers, 1994). In addition, calving difficulty is ranked an extremely painful condition of cattle (Huxley and Way, 2006), which suggests that the impact of this trait also greatly affects animal welfare. It is therefore plausible that there will be a detrimental effect of a difficult calving on the subsequent performance of the animals involved. The objective of this study was to use UK Holstein-Friesian cattle data to phenotypically analyse the effect of calving ease on fertility and milk production, of both cow and calf, in their subsequent lactation.

**Material and methods** This study was restricted to first parity calving ease records only, provided by the Cattle Information Service (CIS) and National Milk Records (NMR) and recorded on a 4 -grade and 5 -grade scale respectively. Categories were defined by CIS as: 1-ease, 2-assisted, 3-difficult, 4 -vet assisted, and by NMR as: 1- normal (not assisted), 2- moderate assistance (farmer), 3- moderate assistance (vet called as precaution) 4 - difficult (extraction by farm staff), 5- very difficult calving (vet assisted). To harmonise scales, category 2 and 3 of the NMR scale were merged; both referring to 'moderate assistance required'. Merging the calving ease and fertility data led to a total of 32,483 and 8,184 records of cow and calf, respectively, originating from 2,486 and 1,410 herds. Fertility data consisted of the calving interval (CI), number of inseminations (NINS), non-return at 56 days (NR) and days to first service (DFS), of the first lactation following the recorded calving. CI was restricted between 300-600 days. Merged calving ease data and production data led to a total of 348,648 and 81,885 records of cow and calf, originating from 2,148 and 1,153 herds, respectively. Production data consisted of the milk yield (kg) recorded at multiple test days in the lactation. The production trait analysed in this study equals the cumulative milk yield of the first three test days, when animals were on average up to 90 days in milk. In all datasets, age of cow was restricted between 18-40 months, to be certain that data included only first parity records. Data were analysed using linear regression and Restricted Maximum Likelihood in ASREML (version 2.00, 2006) fitting fixed effects of calving ease score, month of calving, year of calving, age of cow, data source, days in milk and condition score and a random effect of herd for the fertility traits and herd-testdate for the production trait.

**Results** There was a significant difference in the fertility of cows for different categories of calving ease. Between an easy calving (1) and vet assisted calving (4), there was an increase of 28 days (s.e.d. = 8.04) in CI, 0.7 services (s.e.d. = 0.090) in NINS, and 7 days (s.e.d. = 2.15) in DFS (Figure 1). NR showed a decrease of 9% between an easy and vet assisted calving, though this was not significant. Analyses of the fertility of calves, in their first lactation, did not show significant differences between the easy and vet assisted calving ease categories. The effect of calving ease on the cumulative milk yield of the first three test days is presented in Figure 2 which shows a significant difference between all calving ease categories in the cow analysis. Between an easy and vet assisted calving, milk yield decreased by, on average, 1.67 kg per test day (s.e.d. = 0.85), or 6.1%. The analysis of the calves shows a tendency to decrease in milk yield between a farmer assisted and vet assisted calving of 2.20 kg (s.e.d.= 3.4) on average per test day, or 7.9% (P=0.057).



**Figure 1** Days to first service per category of calving difficulty **Figure 2** Accumulated milk yield per category of calving difficulty

**Conclusions** The results of the study indicate that there is a detrimental effect of a bad calving on the subsequent fertility and production of the cow, thereby supporting the findings of De Maturana *et al.*, 2007. When calving difficulty increases, fertility declines and milk yield in the first stage of lactation decreases. A significant effect of calving ease on the subsequent production and fertility of the calves was not found. Though, calves which were delivered with veterinary assistance showed a tendency to decrease in milk yield in the first stage of their lactation as heifers, in comparison to calves delivered with moderate farmer assistance. Further study is needed to analyse the effect of calving ease on the full subsequent lactation.

**Acknowledgements** The authors gratefully acknowledge funding from The Scottish Government, Defra, NMR, CIS, HUK, Genus, DairyCo, and Cogent

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## Progesterone mediated changes in the composition of cattle oviduct and uterine fluid

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**Introduction** Inadequate systemic concentrations of progesterone in the first few days post insemination are associated with a low probability of embryo survival in cattle. One mechanism by which progesterone can affect embryo survival is by altering the composition of oviduct or uterine fluid on which the embryo is dependent for its normal growth and development. The aim of this study was to characterise the effects of changes in the systemic concentration of progesterone on the ionic, amino acid and energy substrate composition of oviduct and uterine fluid in cattle.

**Materials and methods** All blood sampling, infusion and surgical procedures were carried out under license and in accordance with the European Community Directive, 86-609-EC. Reproductively normal, nulliparous crossbred heifers of similar age (average  $20 \pm 4$  months), live weight Exp 1,  $413\text{kg} \pm 6.39$ ,  $n=11$ ; Exp. 2,  $409\text{kg} \pm 8.51$ ,  $n=9$ ) and body condition score (BCS  $3.2 \pm 0.1$  units) were used in this study. Oestrus was synchronised by two injections of synthetic prostaglandin (PG; 500 $\mu$ l Cloprostenol, Estrumate®, Schering-Plough, Animal Health, Ireland) administered 11 days apart. All heifers were kept indoors and given 4 kg of concentrates and ad-lib silage. In Experiment 1, beginning on Day 1 of the oestrous cycle, heifers were infused, using a jugular venous catheter attached to a peristaltic pump, with 2.5ml ethanol/hr for 48 hr (Control), or with 0.5mg progesterone/2.5ml ethanol/hr for the first 24 hr and then with 1.0mg progesterone/2.5ml ethanol/hr for the next 24 hr (Supplemented). In Experiment 2, beginning on Day 4 of the oestrous cycle, heifers were infused with 2.5ml ethanol/hr for 48 hr (Control), or with 1.5mg progesterone/2.5ml ethanol/hr for the first 24 hr and then with 3.0mg progesterone/2.5ml ethanol/hr for the next 24 hr (Supplemented). Following infusion, oviduct or uterine fluid was collected over a period of 3 hours using an in-dwelling catheter during midline laparotomy. Plasma progesterone and oestradiol were measured by radioimmunoassay (RIA). The concentrations of eighteen amino acids were measured in oviduct fluid and blood plasma by reversed-phase HPLC (Hugentobler *et al.*, 2007a). Anions and cations were measured by ion chromatography (Hugentobler *et al.*, 2007b). The concentrations of glucose, lactate and pyruvate were measured in oviduct fluid and blood plasma using an autoanalyser (Hugentobler *et al.*, 2008). The concentrations of ions, amino acids and energy sources, in oviduct and uterine fluid and blood were analysed by analysis of variance (PROC GLM, SAS v9.10, Cary, NC). Terms included in the model were treatment (control or supplemented), side (ipsilateral or contralateral to corpus luteum) and side x treatment. There was no evidence of a side x treatment interaction or side effect for any ion, amino acid or energy source. Consequently, the data for ipsilateral and contralateral oviducts or uteri were combined and an average value for each animal calculated. A probability of  $P<0.05$  was considered significant.

**Results** In Experiment 1 systemic progesterone concentration increased over 4-fold from  $0.66 \pm 0.11\text{ng/ml}$  ( $n=4$ ) to  $2.88 \pm 0.39\text{ ng/ml}$  ( $n=7$ ) ( $P<0.001$ ) following progesterone supplementation. Systemic oestradiol concentration was not different between control ( $0.35 \pm 0.16\text{ ng/ml}$ ) and supplemented ( $0.38 \pm 0.10\text{ ng/ml}$ ) groups ( $P>0.05$ ). In Experiment 2, systemic progesterone concentration increased almost 2-fold from  $4.94 \pm 0.44\text{ng/ml}$  ( $n=5$ ) to  $8.19 \pm 1.17\text{ ng/ml}$  ( $n=4$ ) ( $P<0.05$ ) following progesterone supplementation. Systemic oestradiol concentration was not different between control ( $0.41 \pm 0.15\text{ ng/ml}$ ) and supplemented ( $0.22 \pm 0.11\text{ ng/ml}$ ) groups ( $P>0.05$ ). Supplementary progesterone had no effect on the energy substrates in oviduct fluid but increased ( $P<0.05$ ) glucose concentration in uterine fluid. Nine of 20 amino acids showed an increase ( $P<0.05$ ) in concentration in oviduct fluid following supplementary progesterone, with glycine showing the largest increase of approximately 2-fold. Progesterone supplementation increased the concentration of valine in uterine fluid. Sulphate concentrations in oviduct fluid decreased ( $P<0.05$ ) following supplementary progesterone, however, there was no effect of supplementary progesterone ( $P>0.05$ ) on the concentrations of any ion in uterine fluid.

**Conclusions** The progesterone mediated changes described in the composition of oviduct and uterine fluid may explain, at least in part, the positive relationship between systemic progesterone and embryo growth, development and viability. It is likely that the embryo response to progesterone is the result of changes in the amino acid composition of the oviduct. These data extend our knowledge of the physiological environments of the cattle oviduct and uterus.

**Acknowledgements** This research was part funded by the Research Stimulus Fund of the Department of Agriculture, Food and Rural Development, Agriculture House, Dublin, Ireland.

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## The effect of supplementation with conjugated linoleic acid on the reproductive performance of lactating dairy cows

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**Introduction** Trans-10, cis-12 conjugated linoleic acid (CLA) is a potent inhibitor of milk fat synthesis (Baumgard *et al.*, 2002). Feeding supplemental CLA may be a means of ameliorating negative energy balance (NEB) post partum, potentially improving subsequent reproductive performance. A recent meta-analysis of 5 controlled studies where CLA had been supplemented to early-lactation dairy cows indicated that CLA supplementation significantly reduced interval to first ovulation and time to conception, and increased the probability of cows becoming pregnant (de Veth *et al.*, 2009). The aim of the present study was to examine the effects of CLA supplementation on milk production and reproductive performance of lactating dairy cows in a field scale situation.

**Materials and methods** 389 spring calving pasture-based dairy cows on a single commercial dairy farm were enrolled on the trial and randomly assigned to one of two dietary treatments (CLA (n=192) or Control (n=197)). The two treatments were balanced for parity and calving date. The cows were milked twice daily in a 60-unit rotary parlour with automatic cow identification, automatic feeding, and electronic milk meters (Dairymaster, Kerry, Ireland). All cows were fed an equal amount of concentrate ration in the parlour. The CLA cows received an additional 50g/day of a lipid supplement (Lutrell, BASF, Germany) from parturition until 60 days in milk. The CLA supplement contained a 50:50 mix of cis-9, trans-11 CLA and trans-10, cis-12 CLA, resulting in a daily intake of 5 g per day of each isomer which was automatically dispensed using a PowerDos® feeding system (Hanskamp AgroTech BV, Zelhem, The Netherlands). Milk samples were collected 3 mornings per week, and each sample was analysed for progesterone using a competitive ELISA test, in order to determine interval to first ovulation. Milk yield and composition were measured fortnightly. Trans-rectal ultrasonography was carried out prior to mating start date to ascertain utero-ovarian status. Heat detection was carried out with the aid of MooMonitor® activity collars (Dairymaster, Kerry, Ireland) and tail paint. Trans-rectal ultrasonography was carried out at 30-36 days and 60-66 days post AI to determine conception rate and embryo loss. Milk yield, milk composition, and interval to first ovulation data were analysed using mixed model analysis. All other reproductive data was analysed using the Chi-squared test.

**Results** Milk yield and composition data are summarized in Table 1. A significant reduction in milk fat concentration and yield was observed in CLA treatment cows during the supplementation period. Milk protein concentration was also reduced. Milk yield was increased by CLA supplementation, although milk solids yield was not affected. Reproductive performance data are summarized in Table 2. There was no effect of CLA on interval to first ovulation, 6-week submission rate, conception rate to first service, embryo loss after first service or 6-week in-calf rate.

**Table 1** Effect of CLA supplementation on milk production and composition

	Control	CLA	S.E.M	P-value
Milk yield (kg/day)	24.7	27.2	0.70	0.003
Milk fat (g/kg)	36.9	30.7	0.60	<0.001
Milk protein (g/kg)	32.8	31.2	0.30	<0.001
Milk fat yield (kg/day)	0.91	0.84	0.02	0.031
Milk protein yield (kg/day)	0.81	0.85	0.02	0.11
Milk solids yield (kg/day)	1.72	1.69	0.05	0.60

**Table 2** Effect of CLA supplementation on reproductive performance

	Control	CLA	P-value
Interval to first ovulation (days)	40.2	44.4	0.12
3-week submission rate (%)	54.8	58.0	0.50
Conception rate to first service (%)	35.1	37.0	0.70
Embryo loss to first service (%)	14.3	21.4	0.30
6-week In-calf rate (%)	43.6	37.0	0.20

**Conclusions** Supplementing dairy cows with CLA reduced milk fat synthesis. Contrary to previous reports, milk protein concentration was also reduced. However, reproductive performance was not improved by CLA supplementation. As reproductive performance was generally poor in this study, it may be that energy status was not the limiting factor and there may have been other problems influencing herd fertility.

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## Effect of feed space allowance on the performance of dairy cows offered grass silage based diets

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**Introduction** Meeting the higher nutrient requirements of high yielding dairy cows remains a key challenge. While many studies have examined nutritional approaches to achieve increased food intakes, for example increasing the nutrient density of the diet, 'non nutritional strategies' may also have an important role in achieving higher intakes. As most food consumed by housed cows is consumed at a feed barrier, options for optimising the feed barrier environment is one of these 'non nutritional strategies'. This is particularly important at present in view of rapidly expanding herd sizes on many farms. As herds expand, some farmers continue to make use of existing feed barriers, thus reducing the feed space per cow, while others add additional barrier space at considerable cost. However, there does not appear to be an agreed 'optimum' space allowance per cow, with recommendations ranging from 20 to 100 cm per cow. If feed space is inadequate, this may have a detrimental effect on food intake, performance and welfare, and it might be expected that primiparous animals would be particularly affected. To address this issue, an experiment was undertaken to examine the effect of feed space allowance on the performance of dairy cows offered grass silage based diets.

**Materials and methods** Forty-two Holstein-Friesian dairy cows were allocated to one of three treatment groups (nine multiparous and five primiparous cows per group) at calving (mean calving date, 1 January). The three groups were housed in adjacent, but visually isolated pens of equal size and similar layout, with 16 cubicles per pen. Within each pen cows accessed food via a 'post and rail' type feed barrier. Treatments examined comprised three horizontal feed space allowances, namely 20, 40 and 60 cm/cow. The experiment commenced with 14 late lactation non-experimental cows occupying each pen. Experimental cows were then transferred into the appropriate experimental pen within 36 hours of calving, and non-experimental cows removed, with the process repeated until each pen contained 14 experimental cows. Cows remained in their experimental groups for a mean of 127 days post-calving, with the period from the last cow calved until the end of the experiment being 88 days. Throughout the experiment cows had *ad libitum* access to a diet comprising grass silage and concentrates (65 : 35 DM ratio). Fresh food was offered at 10.30 h each day in the form of a mixed diet. Group intakes were recorded daily, but were not analysed statistically due to the unreplicated nature of the intake data. The effect of feed space allowance on mean animal performance during the experiment was analysed by ANOVA, with the individual cow used as the experimental unit.

**Results** Mean DM intakes with the 20, 40 and 60 cm/cow treatments were 19.0, 18.7 and 19.3 kg/cow/day. Feed space allowance had no significant effect on milk yield per cow, milk composition, milk somatic cell count, or on cow liveweight or condition score at the end of the experiment ( $P>0.05$ ).

**Table 1** Effect of feed barrier space allowance per cow on the performance of lactating dairy cows

	Feed space allowance per cow			SEM	Sig
	20 cm	40 cm	60 cm		
Total DM intake (kg/day‡)	19.0	18.7	19.3		
Total milk output (kg/day†)	3820	2823	3705	164.4	NS
Milk yield (kg/day†)	32.2	31.5	31.0	1.89	NS
Milk fat (g/kg†)	42.4	42.1	42.2	0.86	NS
Milk protein (g/kg†)	31.3	31.2	32.3	0.46	NS
Milk lactose (g/kg†)	48.6	48.2	48.8	0.39	NS
End of study condition score	2.6	2.6	2.6	0.05	NS
End of study live weight (kg)	585	595	608	8.9	NS

‡Final 88 days on experiment.

† From calving until end of study

**Conclusions** The results of this experiment, in which food was offered *ad libitum*, provide no evidence that the feed space allowances examined had detrimental effect on any of the performance parameters measured. When examined purely from an animal production point of view, a space allowance of 20 cm/cow would appear to be adequate. The number of cows/treatment was not sufficient to allow fertility performance to be examined. At a feed space allowance of 20 cm per cow, there were practical difficulties in being able to place the full allowance of food for a 24 hour period in front of the space available at the barrier.

**Acknowledgement** This study was co-sponsored by DARDNI and AgriSearch.

## Effect of feed space allowance and period of access to food on the performance and behaviour of dairy cows offered a silage based diet

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**Introduction** Meeting the higher nutrient requirements of high yielding dairy cows remains a key challenge on many dairy farms. 'Non-nutritional' approaches which may allow higher food intakes to be achieved involve optimising the feed barrier environment and adopting improved feeding practices. This may involve improving both the availability and accessibility of food. One of the main feed barrier factors which might be expected to have an effect on food intake is available feed space per cow. While feed space allowances per cow have decreased on some farms as herd size has increased, there is considerable variation in optimum feed space allowances cited in the literature. In addition, while it is normally considered prudent to ensure that dairy cows have access to food at all times, management practices on farms may on occasions result in cows running out of food for a period of time before fresh food is offered. To address these issues, an experiment was undertaken to examine the relationship between feed space allowance per cow, and period of access to food, on the performance of dairy cows.

**Material and methods** Forty-eight Holstein-Friesian dairy cows were used in a continuous 2 x 2 factorial design experiment (10 weeks duration). Thirty two cows were multiparous (mean lactation number, 3.5), while the remaining were primiparous. Eight multiparous and four primiparous cows were allocated to each treatment, with cows a mean of 141 days calved when the study commenced. Throughout the experiment cows were kept in four adjacent but visually isolated pens (12 cows per pen) of equal size and similar layout (16 cubicles per pen). Within each pen cows accessed food via a 'post and rail' type feed barrier. Treatments examined comprised two horizontal feed space allowances (15 and 40 cm/cow), and two periods of access to food (unrestricted and restricted). With the former, uneaten feed was removed at 08.00 h, while feeding took place at 09.00 h. With the latter, uneaten feed was removed at 06.00 h, while feeding was delayed until 12.00 h. Fresh food was offered *ad libitum* with all treatments. Food was offered daily in the form of a complete diet comprising forage and concentrates (60 : 40 DM basis), the forage component of the diet comprising grass silage and maize silage (60 : 40 DM basis). Group intakes were recorded daily, but were not analysed statistically due to the unreplicated nature of the intake data. The effect of feed space allowance and period of access to feed on mean animal performance during the experiment was analysed by ANOVA, with individual cows used as the experimental unit.

**Results** Total DM intakes were 18.1 and 18.2 kg/day with the 'restricted feeding time' treatments (15 and 40 cm respectively) and 17.8 and 18.1 kg/day with the 'unrestricted feeding time' treatments (15 and 40 cm respectively). None of milk yield, milk composition, or end of study live weight and condition score were significantly affected by treatment ( $P>0.05$ ).

**Table 1** Effect of feed barrier space allowance per cow, and time of access to feed, on the performance of lactating dairy cows

	Restricted feeding time		Unrestricted feeding time		SEM	Significance		
	15 cm	40 cm	15 cm	40 cm		Space	Time	Interaction
Total DM intake (kg/day)	18.1	18.2	17.8	18.1				
Milk yield (kg/day)	29.8	30.7	29.2	29.5	0.61	NS	NS	NS
Milk fat (g/kg)	39.4	41.0	40.5	41.2	0.68	NS	NS	NS
Milk protein (g/kg)	32.9	32.6	32.5	33.6	0.42	NS	NS	NS
Milk lactose (g/kg)	47.3	47.7	46.6	46.9	0.59	NS	NS	NS
Somatic cell count (000/ml)	354	230	470	585	123.2	NS	NS	NS
End of study condition score	2.5	2.5	2.6	2.5	0.06	NS	NS	NS
End of study live weight (kg)	620	618	636	628	9.2	NS	NS	NS

**Conclusions** Feed space allowance had no significant effect on any of the performance parameters examined within this experiment. Thus from a cow performance point of view, it would appear that a feed space allowance of 15 cm per cow may be adequate for mid lactation cows. In addition, restricting the period of time during which cows had access to food had no effect on cow performance, even at a space allowance of 15 cm/cow. However, within the current study this feeding time restriction was applied continuously throughout the experiment, and cows appeared to become accustomed to this scenario. It is possible that not having access to food on random occasions (ie occasionally running out of food) may actually be more stressful for cows than a regular period without access to food.

**Acknowledgement** This study was co-sponsored by DARDNI and AgriSearch.



## Key housing and management factors affecting lameness levels on Northern Ireland dairy farms

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**Introduction** Lameness in dairy cows continues to have a negative effect on the welfare of affected animals, on the economics of dairy production and on consumer perception of the dairy industry. The aim of the current study was to identify the farm management and housing factors most associated with lameness in dairy cows.

**Materials and methods** Fifty-nine dairy farms in Northern Ireland were visited once during the winter housing period of either 2005/6 or 2006/7. Farms were selected on the basis of being participants in a previous postal survey, and through recruitment at dairy discussion groups and exhibitions. Farms were located in each of the 6 counties in Northern Ireland, and were selected with no prior knowledge of lameness levels. The farms had an average milking herd size of  $138 \pm 53$  cows (mean  $\pm$  SD), and average 305-day milk yield of  $8284 \pm 1543$  litres. Forty-nine farms had at least 90 % purebred Holstein cattle. A one-hour interview with a set questionnaire was carried out with the person who made the majority of lameness management decisions. The questionnaire gathered the following farm information: mixed enterprise (yes or no), herd size ( $\geq 135$  or  $< 135$  cows), amount of concentrates fed ( $> 2.2$  or  $\leq 2.2$  tonnes/cow/year), average milk yield ( $>8500$  or  $\leq 8500$  l/lactation), % Holstein in herd (100% or  $<100\%$ ), winter forage (grass silage only or alternative forages included), floor type used in cubicle houses (slats, solid or a mix of slats and solid), whether or cubicle houses were overstocked (yes or no), whether or not flooring of cubicles was concrete (i.e. no mat/mattress) (yes or no), the foot trimming regime used (once per year, as needed, only lame cows, never), if a footbath is used on the farm (yes or no), how soon mild cases of lameness are treated (within 1 day, 2 days, 3-4 days, 5-7 days, or after more than 7 days/ never). The following housing factors were judged by visual assessment: floor maintenance (excellent, good, average/poor), if adequate bedding was provided in cubicles (yes or no), the overall comfort level of cubicles (excellent, good, average/poor), the overall cleanliness of passageways (excellent, good, average/poor). All cows in the milking herd were assigned a locomotion score of between 1 and 5 during the same visit (Flower and Weary, 2006). Cows assigned a score of 3 or higher were considered clinically lame (score 3 = slight limp detected). Two farms were excluded from final analysis, and the locomotion score of 6292 cows was included in final analysis. The response variables assessed were % of cows with a locomotion score greater than or equal to 3 or 4. Associations with explanatory variables detailed above were assessed using linear regression analysis. Any relationship with a probability value less than 0.25 was allowed to go forward to stage 2 ("Best Subsets Regression"). The Akaike Information Criterion was used to select the best model for each response variable.

**Results** The number of lame cows (locomotion score 3 or higher) as a percentage of the cows scored on each farm ranged from 1.5 to 74.7 % (mean: 32.6, SD: 14.1). The mean prevalence for lameness score 4 or higher was 3.9 % (SD: 4.03). The main variables associated with lameness (score 3+) are listed below (Table 1).

**Table 1** Influence of management and housing factors on the percentage of lame cows (score 3+) in herds

Variable	Levels	Predicted mean	Lower C.I.	Upper C.I.	P
Concentrate feeding level	High (above 2.2 tonnes/animal/yr)	39.41	31.70	47.39	
	Low (less/equal to 2.2 tonnes/animal/yr)	28.94	23.71	34.47	<0.05
Flooring	Slats <sup>b</sup>	41.84	35.28	48.56	
	Solid <sup>ab</sup>	32.21	22.16	43.17	
	Slats/solid <sup>a</sup>	28.49	22.57	34.81	<0.01
Concrete in cubicle	No	29.78	25.20	34.58	
	Yes	38.51	29.87	47.53	<0.06
Cubicle comfort	Excellent <sup>a</sup>	28.77	19.72	38.77	
	Good <sup>a</sup>	30.97	25.11	37.16	
	Average/poor <sup>b</sup>	42.86	35.32	50.57	<0.05

<sup>ab</sup>Levels within the same variable with a different superscript differ significantly

The variables associated with severe lameness (score 4+) were the same as those listed above, except that 'concentrate feeding level' was replaced in the model by 'winter forage'. The predicted mean level of severe lameness was higher when alternative forages were used rather than grass silage as the sole forage ( $P < 0.07$ ).

**Conclusions** These results suggest that farmers should ensure that cubicles are comfortable by providing mattresses or mats and adequate bedding, and by replacing outdated designs. The negative effect of slats may have been related to the fact that often they were not cleaned. Housing and feeding factors appeared relatively more important than the lameness management strategy used on farm.

**Acknowledgements** Funding from AgriSearch and DARDNI is gratefully acknowledged

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## Selection of sires with good lambing and lamb vigour characteristics within three Suffolk strains

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**Introduction** Two of the causes of lamb mortality are (1) dystocia and (2) low vigour lambs. Both of these problems require high levels of human intervention to ensure survival of the lambs. Therefore, selection for traits requiring lower levels of input is desirable. The New Zealand strain of Suffolks has purportedly been selected for low input 'easy care' traits and represents the possibility of introducing genes for improved lambing ease and lamb vigour into the British Suffolk strains. The aim of this study was to compare birth, neonatal behaviour traits and dag scores of lambs sired by one of three main Suffolk strains.

**Materials and methods** Welsh Mule ewes were synchronised in oestrus and mated with sires from 3 different Suffolk strains: New Zealand sires (NZ, n 4) – selected upon 'survivability', ease of lambing and performance data, UK High Index-selected sires (UKH, n 3) – selected on performance data, or UK Traditional sires (UKL, n 3) – unselected. In total, 333 ewes give birth to 655 lambs (255 NZ, 205 UKH, & 205 UKL). Each lamb was scored on: Birth assistance (BA), Lamb vigour (LV, at 10 minutes of age), and, Sucking assistance (SA, Table 1). In addition, birth weights and weight and dag score at 8 weeks of age were recorded. All recording was done blind to the knowledge of lamb sire. Score data were ranked then analysed using the Linear Mixed Model procedure in SAS, all analyses had sire type, ewe age, ewe condition score, lamb sex and litter size as fixed effects and sire ID, fitted as a random factor nested within sire type, and ewe ID as random factors. In addition, analysis of BA score had birth weight as a covariate.

**Table 1** Description of scores for the three neonatal behaviour traits

BA	LV	SA
0 -	extremely active lamb, been standing on all feet	lamb sucking well <1 hour, no help
1 unassisted or easy uncomplicated delivery	very active lamb, on back legs	lamb sucking well <2 hours, no help
2 minor assistance required, presentation corrected with little effort needed for delivery	active lamb, lying on chest and holding head up	lamb given help <3 times in first 24 hours after birth
3 major assistance required, difficult delivery	weak lamb, flat, holding head up	lamb given help >1 day <3 days
4 veterinary assistance required	very weak lamb, unable to lift head	lamb still needing help > 3 days

**Results** Table 2 shows the least square means and s.e. for the three neonatal scores and weights. There was no effect of sire type on BA rank ( $P>0.1$ ), however, there was a tendency for NZ lambs to have fewer difficult births than UKH lambs ( $P=0.089$ ) but no difference was found between NZ & UKL nor between UKL and UKH. Lamb birth weight affected the BA score with lambs requiring minor birth assistance being heaviest (mean weights $\pm$ s.e. (kg); score 1= $4.52\pm0.051$ ; score 2= $5.46\pm0.091$ ; score 3= $4.94\pm0.277$ ; score 4= $4.30\pm0.300$ ;  $P<0.001$ ), however, there was no interaction of sire type and birth weight. There were no effects of sire type on LV rank and SA rank, with no differences found between any of the groups. No effects of sire type were found within dag rank, however, there was a trend for NZ lambs to have less faecal soiling than UKH ( $P=0.053$ ). No differences in dag rank were found between NZ & UKL or UKH & UKL. There were no overall effects of sire type on birth weight, however, NZ lambs tended to weigh less than UKH ( $P=0.070$ ) and UKL ( $P=0.071$ ). There was no effect of sire type on the 8 week weight and no differences were found between any of the groups.

**Table 2** Least square means and standard errors for BA, LV and SA, birth weight, 8 week weight and dag rank (s.e. as subscript)

	BA rank	LV rank	SA rank	Dag rank	Birth weight	8 week weight
NZ	305.7 <sub>21.2</sub>	269.8 <sub>23.3</sub>	292.8 <sub>19.0</sub>	237.3 <sub>29.8</sub>	4.43 <sub>0.13</sub>	20.9 <sub>0.53</sub>
UKH	338.3 <sub>22.3</sub>	273.5 <sub>25.0</sub>	271.0 <sub>20.6</sub>	294.4 <sub>31.6</sub>	4.65 <sub>0.14</sub>	21.6 <sub>0.55</sub>
UKL	315.4 <sub>22.4</sub>	245.1 <sub>25.2</sub>	273.2 <sub>18.4</sub>	263.4 <sub>31.4</sub>	4.64 <sub>0.14</sub>	21.4 <sub>0.56</sub>

**Conclusions** This study shows that Traditional British and New Zealand lambs were similar in performance for birth assistance and lamb vigour traits (LV and SA). High Index-Selected lambs required more assistance at birth than NZ lambs but have similar levels of vigour when compared with Traditional and NZ strains. This infers that British Suffolks could have the rates of dystocia slightly improved by introduction of NZ genes with no change in lamb vigour traits. However, greater differences between the strains may be found if the study was repeated with pure-breds lambs. The high numbers of tendencies suggests that the number of lambs was low. Also, the number of individual sires used in this study was low, which may not be representative of the variation within the broader strain populations. A repeat experiment with a greater number of sires from each strain may provide more information.

**Acknowledgements** This study was funded by HCC, Innovis and the Suffolk Sheep Society. Thanks to farm and technical staff at Morfa Mawr sheep unit of the Institute of Biological, Environmental & Rural Sciences, Aberystwyth University. SMM is supported by a BBSRC CASE studentship in association with Genesis Faraday and the Suffolk Sheep Society.

## Grazing patterns and habitat selection of the Scottish Blackface compared with a crossbred, using GPS Satellite telemetry collars

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**Introduction** Environmentally sustainable grazing regimes are an important aspect of domestic livestock farming in upland hill areas. In order to achieve such grazing regimes an understanding of the relationship between plants and animals must be established. This requires knowledge of the foraging behaviour of the animal, such as diet selection spatial distribution, foraging patterns. The collection of such can be the most difficult aspect of the study however; global positioning system (GPS) technology can provide a suitable solution (Turner *et al.*, 2000). The aim of this study was to investigate grazing patterns, habitat selection and spatial distribution of the Scottish Blackface, a traditional hill sheep breed, compared with a crossbred, Texel X Scottish Blackface in a natural free ranging environment with the use of GPS telemetry collars.

**Materials and methods** The study was carried out at Glenwherry hill farm, animals involved in this study were two year old females, pure Scottish Blackface and Texel X Scottish Blackface (Texel XBF) reared on the hill farm. The flock was a combination of both breeds and contained up to 120 females, grazing an area of the hill (163 hectares) during May-October. Randomly selected ewes were fitted with GPS collars, two ewes from each breed and returned to flock and grazed as normal. The collars were programmed to record GPS locations at 30 minutes intervals between 06:00am-22:00pm (day) and 60 minutes intervals between 22:00pm-06:00am (night). Monitoring took place during May to October 2008. Four GPS collars were available, collars were changed on to different ewes approximately every 4 weeks. Results were obtained from 24 ewes, from which the results from eighteen ewes, nine per breed were suitable for analysis. Habitat mapping was carried out, aerial photos were used as a base, followed by ground mapping. Information was entered into ArcView GIS 3.2. habitat maps were created. Collar data was also entered into ArcView GIS 3.2. and combine with habitat data. Occurrence and frequency of habitats visited by ewes was identified. Results were recorded for, percentage occurrence on habitats, altitude, area covered and distance moved. Data were analysed by Analysis of Variance using Genstat. Analysing occurrence on habitats present over 24 hour periods. Area covered and distance travelled were analysed by two sided t test using Genstat.

**Table 1** Percentage occurrence on habitats, altitude, area covered and distance travelled by Scottish blackface and Texel XBF ewes, monitored by GPS Collars

	Ewe genotype		SEM	P-value	Sig
	Scottish Blackface	Texel XBF			
Blanket bog	12.58	9.60	1.37	0.005	**
Degraded blanket bog	20.67	18.78	1.96	0.499	NS
Marshy Grassland	40.02	52.47	3.57	0.025	*
Unimproved	26.73	19.15	3.79	0.175	NS
Altitude (m)	306.81	299.49	1.42	0.006	**
Area covered (ha)	5.99	4.62	0.59	0.035	*
Distance Travelled (m)	1.89	1.20	0.21	0.005	**

**Table 2** Area of habitats present on hill as percentage of total area of hill habitats ranked in order of preference of habitat by grazing ewes

Habitat	Area %	Rank
Blanket bog	49	4
Degraded blanket bog	27	3
Marshy Grassland	22	1
Unimproved	2	2

**Results** Scottish Blackface occurred more often in blanket bog habitats than the Texel XBF, ( $P > 0.005$ ). Texel XBF occurred more often in Marshy Grassland habitats ( $P > 0.025$ ) than the Scottish blackface. No significant differences were found between breeds on degraded blanket bog or unimproved habitats. Both Scottish Blackface and Texel XBF occurred most often in Marshy habitats 40.02% and 52.47% respectively and occurred least often in Blanket bog habitats 12.58% and 9.60% respectively. Scottish Blackface were found to grazed at higher altitudes, on average 7 m higher than Texel XBF ( $P > 0.006$ ). Significant differences were found between breeds for both area covered and distance travelled per day, ( $P > 0.035$ ) and ( $P > 0.005$ ) respectively.

**Conclusion** Both breeds show higher preferences for grass based habitats, with Scottish blackface ewes showing higher preferences for upland habitats such as blanket bog than Texel XBF, this supports previous work (McCloskey *et al.*, 2009). Scottish Blackface ewes display better characteristics for grazing and managing vegetation on hill environments as they graze over a larger area and move higher up the hill, they also spend more time grazing on the various habitats present such as blanket bog, this is a consideration for upland management. The use of GPS collars has enabled the collection of a range of spatio-temporal information over an extensive area. This shows the potential for developing a more detailed analysis of animals grazing in extensive environments through the use of GPS equipment.

**Acknowledgements** Scholarship funding from DARD is gratefully acknowledged

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## Remote physiological monitoring in livestock: Assessment of stress in transit to improve welfare

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**Introduction** Modern intensive livestock production systems may impose a number of potential stressors upon the animals that may compromise welfare, health and productivity. Thus, during livestock transportation the “on-board” thermal micro-environment may pose a major threat to animal welfare and may be associated with reduced production efficiency and mortality (Mitchell and Kettlewell 2008). The quantification of physiological responses in the face of environmental challenges presented by production, handling and transport environments may be used to characterise the extent of physiological stress imposed and when incorporated in to the process of “physiological stress modelling” such data may constitute the basis of definition of acceptable ranges and limits for environmental variables such as temperature and humidity (Mitchell 2005). The transportation thermal environment presents some particular difficulties as it may be continuously changing during a long journey in response to external climatic conditions as well as being influenced by vehicle ventilation and animal metabolic heat and water production. In these circumstances continuous monitoring of the animals physiological responses is required but this is made difficult by the complex nature of vehicle environments and restricted access to the animals under commercial transport conditions and vehicle configurations. Ideally a method for the continuous remote monitoring of meaningful physiological variables or signals is required that does not necessitate the presence of an observer or any human intervention in order to make the measurements. It may be proposed that both radio-telemetry and physiological data logging represent potential methodologies fulfilling these criteria. In the present study both techniques have been employed to monitor and record deep body temperature responses of pigs and lambs to journeys under hot weather conditions typical of those encountered in southern and central Europe in summer

**Materials and methods** The journeys employed were of 8 hours duration and are typical of those associated with the transportation of animals to slaughter. Four journeys were undertaken with pigs and two with lambs. On each journey up to 180 pigs (mainly gilts) were transported (average body weight  $102 \pm 6$  kg) at a space allowance of  $0.52 \text{ m}^2$  per animal. For the lambs 600 mixed sex animals (average body weight  $22 \pm 2$  kg) were transported (200 per deck) using a space allowance of  $0.15 \text{ m}^2$  per animal. The experimental journeys were undertaken in the Spanish province of Aragon in August in daylight between 07:00 and 15:00 with average journey times of 8.1 hours and 7.5 hours for pigs and lambs respectively. A mid-journey break (vehicle stationary) of around 30 minutes duration was taken approximately 3 hours in to the journey. In each case a circular route from and back to the farm or collection centre of origin was employed. Temperatures and relative humidities were recorded on each of the three decks of the vehicle and at air inlets and fan outlets where appropriate. Ambient conditions were recorded by sensors mounted on the exterior of the vehicle. For each journey 8 pigs were previously surgically implanted with a radio-telemetry package to continuously record abdominal deep body temperature (DBT) 14 days prior to travel. In preparation for each lamb journey 8 animals were surgically implanted with modified temperature data loggers for continuous recording of intra-peritoneal temperature at least 2 weeks before the journey. Mean DBT values obtained at 1 minute intervals were determined in pre, post-transport and journey periods and were compared with control body temperature (the pre-journey period) by analysis of variance.

**Results** The average ambient temperature during journeys transporting pigs was  $32^\circ\text{C}$  and the water vapour density was  $9.2 \text{ g/m}^3$ . The corresponding values for the journeys involving lambs were  $31.4^\circ\text{C}$  and  $10.4 \text{ g/m}^3$ . These mean temperatures are equal to or exceed the EU recommended limits (without tolerances) in current and proposed legislation and may thus be deemed to represent potential heat stress conditions for both species. The associated ambient and “on-board” water vapour densities are representative of relative humidities in the range of approximately 30-45% and thus indicate relatively “dry” conditions”. The patterns of DBT before, during and after typical journeys for pigs and lambs indicate that despite the elevated ambient temperatures during the journey and an apparent associated thermal challenge the DBT values for both pigs and lambs did not increase during the journeys and in fact decreased indicating some cooling in transit (see Table 1). It is proposed that these apparent decreases in core temperature result from convective cooling in the moving vehicle.

**Table 1** Mean deep body temperatures during the pre-journey control period and in transit (mean  $\pm$  SD)

Journey	Control body temperature ( $^\circ\text{C}$ )	Mean body temperature in transit ( $^\circ\text{C}$ )	Change ( $^\circ\text{C}$ )	Significance
Pigs	$39.2 \pm 0.41$	$38.9 \pm 0.24$	-0.3	NS
Lambs	$39.8 \pm 0.23$	$39.3 \pm 0.37$	- 0.5	P=0.006

**Conclusions** The results demonstrate that continuous monitoring of physiological variables in “real” animal production and transport conditions is an essential tool for assessing physiological stress and welfare and that more detailed physiological information is essential when assessing the effects of the thermal microenvironments in transit in relation to the adequacy and pertinence of current and proposed animal transport welfare legislation.

**Acknowledgements** This research was financially supported by Defra

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## Effectiveness of different footbath solutions in the treatment of digital dermatitis in dairy cows

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**Introduction** Digital dermatitis is a world-wide problem in dairy herds that accounts for approximately 20-25% of all cases of lameness (Laven, 2003). It is not only a serious welfare issue in dairy herds, but it also has serious financial implications. For example, each case of digital dermatitis is estimated to cost between £75-£82 in the UK (Esslemont, 2005). Often the most practical solution for controlling dermatitis is group topical therapy (i.e. footbathing). For this to be successful, an effective antibacterial product needs to be used. Neither antibiotics nor formalin can be recommended for use in footbaths. This is because antibiotics are expensive and their long-term use may lead to increased antibiotic resistance in cattle, and because formalin is both toxic and carcinogenic. Copper sulphate solutions are used extensively in footbaths for cattle, but long-term use may have adverse effects on the environment through increasing soil copper levels. The aim of this study was to compare the effectiveness of different footbathing regimes using different copper sulphate concentrations in the treatment of digital dermatitis.

**Material and methods** Lactating cows (n = 120) from the experimental herd at the Agri-Food and Biosciences Institute were allocated to one of four treatment regimes: 1) weekly footbathing with 5% copper sulphate (n = 40), 2) weekly footbathing with 2% copper sulphate (n = 40), 3) fortnightly footbathing with 5% copper sulphate (n = 20), or 4) fortnightly footbathing with 2% copper sulphate (n = 20). The cows were balanced for experiment, milk yield, body weight and condition score. Cows allocated to the weekly footbathing regime had on average a high prevalence of active digital dermatitis (DD) at start of the trial (>60%), whereas cows allocated to fortnightly footbathing had a lower prevalence of active DD (≤25%). During the study period (7 weeks) the cows walked through a water bath and then the allocated footbath solution on four consecutive milkings (weekly or fortnightly, respectively). Digital dermatitis was scored on the hind claws of all animals during milking on a weekly basis using a 5-point nominal scale developed by Döpfer *et al.* (1997). The data were analysed as binomial repeated measures analysis using GenStat. Only active lesions (i.e. early stage lesions and painful classical ulcerative stage lesions) are presented. For each cow DD lesions were scored as 'healed' when lesions were improving or became absent on both left and hind feet, and as 'not healed' when either or both hind feet had lesions that were getting worse or not improving.

**Results** For cows on the weekly footbathing regime (i.e. with high levels of DD) the prevalence of active DD lesions decreased faster when the 5% rather than the 2% copper sulphate solution was used (See Fig 1). Significantly improved healing of DD lesions was also shown with the weekly 5% rather than 2% copper sulphate footbathing regime ( $P < 0.05$ ). For cows on the fortnightly footbathing regime (i.e. with low levels of DD) there was no significant difference in number of active lesions, or in healing of lesions, between the 2% and 5% copper sulphate solutions ( $P > 0.05$ ).

**Table 1** Proportion of cows that showed healing of DD lesions on different footbathing regimes.

Regime	CuSO <sub>4</sub> 5%	CuSO <sub>4</sub> 2%	SED
Weekly	0.65 <sup>b</sup>	0.50 <sup>a</sup>	0.054
Fortnightly	0.63	0.60	0.054

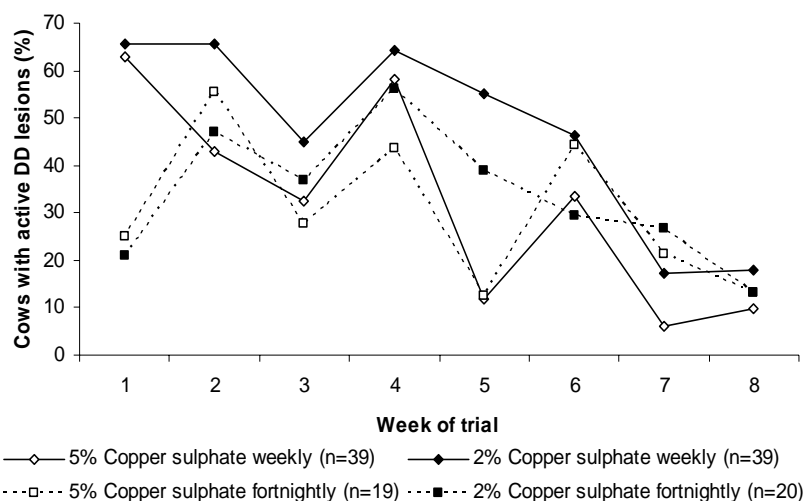
Rows with different superscripts are significantly different at  $P < 0.05$

**Conclusions** It is more effective to treat herds with a high prevalence of digital dermatitis with a 5% rather than a 2% copper sulphate solution in a weekly footbathing regime. It appears that when prevalence of digital dermatitis is medium (i.e. 25% of the herd with active digital dermatitis lesions), fortnightly footbathing with 5% or 2% copper sulphate will control the disease.

**Acknowledgements** The authors gratefully acknowledge funding from AgriSearch and DARDNI.

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**Figure 1** Percentage of cows with active digital dermatitis (DD) lesions on the different footbathing regimes.

## Exploration of potential mechanisms for parasite induced anorexia of sheep through modelling

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**Introduction** Gastrointestinal (GI) parasitism results in a reduction of the voluntary food intake, and hence productivity, of sheep. Previously an *in silico* simulation model of *Teladorsagia circumcincta* infections was developed (Vagenas *et al.* 2007), describing nutrient utilisation, host-parasite interactions and the development of immunity, for the purpose of predicting effects of host nutrition and genotype on the progression of GI infections. However, a difficult feature of such models is appropriately modelling parasite-induced anorexia. Although the precise causes of anorexia are unknown, two mechanisms for modelling anorexia can be invoked; a reduction in intrinsic growth rate (IGR) and a reduction in food intake (FIR), both as a function of parasite burden. This paper explores these mechanisms, and their consequences on predicted live weight (LW), food intake (FI) and faecal egg count (FEC).

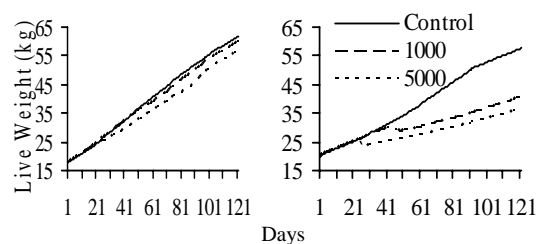
**Materials and methods** For both mechanisms the model of Vagenas *et al.* (2007) was used to simulate an immunologically naïve lamb from 2 to 6 months of age. The lamb modelled was similar to a Scottish Blackface, with an initial and mature live weight of 18kg and 85kg, respectively. The lamb was offered, *in silico*, one of two qualities of grass *ad libitum*; viz. good quality (12.6MJ/kg of dry matter (DM) metabolizable energy (ME) and 190g/kg DM crude protein (CP)) and poor quality (7.5MJ ME/kg DM and 97g CP/kg DM), respectively. The good quality grass is presumed to be of sufficient quality to be non-limiting (AFRC, 1993). The lamb was challenged *in silico* with either control, 1000 or 5000 infective larvae per day, from day one, corresponding to a range of challenge levels that normally lead to subclinical *T. circumcincta* infections (Coop *et al.* 1985). Predictions of FI, LW and FEC were assessed over time, with detailed comparisons presented here at day 60, a time point when impacts of anorexia are clearly observed across all scenarios investigated in this study.

**Results** Predicted trait values, assessed 60 days post infection, are summarised in Table 1, with anorexia mechanisms, grass quality and larval challenge all affecting predicted performance. For the good quality grass there was little difference in the FEC between the two mechanisms, however, there were notable differences in predicted FI and LW. With the FIR mechanism, reductions were predicted for LW and FI, compared to the control, which increased with higher larval challenges, whereas with the IGR mechanism similar reductions for both larval doses were predicted. For the poor quality grass, with the IGR mechanism FI is determined by the bulkiness of the food and as a consequence predicted FI was not reduced below the maximum gut fill; i.e. pathogen challenge does not further reduce the already constrained resources and hence little impact of parasitism on poor quality grass was observed for IGR mechanism. With the FIR mechanism, predicted FI displayed a reduction greater than that imposed by the maximum gut fill, resulting in a large reduction in predicted LW and an increase in FEC. Therefore, the model containing the FIR mechanism predicted a severe effect of parasitism in lambs offered poor quality grass. Fig 1 shows the progression of the impact of larval dose and grass quality on LW for the FIR mechanism over time, and the large temporal impacts on poor quality grass can be seen.

**Table 1** Model predictions 60 days post infection, for actual FEC, FI and LW as a proportion of the control lamb

Grass qual.	Larval dose <sup>†</sup>	IGR Mechanism			FIR Mechanism		
		FI <sup>‡</sup>	FEC	LW	FI	FEC	LW
Good	1000	0.82	130	0.89	0.89	120	0.96
	5000	0.81	237	0.89	0.78	255	0.88
Poor	1000	0.95	123	0.94	0.64	197	0.80
	5000	0.94	241	0.94	0.58	561	0.72

<sup>†</sup> units = larvae/day, <sup>‡</sup> all values for FI (kg), FEC (eggs/g), and LW (kg) are expressed as a proportion of the control lamb.



a. Good quality grass

b. Poor quality grass

**Figure 1** LW for different challenge levels, FIR mechanism

**Conclusions** The impacts of the different anorexia mechanisms differ with both larval challenge rate and food quality, leading to different predicted impacts of parasitism. However, predictions of the impact of parasitism on LW, across different challenge levels, using the FIR mechanism are very similar to the experimental results reported by Coop *et al.* (1985) for good quality grass. These predictions impact on our understanding of the nature and implications of anorexia, and provide testable hypotheses for experimental verification.

**Acknowledgements** We thank BBSRC, Merial and the Bioscience KTP (formerly Genesis Faraday) for funding.

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## A multimedia-based cognitive-behavioural intervention program improves attitudes and handling behaviours of stockpeople in livestock farming

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**Introduction** The amount and quality of human-animal interactions were shown to strongly affect animal welfare and productivity of several farm animal species via the strength of positive or negative (e.g. fear) emotions involved in the perception of humans (for review: Hemsworth and Coleman, 1998). In a recent survey on Dutch pig farms it was shown that in general, a negative attitude is associated with rough or aversive handling practices, whereas more positive ideas on handling are linked with more quiet and gentle handling practices (Visser *et al.*, 2006). Such relationships between attitudes of stockpeople and their behaviour towards animals have now been shown in several species, and several studies also show a subsequent relation with the behavioural response of the animals to humans and their productivity (e.g. in cattle: Waiblinger *et al.*, 2002). In Australia and United States, cognitive-behavioural intervention programmes have been designed to specifically target key attitudes and behaviours of stockpeople. These training programmes have produced substantial improvements in the attitude and behaviour of stockpeople and a marked reduction in the level of fear of humans by pigs and cattle (e.g. Coleman *et al.*, 2000). Based on the Australian experiences, within Welfare Quality® the multimedia training program 'Quality Handling' was developed specifically for the European context. The present study investigated whether this training improved attitude and behaviour of European stockpeople towards their animals, and also changed behavioural and fear responses of the animals.

**Materials and methods** Three species were involved: cattle, pigs and laying hens. Following development of the training packages, their effectiveness in achieving changes in attitudes and behaviour of stockpeople was tested in field tests. The field tests were carried out in The Netherlands (laying hens and pigs), and Austria (dairy cattle). Stockpeople were randomly allocated to training groups (dairy cattle: 10 farms, 14 people; pigs: 8 farms, 12 people; laying hens: 7 farms, 10 people) or control groups (dairy cattle: 9 farms, 9 people; pigs: 9 farms, 12 people; laying hens: 8 farms, 11 people). All farms were visited twice. Only stockpeople of the training group were trained, within 2 weeks after the first visit. The period between the two visits was approximately 6-8 weeks. Human attitudes towards animals were determined by means of a questionnaire filled in during the visits. Average scores were obtained for beliefs about animal characteristics (general attitude) and handling situations (behavioural attitude). Stockpeople's behaviour was assessed by means of behavioural observations during handling, and expressed in % of positive behaviours per unit or animal. Finally, the animal's avoidance behaviour to the approach of an unfamiliar person was measured to assess fear for humans.

**Results** To analyze the results of the field tests, a combined analysis was performed for the three species with stockperson as the replicate. Sixty four stockpeople participated although some missing data resulted in varying sample sizes for the analyses. Data were first standardized within each species to remove the effects of the species-specific units of measurement of each variable. Data were analyzed by a 3 (species) by 2 (treatment group) analysis of covariance with the post training score as the dependent variable and the pre training score as the covariate. There was a significant increase in positive general attitude ( $F_{1,57}=4.77$ ,  $p<0.05$ ) and in positive behavioural attitude towards animals under care ( $F_{1,57}=7.03$ ,  $p<0.01$ ) for the trained group compared to the control group. Moreover, the percentage of positive behaviours towards animals under care increased significantly in the trained group compared to the controls ( $F_{1,49}=9.48$ ,  $p<0.01$ ). The training did not significantly affect avoidance behaviour upon human approach ( $F_{1,43}=3.52$ ,  $p=.07$ ).

**Conclusions** The results demonstrate that the multimedia training program 'Quality Handling' is a promising tool to improve the attitudes and handling behaviours of stockpeople in European livestock farming. The period between the training and second visit may have been too short to result in an effect on animal fear and behaviour. The training packages were finalized in 2009 and are now available for training sessions in English (pig and laying hen programmes also in Dutch; cattle program also in French and German).

**Acknowledgements** The study was supported by the European research project Welfare Quality®, and co-financed by the European Commission within the 6th Framework Programme, contract no. FOOD-CT-2004-506508. For development and testing of the training packages for pig and laying hen stockpeople, funding was also obtained from the Dutch Ministry of Agriculture, Nature and Food Quality.

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## The effect of birth weight on the variation in live weight of pigs at weaning

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**Introduction** A high variation in birth weight has been found to contribute to high variations in survivability (Akdag *et al.* 2009). However, few studies have determined the effect of birth weight on variation in wean weight. Using the historical records of the research herd at AFBI Hillsborough, which include the birth and wean weight of all pigs born, the objective of this study was to investigate the effect of birth weight on the variability in pig wean weight.

**Materials and methods** Data from 96 batches of farrowings, (on average 14 sows farrowed per batch giving 1,351 sows in total) which took place between January 2003 and April 2008 on the AFBI Hillsborough herd, were used. On average, each sow had 3.9 farrowings and the average number of pigs weaned per sow was 9.7. All pigs born (13,158) were Landrace x Large White and were weighed within 2 hours of birth, and again at weaning (28 days  $\pm$  2 days). The coefficient of variation (CV) for birth and wean weight of the pigs reared within each batch of farrowing was calculated and analysis of variance was used to test for differences. Regression analysis investigated the relationship between birth weight and 28 day weight. Furthermore, the data were grouped into weight categories: 0.75kg (120 pigs), 1.00kg (486 pigs), 1.25kg (1,458 pigs), 1.50kg (1,878 pigs), 1.75kg (1,824 pigs), 2.00kg (786 pigs), and 2.25kg (294 pigs) with a tolerance of  $\pm$ 0.05kg within each weight category. Within each weight category, the data were randomised into 6 groups which represented 6 replicates. For each 'replicate' within each weight category, the coefficient of variation (CV), standard deviation (SD) and average of the 28 day weight was calculated, and then regressed against birth weight. The effect of birth weight category on average wean weight, SD and CV of wean weight were analysed by Analysis of Variance. Using the same weight categories described above, the probability of pigs being above 8kg, 8.5kg or 9kg at weaning was calculated.

**Results** The CV of wean weight (0.18) (representative of all pigs born within a batch of farrowings) was found to be significantly lower ( $P < 0.001$ ) than the CV of birth weight (0.21). The relationship between birth weight and 28 day weight was found to be significant ( $P < 0.001$ ) but weak ( $R^2 = 0.243$ ). As birth weight increased, wean weight also increased ( $P < 0.001$ ) (Table 1). Birth weight had a significant effect on the SD and CV of wean weight (both  $P < 0.001$ ) (Table 1). In general as the birth weight of pigs increased the SD and CV for their wean weight decreased. The relationship between birth weight and the CV of wean weight was found to be strong ( $R^2 = 0.927$ ) and fitted an inverse quadratic curve (Figure 1). It was also found that a pig with a birth weight under 1kg at birth had a 28% probability of being over 8kg at weaning, compared with a 64% probability for a pig with a birth weight between 1 and 1.5kg (Table 2).

**Table 1** The effect of birth weight on the average, SD and CV of wean weight

	0.75kg	1.00kg	1.25kg	1.50kg	1.75kg	2.00kg	2.25kg	LSD	P
Average (kg)	6.82 <sup>a</sup>	7.72 <sup>b</sup>	8.3 <sup>c</sup>	9.08 <sup>d</sup>	9.6 <sup>e</sup>	10.25 <sup>f</sup>	10.7 <sup>g</sup>	0.05	<0.001
SD	1.71 <sup>a</sup>	1.58 <sup>b</sup>	1.5 <sup>bc</sup>	1.54 <sup>bc</sup>	1.46 <sup>c</sup>	1.46 <sup>c</sup>	1.52 <sup>bc</sup>	0.10	<0.001
CV	0.250 <sup>a</sup>	0.205 <sup>b</sup>	0.181 <sup>c</sup>	0.170 <sup>d</sup>	0.152 <sup>e</sup>	0.143 <sup>f</sup>	0.142 <sup>f</sup>	0.0012	<0.001

<sup>a,b,c</sup> numbers with the same superscripts are not significantly different ( $P > 0.05$ )

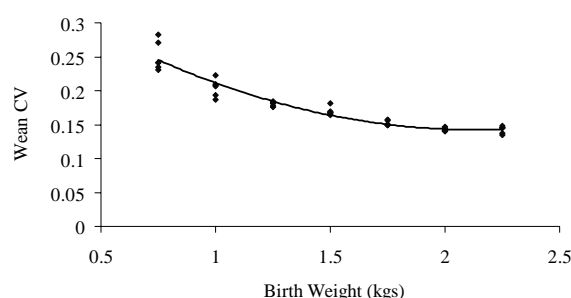
**Table 2** Probability (%) of being over 8.0, 8.5 or 9.0kg when born at different birth weights

Weight at birth:	Weight at weaning:		
	>8.0kg	>8.5kg	>9.0kg
<1kg	28.0	18.3	11.8
1.0 – 1.5kg	64.1	51.3	37.0
1.5 – 2.0kg	85.6	76.4	66.0
>2kg	93.5	89.9	85.3

**Conclusion** This study highlighted that as birth weight increased, variable weight of pigs at weaning decreased. This suggests that light birth weight pigs are 'less predictable' in terms of weaning weight than heavier pigs. This is further highlighted by 28% of pigs born under 1kg achieving weaning weights over 8kg. However, this probability is based on an average litter size of 9.7. Competition for resources, e.g. milk, in larger litters would be greater and it would be of interest to investigate the effect of litter size on the above probability. Unfortunately it was not within the scope of this study to investigate the characteristics of the light birth weight pigs which were over 8kg at wean, however these results suggest that light weight pigs at birth could be encouraged through managerial practices to achieve better weaning weights.

**Acknowledgements** The authors gratefully acknowledge funding from Devenish Nutrition Ltd.

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**Figure 1** Relationship between birth weight and wean CV



## Can Hampshires tolerate low lysine diets post weaning?

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**Introduction** The UK pig industry has traditionally used a Large White sire over a Large White X Landrace dam (back cross). More recently there has been an increase in the use of three way crosses due to the improvements associated with hybrid vigour. Preliminary studies at the University of Leeds have shown that the Hampshire X (Large White X Landrace) pigs outperform Large White X (Large White X Landrace) pigs in terms of ADG due to a higher ADI. Due to the high cost of dietary protein, feeding strategies that can reduce protein in the diet are often used to reduce overall feed costs. As the Hampshire has a higher ADI compared to the Large White it may be that Hampshires can cope better with a low lysine diet. They may be able to compensate for the lower dietary lysine through a higher ADI. The aim of this study is to identify whether Hampshires can cope better when fed a low lysine diet post weaning compared to Large Whites.

**Materials and methods** A 2x2 factorial design compared growth of pigs on two lysine diets (low and high) and the growth performance of two cross bred genotypes (Hampshire X (Large White X Landrace) and Large White X (Large White X Landrace)). A total of 264 pigs (132 Hampshire X, 132 Large White X) were weaned at 28±4 days, 8.2 ± 0.16 kg and remained on trial for 20 days. Pigs were allocated to pens based on their genotype and balanced for weight and sex. Pigs were given *ad-libitum* access to either a low lysine diet (0.80%) or a high lysine diet (1.75%), above that recommended by the NRC (1998) to ensure performance was not limited. Diets were iso-energetic and formulated to the same amino acid:lysine ratios. Pigs were individually weighed at weaning (day 0) and then at 7, 14, and 20 days post weaning. Weekly pen feed intakes were recorded and feed conversion ratios (FCR) calculated throughout the trial. All pigs were checked for health daily. A General Linear Model (Minitab version 14.0) was used to analyse any differences in growth performance, health performance and feed intake between the pigs.

**Results** The results have shown that a reduction in dietary lysine reduced growth performance in both Hampshires and Large Whites. Pigs fed the high lysine diet were 2.1 ± 0.12 kg heavier than pigs fed the low lysine diet at day 20 post weaning ( $P<0.001$ ). Pigs fed the high lysine diet had a higher ADG and a more efficient FCR. ADI was not significantly different between pigs on the two diets (Table 1). From days 0-20 post weaning Hampshires grew faster in comparison to Large Whites due to a higher ADI. There was no difference in FCR between genotypes. The difference between the day 20 weights for Hampshires on the two diets was larger than the difference between the day 20 weights for Large Whites ( $P<0.001$ ). Hampshires had a difference of 2.7±0.16 kg and Large Whites had a difference of 1.5±0.16kg (Table 1). There was no difference in health performance between any of the treatments.

**Table 1** Growth performance 0 – 20 days post weaning

Post Weaning Performance	H		LW		SEM	Diet	P-Value	
	High Lysine	Low Lysine	High Lysine	Low Lysine			Genotype	Interaction
Wean weight (kg)	8.4	8.4	8.0	8.0	0.21	0.970	<0.005	0.979
20 d weight (kg)	15.8 <sup>a</sup>	13.1 <sup>c</sup>	14.1 <sup>b</sup>	12.6 <sup>c</sup>	0.16	<0.001	0.000	0.001
0-20 d ADI (kg/d)	0.411 <sup>a</sup>	0.383 <sup>a</sup>	0.321 <sup>b</sup>	0.339 <sup>b</sup>	0.0097	0.575	<0.001	0.021
0-20 d ADG (kg/d)	0.381 <sup>a</sup>	0.248 <sup>b</sup>	0.296 <sup>c</sup>	0.222 <sup>b</sup>	0.0085	<0.001	<0.001	0.001
0-20 d FCR	1.10 <sup>a</sup>	1.56 <sup>b</sup>	1.08 <sup>a</sup>	1.59 <sup>b</sup>	0.036	<0.001	0.894	0.520

**Conclusions** A reduction in dietary lysine reduced growth performance in both Hampshires and Large Whites. Hampshires were more sensitive to a low lysine diet post weaning. The different diets fed during the trial had an effect on the degree of growth reduction between the two genotypes. There was a bigger difference in weight between Hampshires fed the high and low diets compared to the weight difference between Large Whites on the two dietary treatments. ADI was not significantly different between genotypes on the two diets, suggesting that neither genotype could increase their feed intake to compensate for the lower lysine level. As in a preliminary trial carried out at the University of Leeds, Hampshires grew faster in comparison to Large Whites due to a higher ADI resulting in a higher ADG. Overall the results suggest Hampshires were less tolerant to a low lysine diet; they were faster growers and therefore may have a greater requirement for lysine.

**Acknowledgements** This research is supported by BBSRC and ABN.

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## The post weaning and finishing performance of pigs with different wean weights when offered a high or low allowance of starter diets

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**Introduction** Previous research comparing pig performance using different levels of starter diets has commonly examined the effect on the average weight of the group (e.g. Lawlor *et al.* 2002). In the few studies that have examined the effects of starter diets on pigs with different wean weights, the pigs were weaned at three weeks of age (e.g. Mahan *et al.* 1998). The main aim of this study was to investigate the effect of starter diet allowance on the post weaning performance of light, medium and heavy weight weaned pigs.

**Materials and methods** The experiment was designed as a 3 (light vs medium vs heavy weight pigs at weaning) x 2 (high vs low starter diet allowance) factorial design. Over six time replicates 720 LR x LW pigs were weaned at 4 weeks of age and penned in groups of 20. The average pen weight of light, medium, and heavy weight pigs was 7.1 (SEM 0.07), 9 (SEM 0.03) and 10.5kg (SEM 0.06) respectively. Pens of pigs were randomly allocated to either a 'High' or 'Low' starter diet allowance. Groups were balanced for sex and the weight of pigs was balanced between starter diet allowance. The 'Low' starter diet allowance regime offered pigs 2kg/pig of starter 1 diet followed by 4 kg/pig of starter 2 diet. The 'High' starter diet allowance regime offered pigs 4kg/pig of starter 1 diet followed by 8 kg/pig of starter 2 diet. Starter 1 and Starter 2 diets were commercial diets (Devenish Nutrition Ltd) with digestible energy content (DE) of 15.8 and 15.5MJ/kg respectively, total lysine 16 and 15 g/kg respectively and both had a crude protein (CP) content of 200 g/kg. In both regimes, pigs were offered grower diet (DE 14MJ/kg, total lysine 12 g/kg and CP 186 g/kg) *ad libitum* after they finished their allocation of starter 2. Pigs were transferred to finishing accommodation at 10 weeks of age and were offered the grower diet to 11 weeks of age followed by a finish diet until 20 weeks of age. Pigs were weighed and feed intakes were recorded at 7 and 10 weeks of age. Light and heavy weight pigs were also weighed at 15 and 20 weeks of age. The average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and feed intake per kg of body weight (FI/kg) was calculated between weaning and 10 weeks of age. Analysis of variance was used to test for the effects of treatment according to the 3 x 2 factorial design.

**Results** There were no significant interactions between the weight of pigs and starter diet allowance on pig weight or performance and the direct effects of starter diet allowance and weight of pig are presented in Table 1. The ADG and ADFI of medium and heavy pigs was similar but that of light weight pigs was significantly lower (Table 1). The FCR of light weight pigs was significantly ( $P<0.001$ ) better than that of heavy weight pigs with that of medium weight pigs being intermediate. The FI/kg of body weight was highest ( $P<0.001$ ) for light weight pigs and lowest for heavy weight pigs. Overall, pigs offered a high allowance of starter diets had a higher 10 week weight, ADG, lower ADFI and FI/kg bodyweight and an improved FCR compared with pigs offered a low allowance. However, the 10 week weight and ADG (between weaning and 10 weeks of age) of light and medium weight pigs was similar when they were offered either a low or high allowance of starter diets but that of heavy weight pigs was 1kg and 26g/day significantly higher when they were offered a high allowance of starter diets compared with a low allowance. The 15 and 20 week weight of heavy weight pigs was similar whether they were offered a high or low allowance of starter diets post weaning (56.3 and 57.8kg respectively). However, the 15 week weight of light weight pigs was significantly ( $P<0.01$ ) greater when they were offered a high allowance of starter diets post weaning (52.4kg) compared with a low allowance (49.4kg). In addition, the 20 week weight of light weight pigs tended ( $P=0.055$ ) to follow the same pattern with the weights of those which were offered a high allowance being 83.5kg and those offered a low allowance being 80.9kg.

**Table 1** Pig performance between wean and 10 weeks of age

	Low allowance			High allowance			SEM	Effect of diet	Effect of weight
	Light	Medium	Heavy	Light	Medium	Heavy			
10 week weight (kg)	25.9	29.4	31.1	26.2	29.9	32.1	0.27	<0.01	<0.001
ADG (g/day)	459	499	504	468	509	530	6.2	<0.01	<0.001
ADFI (g/day)	684	743	753	657	730	743	8.4	<0.05	<0.001
FCR	1.52	1.54	1.57	1.42	1.45	1.47	0.011	<0.001	<0.001
FI/kg (g/kg)	42.1	39	36.6	40.1	37.9	35.3	0.33	<0.001	<0.001

**Conclusions** Higher allowances of starter diets improved the FCR of pigs in all weight categories. Heavy weight pigs had better growth when offered higher allowances of starter diets. There was a carry over effect into the finishing period for light pigs with higher allowances of starter diets improving their 15 and 20 week weight. Light weight pigs ate more per kg of their body weight in the post weaning period than medium and heavy weight pigs.

**Acknowledgements** This work was jointly funded by DARD, Devenish Nutrition Ltd and John Thompson and Sons Ltd

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## The effect of terminal sire breed and slaughter weight on pig production and carcass performance

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**Introduction** Magowan *et al.* (2007) found that the growth performance of Landrace x Large White (LR x LW) pigs between commercial herds in Northern Ireland varied significantly. Since that time, the use of other breeds in NI has increased greatly. The current study investigated the effect of breed on pig performance and carcass quality of pigs when taken to different slaughter weights.

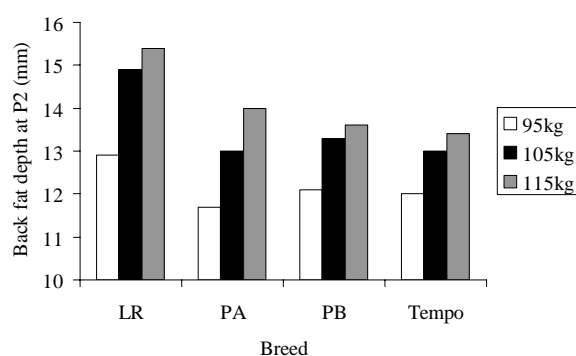
**Materials and methods** Over 12 time replicates, a total of 192 LR x LW F1 dams were artificially inseminated with semen from either the Tempo, Pietrain Austrian (PA), Pietrain Belgium (PB) or Landrace (LR) breed. The semen supplier was asked for semen from two of the top performing boars within each breed. The production performance of 240 pigs per breed, over 12 time replicates was compared between weaning and slaughter. All pigs were weighed at weaning (28 days of age) and pigs of the same breed were penned together in groups of 20 from weaning to finish. Each pen was balanced for weight and sex within each replicate. Pigs were weighed and feed intakes were recorded at 10 and 20 weeks of age and finish (target weight of 105kg). Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and the coefficient of variation (CV) for weight and growth rate was subsequently calculated. The back fat depth at P<sub>2</sub> and hot weight of carcasses were measured 45 minutes after slaughter. Carcass FCR was calculated by dividing the total feed used from weaning to finish by the hot weight of the carcass. Six boars and six gilts within each breed (and balanced for sire) were taken to slaughter weights of either 95, 105 or 115kg. Their back fat depth at P<sub>2</sub> and hot weight was measured 45 minutes after slaughter. Analysis of variance was used to test for the effects of breed on pig and carcass performance measurements and the effect of slaughter weight on back fat depth at P<sub>2</sub> and kill out percentage

**Results** Breed had no significant ( $P>0.05$ ) effect on pig performance post weaning (4 to 10 weeks of age). However, during the finishing period (10 weeks of age to approximately 105kg) Tempo pigs had the highest ADG, highest ADFI and lowest FCR (Table 1). The CV of 20 week weight and CV for growth rate (10 to 20 weeks of age) were highest in LR pigs (Table 1). LR pigs also had the greatest back fat depth at P<sub>2</sub> but a similar kill out % to Tempo pigs while PA and PB pigs had the highest kill out % (Table 1). Breed had a significant effect on carcass FCR ( $P<0.01$ , SED 0.048) with that of the Tempo pigs being best (2.59), that of LR pigs being poorest (2.76) and that of the PA and PB pigs being intermediate (2.64 and 2.68 respectively). When slaughter weights were increased to 115kg, the back fat depth of all pigs increased significantly (Figure 1). However, at these heavy carcass weights, only LR pigs fell into code 2 (average 14.9mm and 15.4mm for slaughter weights of 105 and 115kg respectively) (Figure 1). Slaughter weight did not affect the kill out percentage of pigs ( $P>0.05$ ).

**Table 2** Pig performance (10 weeks of age to finish (105kg)) and carcass quality of pigs from different breeds

	LR	PA	PB	Tempo	SED	Sig
ADG (g/day)	804 <sup>a</sup>	815 <sup>a</sup>	794 <sup>a</sup>	888 <sup>b</sup>	11.6	<0.001
ADFI (g/day)	2083 <sup>bc</sup>	2027 <sup>ab</sup>	2006 <sup>a</sup>	2119 <sup>c</sup>	35.6	<0.05
FCR	2.68 <sup>c</sup>	2.52 <sup>ab</sup>	2.59 <sup>bc</sup>	2.44 <sup>a</sup>	0.047	<0.001
Back fat depth at P <sub>2</sub> (mm)	13.9 <sup>b</sup>	12.8 <sup>a</sup>	12.6 <sup>a</sup>	12.9 <sup>a</sup>	0.23	<0.001
Kill Out %	75.8 <sup>a</sup>	77.4 <sup>b</sup>	77.0 <sup>b</sup>	76.1 <sup>a</sup>	0.28	<0.001
CV 20 week weight	0.12 <sup>b</sup>	0.10 <sup>a</sup>	0.11 <sup>ab</sup>	0.09 <sup>a</sup>	0.011	<0.05
CV ADG 10-20 weeks of age	0.17 <sup>b</sup>	0.12 <sup>a</sup>	0.14 <sup>ab</sup>	0.11 <sup>a</sup>	0.017	<0.01

<sup>a,b,c</sup>, numbers with the same superscripts are not significantly different ( $P>0.05$ )



**Conclusion.** Tempo pigs grew faster and were more efficient than PA, PB or LR pigs. However, the carcass performance of PA and PB pigs was superior to Tempo and LR pigs. It is likely that the economic value of using Tempo and Pietrain Austrian pigs is similar and greater than that of Landrace pigs. This study highlights the variation in pig performance and carcass quality that can arise from different breeds. When the slaughter weights of pigs were increased, it was found that although back fat depth increased across all genetic sources, only Landrace pigs would have been penalised at the heavier slaughter weights.

**Acknowledgements** This work was jointly funded by Pig ReGen Ltd

**Figure 1** The effect of breed and slaughter weight on back fat depth at P<sub>2</sub>

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## Effect of short-term feeding of genetically modified Bt maize (MON810) on gut microbiota, intestinal morphology and immune status of weanling pigs

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**Introduction** The inclusion of genetically modified (GM) plants in animal feed and for human consumption has consistently increased over the past thirteen years since they were first cultivated in 1996 (Schnepf *et al.*, 1998). Since the introduction of GM crops much debate, has centred around issues relating to their safety for consumption. Consumer concerns are mostly related to a perceived risk to health, allergenicity of the transgenic proteins or the transfer of antibiotic resistance from the plant to bacteria residing in the human gastrointestinal tract (Bertoni and Marsan, 2005). The objective of the current study was to identify any effects that short-term feeding of transgenic MON810 maize may have on gut microbiota, intestinal morphology and immune status of weanling pigs.

**Materials and methods** Crossbred weanling pigs (entire males; n=32) were used in a 36 day experiment. Pigs were weaned at ~ 28 d of age, blocked by weight and ancestry, and randomly assigned to one of two treatments. Pigs were housed individually in a total of 4 rooms with 8 pigs per room (16 pigs/treatment). Diets were non-medicated weanling diets and experimental treatments were as follows; diet 1 - Non-GM isogenic parent line of maize and diet 2 - transgenic MON810 maize. Faecal samples were taken from 32 pigs (n = 16 pigs/treatment) before (d -1) and at the end of feeding experimental treatments (d 30) for microbiological analysis. On d 31, 10 pigs/treatment were slaughtered by captive bolt stunning followed by exsanguination. Immediately post-mortem terminal ileal and cecal digesta were collected from 10 pigs/treatment for microbiological analysis. Tissue samples were also taken from the small intestine for the determination of gross morphology. Whole blood samples were taken from 10 pigs/treatment on d 0, and 29. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood, stimulated with the mitogen phorbol myristate acetate (PMA) and cytokine production by the cells measured by ELISA. All data was analyzed as a complete randomised block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). For all response criteria, an individual pig was the experimental unit. Microbiology data were log-transformed prior to analysis to ensure data points were normally distributed.

**Results** To identify if transgenic MON810 maize consumption had an influence on culturable faecal or intestinal microbial populations, total *Lactobacillus*, *Enterobacteriaceae* and total anaerobes were enumerated. The effect of short-term feeding of MON810 maize on gut microbiota is shown in Table 1. Short-term feeding of transgenic MON810 maize to weanling pigs had no effect on duodenal, jejunal or ileal villus height, crypt depth, villus height: crypt depth ratio or number of goblet cells/villus in the duodenum, jejunum and ileum. However, non-GM fed pigs tended (P=0.10) to have more goblets cells/µm of duodenal villus compared to GM fed pigs. To assess the effects of short-term feeding of transgenic MON810 maize on the systemic immune response, cytokine production by PBMCs was measured following mitogen stimulation. Mitogen stimulated PBMCs isolated from pigs fed transgenic MON810 on d 30 tended to produce less IL-12 than cells isolated from control pigs at the same timepoint (312.7 vs 1321 pg/mL; P<0.09). There was no effect of treatment on IL-10, IL-6, IL-4 or TNFα production either from resting or stimulated PBMCs.

**Table 1** The effects of short-term feeding of MON810 maize on gut microbiota

	Non-GM maize	GM maize	SE	P-value
Faecal <i>Enterobacteriaceae</i> – d 0, log <sub>10</sub> CFU/g	7.96	7.80	0.071	0.182
Faecal <i>Enterobacteriaceae</i> – d 30, log <sub>10</sub> CFU/g	7.05	6.37	0.290	0.105
Faecal <i>Lactobacillus</i> – d 30, log <sub>10</sub> CFU/g	9.02	9.41	0.146	0.106
Faecal Total Anaerobes – d 0, log <sub>10</sub> CFU/g	9.39	9.48	0.072	0.395
Faecal Total Anaerobes – d 30, log <sub>10</sub> CFU/g	9.26	9.26	0.111	0.980
Ileal <i>Enterobacteriaceae</i> – d 31, log <sub>10</sub> CFU/g	5.61	5.80	0.387	0.749
Ileal <i>Lactobacillus</i> – d 31, log <sub>10</sub> CFU/g	6.21	6.31	0.117	0.574
Ileal Total Anaerobes – d 31, log <sub>10</sub> CFU/g	7.18	6.99	0.214	0.556
Cecal <i>Enterobacteriaceae</i> – d 31, log <sub>10</sub> CFU/g	6.31	6.65	0.194	0.261
Cecal <i>Lactobacillus</i> – d 31, log <sub>10</sub> CFU/g	7.78	7.91	0.152	0.574
Cecal Total Anaerobes – d 31, log <sub>10</sub> CFU/g	9.29	9.38	0.101	0.568

**Conclusions** Results obtained from short-term feeding of transgenic MON810 maize to weanling pigs have demonstrated no adverse effects on intestinal morphology, no changes in selected culturable gastrointestinal microbial populations with the exception of a trend for reducing a potentially pathogenic population in the faeces, and a lack of systemic immune stimulation. Overall, to date we have found that transgenic MON810 maize has failed to adversely alter the physiology of the weaned pig in any parameter measured; however continued research in this area will provide more definitive answers.

**Acknowledgements** The authors gratefully acknowledge funding from the EU Commission 7<sup>th</sup> Framework Programme

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## Effect of dietary chicory on boar taint

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**Introduction** Entire male pigs often have a higher incidence of odours and flavours, found unpleasant by some consumers, known as boar taint. Boar taint is due to an excessive accumulation of two major compounds, skatole and androstenone, in adipose tissue. Skatole is a product of bacterial activity in the large intestine. Its levels in fat are influenced by diet, possibly through altering the bacterial activity or availability of the substrate, tryptophan (Jensen *et al.* 1997). Additions of non-digestible oligosaccharides, for example inulin, in the diet, have reduced skatole levels in faeces, backfat and blood (Jensen and Jensen 1998). The current project was therefore undertaken to see if a short feeding period with inclusion of chicory, a source of inulin, before slaughter will be sufficient to significantly reduce the level of skatole.

**Materials and methods** In a preliminary study 30 farms supplying a commercial abattoir have been sampled after the pigs have been slaughtered. Each farm provided 50 samples of backfat from entire pigs that have been minced to obtain a single sample tested for androstenone and skatole levels using the procedures of Whittington *et al.* (2004). In a first feeding trial, on 7 farms, 50 g/kg dried chicory was incorporated in the finishing diet for 2 weeks, 6 farms were used as controls. The farms were tested with the same method as the preliminary study. In the main feeding trial only one of the 30 farms was tested. The pigs had been divided into 4 groups fed different levels of chicory: 0, 30, 60 and 90g/kg DM. For each group 30 entire pigs were sampled at 3 different times: a first time (called week 0) to measure the base level of skatole and androstenone in all the pigs, then the supplement of chicory was introduced and the pigs were sampled after 1 and 2 weeks on the test diet. All 360 backfat samples had been tested for skatole concentration; androstenone had been measured in 110 pigs (all 90 g/kg pigs and 20 pigs of 0 chicory, week 2). All the samples had been presented to a 10 member taste panel for “sniff” tests to determine if reducing skatole had also reduced boar taint. Data were statistically analysed using general linear models (GLM), comparing the different levels of chicory in the diet.

**Results** The preliminary study showed a high variation in the concentration of skatole and androstenone between farms, with levels generally high in comparison with the normally accepted thresholds for the taint compounds (0.2 µg/g for skatole and 1 µg/g for androstenone). On average the androstenone concentration was 0.71µg/g, and skatole was 0.19 µg/g. In the first feeding trial the skatole level was reduced in the farms with the chicory diet, so we proceeded to the final stage of the project. In the main trial, 90 g/kg chicory fed for 2 weeks was successful in reducing skatole to a level well below the ‘threshold’ for this compound, with only 1 pig with a skatole value over the threshold. In the 90 g/kg group there was a downward trend in skatole by 1 week and 0.55 of pigs had levels between 0 and 0.05, typical of levels in castrated males. The other levels of chicory (30 and 60 g/kg) were not effective (Table 1). The concentration of androstenone increased slightly in the pigs fed 90 g/kg chicory after 2 weeks. Table 2 shows the sensory results after 2 weeks feeding. However the values for abnormal odour are higher than in these other studies. There was no trend in the abnormal odour scores at 2 weeks. The 90 g/kg chicory group, in which skatole had been reduced, had values as high as in the other treatments. A clue to the reason for this is shown by the increase in the score for the term ‘parsnips’ used to describe the odour of androstenone.

**Table 1** Effect of feeding chicory on skatole levels (µg/g)

Week	0 g/kg	30 g/kg	60 g/kg	90 g/kg	p-val
0	0.149 <sup>a</sup>	0.226 <sup>b</sup>	0.131 <sup>a</sup>	0.137 <sup>a</sup>	<0.05
1	0.111	0.085	0.080	0.108	ns
2	0.237 <sup>b</sup>	0.129 <sup>b</sup>	0.124 <sup>b</sup>	0.047	<0.001

**Table2** Main sensory results after 2 weeks

	0	30	60	90	p-val
Pork odour <sup>x</sup>	3.53	3.72	3.58	3.70	ns
Abnormal <sup>x</sup>	4.30 <sup>a</sup>	3.90 <sup>c</sup>	4.22 <sup>ab</sup>	4.04 <sup>bc</sup>	<0.001
Mothballs <sup>y</sup>	11.2 <sup>a</sup>	8.2 <sup>b</sup>	9.2 <sup>ab</sup>	7.4 <sup>b</sup>	<0.05
Parsnip <sup>y</sup>	16.3	17.7	18.4	19.6	ns

<sup>x</sup>1-8 scales, <sup>y</sup>0-100 scales

**Conclusions** The results show that the inclusion of dried chicory in the diet for 2 weeks before finishing reduced skatole concentrations in backfat to a level typical of castrates. However no improvement in odour scores occurred, probably because androstenone remained high. It is possible that as skatole declined, the perception of androstenone increased causing no change in overall abnormal odours.

**Acknowledgements** This project was funded by BPEX.

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## A note on the effect of breed on the feeding behaviour of pigs and variation in feed intake, feed conversion ratio and feeding behaviour between pigs of the same breed

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**Introduction** In scientific studies the effect of breed on feed intake is often determined on groups of pigs (e.g. McCann *et al.* 2008). However, there is a lack of information regarding the feed intake or feeding behaviour of individual pigs in group housing and from different breeds. The objective of this study was to investigate the variation in feed intake and feeding behaviour of group housed pigs representing three commercially available breeds.

**Materials and methods** Twelve LR x LW F1 dams were inseminated with mixed semen from either the PIC 337, PIC 327 or Tempo breed. Piglets were weaned (28 days of age) and penned together in groups of 20, respective of breed. At 10 weeks of age, 72 pigs (12 boars and 12 gilts from each breed) were selected according to weight and placed in groups of 8 (which were balanced for breed and weight) in the finishing accommodation. Pigs were offered feed from ACEMO 54 electronic feeders from 10 weeks of age. At 12 weeks of age the average weight of PIC 327, PIC 337 and Tempo pigs was 38.9, 38.8 and 38.0kg respectively. Feed intake, time of feed and duration of feeding was recorded on an ongoing basis between 12 weeks of age and at finish (152 days of age). Pigs were weighed again at finish. The effect of breed and gender on average daily feed intake (ADFI), feed conversion ratio (FCR) and feeding behaviour (intake/visit/24h, total visits/24h, average time spent/visit and total time spent feeding/24hr) was tested using analysis of variance (Genstat version 10) with 12 week weight used as a covariate. Feeding behaviour data where pigs were found to eat less than 30g but were in the feeder for 30 minutes or more were excluded. The standard deviation (SD) and coefficient of variation (CV) for ADFI, FCR and feeding behaviour data were also calculated for the 24 pigs representing each breed.

**Results** Breed had no significant effect on the ADFI, FCR or the total time spent feeding/24hr (Table 1). However, Tempo pigs had fewer visits to the feeder/24hr but each visit was longer and they ate more feed within that visit compared with pigs from the PIC 327 or PIC 337 breeds (Table 1). The CV of ADFI was higher in the group of Tempo pigs compared with that of the PIC 327 and PIC 337 pigs (Table 2). The CV of FCR was lowest in PIC 337 pigs. The CV of feeding behaviour (intake/visit/24h, total number of visits/24h and average time spent/visit) was markedly greater than that of ADFI and FCR. The SD and CV of feeding behaviour also differed across the groups of pigs from the different breeds (Table 2). There were no significant interactions between breed and gender. Furthermore, there was no significant effect of gender on feeding behaviour (intake/visit/24h (average 248g); total visits/24h (average 10.9); average time spent/visit (average 429s) and total time spent feeding/24h (average 61.7min)). Gender did not significantly effect the ADFI of pigs but gilts had a significantly ( $P<0.01$ ) poorer FCR (2.31) than boars (2.18).

**Table 1** The effect of breed on ADFI, FCR and feeding behaviour between 12 weeks of age and finish

	PIC 327	PIC 337	Tempo	SEM	Sig
ADFI (g/day)	2073	2240	2139	53.7	NS
FCR	2.23	2.20	2.31	0.041	NS
Intake/visit/24h (g)	190 <sup>a</sup>	230 <sup>a</sup>	320 <sup>b</sup>	19.9	<0.001
Total visits/24h	13.8 <sup>b</sup>	11.8 <sup>b</sup>	7.3 <sup>a</sup>	1.11	<0.001
Av Time spent/visit (s)	307 <sup>a</sup>	375 <sup>a</sup>	599 <sup>b</sup>	40.0	<0.001
Total time spent feeding/24h (minutes)	59.4	60.0	65.7	2.65	NS

<sup>a,b,c</sup>, numbers with the same superscripts are not significantly different ( $P>0.05$ )

**Table 2** The SD and CV of ADFI, FCR and feeding behaviour within each group of pigs (24) from the different breeds.

	PIC 327		PIC 337		Tempo	
	SD	CV	SD	CV	SD	CV
ADFI (g/day)	233	0.112	251	0.112	335	0.157
FCR	0.229	0.102	0.131	0.059	0.237	0.103
Intake /visit/24hr (g)	96.2	0.513	94.9	0.394	96.6	0.309
Total visits/24h	6.1	0.434	6.1	0.534	3.4	0.454
Av Time spent/visit (s)	160	0.524	163	0.430	232	0.390
Total time spent feeding/24h (minutes)	9.3	0.157	13.2	0.221	14.3	0.217

**Conclusion** This study suggests that although the average daily feed intake and feed conversion ratio of pigs between breeds may not differ significantly, their feeding behaviour can be very different. Furthermore the variation in feeding behaviour across all breeds was greater than the variation in feed intake and feed conversion ratio observed in the same group of pigs. Further studies with increased replication are required to validate these conclusions.

**Acknowledgements** The co-operation of PCM regards the use of the research facility to conduct this study is appreciated.

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## Comparison of a $\beta$ -glucan-supplemented wheat-based diet with an oat-based diet on apparent total tract nutrient digestibility, nitrogen utilisation and accompanying manure odour and ammonia emissions from finisher pigs

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**Introduction** Increasing consumption of  $\beta$ -glucans in finisher pig diets through dietary provision of oats has been demonstrated to promote carbohydrate fermentation in the distal gastrointestinal tract, with accompanying reductions in manure ammonia and odour emissions as compared with conventional wheat-based diets (O'Shea *et al.*, 2009a). However, offering a source of endogenous  $\beta$ -glucans is associated with decreased nutrient digestibility, due to the presence of other poorly digestible carbohydrates present in the parent oat grain (O'Shea *et al.*, 2009a). The objective of the current study was to investigate the influence of supplementing a wheat-based diet with oat-derived  $\beta$ -glucans compared with an intact source of  $\beta$ -glucans (oat-based diet) on apparent nutrient digestibility and manure odour and ammonia emissions from finisher pigs. An enzyme composite containing  $\beta$ -glucanase and  $\beta$ -xylanase was included to elucidate the effect of both endogenous and exogenous  $\beta$ -glucans on the aforementioned selected parameters.

**Materials and methods** A complete randomized design experiment was conducted to investigate the source of dietary  $\beta$ -glucans and the inclusion or not of an enzyme containing  $\beta$ -glucanase on nutrient digestibility and manure emissions from finisher boars. Experimental diets were as follows: 1) oat-based diet (O), 2) oat-based diet + enzyme (OE), 3) wheat-based diet +  $\beta$ -glucans (WG) and 4) wheat-based diet +  $\beta$ -glucans + enzyme (WGE). All diets were formulated to contain similar concentrations of digestible energy (DE; 13.7MJ/kg), and apparent ileal digestible lysine (8.6g/kg) and total  $\beta$ -glucans. Purified  $\beta$ -glucans were oat-derived (Cambridge Commodities, Cambridgeshire, UK). The enzyme supplement was derived from *Penicillium funiculosum* (IMI SD 101) and contained Endo-1,3 (4)- $\beta$ -glucanase (EC 3.2.1.6) and Endo-1,4- $\beta$ -xylanase (EC 3.2.1.8). Sixteen boars were blocked based on live-weight (60.5kg sd 2 kg) and assigned to one of four dietary treatments (n=4). After a two week dietary adaption period pigs were transferred to metabolism crates for a 7-day apparent total tract nutrient digestibility, nitrogen utilisation (O'Shea *et al.*, 2009b) and manure collection study (n=4). *In vitro* manure ammonia and odour emissions were conducted as described previously (O'Shea *et al.*, 2009b). Briefly, separate urine and faeces collections were amalgamated in original production ratios. Ammonia emissions were determined by microdiffusion. Odour concentrations were determined by olfactometry (Hayes *et al.*, 2004). Experimental data were analysed as a complete randomised design using the GLM procedure of the SAS institute (1985). Contrast statements were used to compare 1) O vs WG, 2) O vs OE and 3) WG vs WGE.

**Results** Consumption of the O diet significantly decreased dry matter ( $P<0.001$ ) digestibility and increased the urine:faeces N excretion ratio ( $P<0.05$ ) compared with the WG diet. Consumption of the O diet significantly decreased manure ammonia ( $P<0.05$ ) and odour emissions ( $P<0.05$ ) compared with the WG diet.

**Table 1** Effect of dietary  $\beta$ -glucan type and enzyme supplementation on nutrient digestibility and manure emissions (LSM $\pm$ sem)

Diet	O	OE	WG	WGE	sem	Contrast 1	Contrast 2	Contrast 3
Dry matter digestibility	0.793	0.788	0.903	0.893	0.006	***	ns	ns
Urine:faeces N excretion	3.0	3.9	4.4	6.1	1.4	*	ns	*
Ammonia 0-240h (mg/g N intake)	71.6	75.4	87.2	87.2	5.1	*	ns	ns
Odour 72h Ou <sub>E</sub> /m <sup>3</sup>	2366	1993	5212	3461	765.0	*	ns	ns

Contrast 1 (O+OE vs WG+WGE); Contrast 2 (O+WG vs OE+WGE); Contrast 3 (interaction between  $\beta$ -glucans and enzyme)

**Discussion and conclusions** In the current study, supplementation of oat-derived  $\beta$ -glucans to a wheat-based pig diet did not depress dry matter digestibility to levels comparable with the oat-based diet. This suggests that  $\beta$ -glucan consumption may be increased without depressive implications for nutrient availability, as has been typically observed where  $\beta$ -glucans are offered in an intact form in oats (O'Shea *et al.*, 2009a). However, the supplementation of purified  $\beta$ -glucans to a wheat-based diet was ineffectual in reducing pig manure odour and ammonia emissions to levels comparable with consumption of the oat-based diet, possibly reflecting the role of other fermentable constituents within oats in functionally mitigating these indices of environmental pollution.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Commercialisation Fund administered by Enterprise Ireland.

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## Influence of within pen gender composition and weight variation on the welfare and growth performance of finishing pigs

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**Introduction** Research shows that entire male pigs are more aggressive during the finishing period than female pigs (Boyle and Björklund 2007). It is not clear to what extent this aggression is influenced by the gender composition of the group. The level of within-group weight variation is also likely to influence aggression and productivity in pigs (O'Connell *et al.* 2005). The aim of this study was to assess the effects of housing entire male finishing pigs in single-sex or mixed-sex groups with high or low within-group weight variation. Effects on aggressive and sexual behaviour and on injury levels and productivity were assessed.

**Material and methods** At the start of the finishing period, 200 pigs were allocated to one of 4 treatments (over 5 replicates) according to group gender composition (all-male group of 10 pigs, or mixed-gender group (5 males, 5 females)) and within-group weight variation (high variation,  $cv \sim 0.18$  or low variation,  $cv \sim 0.05$ ). Treatments were: all-male with high variation [MaH], all-male with low variation [MaL], mixed-sex with high variation [MiH] and mixed-sex with low variation [MiL]. The initial mean body weight in the pen was balanced across treatments ( $29 \pm 0.3$  kg). Pigs were provided with *ad-libitum* access to food. The frequency of fighting and mounting was recorded during 12 focal 3-min observations each week across the 11 week finishing period. Skin lesions were scored at 5, 35 and 70 days after mixing, using a scale of 0 to 4 (0: no lesion, 1: one superficial lesion, 2: more than one superficial or one red lesion, 3: more than one red lesion, 4: one red open wound) on 16 areas of the body of each pig. Scores for each area of the body were combined. Pigs were weighed at 10 and 21 weeks of age, and daily feed intake (DFI) was recorded. The DFI, the average daily gain (ADG) and the food conversion ratio (FCR) were calculated. Pigs were slaughtered at 21 weeks of age and data on carcass weight, P<sub>2</sub> fat depth, lean meat and kill out % were collected. The within-group variation in slaughter weight, carcass weight, P<sub>2</sub> fat depth and lean meat % was calculated. For all data, the pen was considered as the experimental unit. Skin lesion scores and frequency of fighting and mounting were analysed by REML variance components analysis with day, gender composition and within-group weight variation as main effects. Data on performance were analysed by ANOVA with gender composition and within-group weight variation as main effects. For the analysis of growth performance, initial mean body weight was used as a covariate.

**Results** The average frequency (per 3-min observation) of mounting was 2-fold higher in all-male than in mixed-sex groups (all-male group: 0.22, mixed-sex group: 0.11; s.e. 0.031;  $P < 0.001$ ). There was no effect of within-group weight variation on the frequency of mounting ( $P > 0.05$ ), and no effect of treatments on the frequency of fighting ( $P > 0.05$ ). Skin lesion scores were highest in MaL groups and lowest in MiH group (MaL: 1.25, MaH: 1.15, MiL: 1.12, MiH: 1.09; s.e. 0.056,  $P < 0.05$ ). There was a tendency for interactive effects between group gender composition and within-group weight variation on slaughter weight, carcass weight, DFI and ADG ( $P < 0.10$ ), but no effect on the FCR ( $P > 0.05$ , Table 1). Reducing within-group starting weight variation led to a reduction in within-group variation in weight at slaughter (high variation: 0.15, low variation: 0.08; s.e. 0.010,  $P < 0.001$ ) and carcass weight (high variation: 0.16, low variation: 0.09; s.e. 0.010,  $P < 0.001$ ). The P<sub>2</sub> fat depth and lean meat % were not affected by treatment ( $P > 0.05$ , Table 1).

**Table 1** Interactive effects between group gender composition and within-group weight variation on production performance

	MaH	MaL	MiH	MiL	s.e	P
Slaughter weight, kg	87.1	89.0	88.5	86.0	1.27	<0.1
Carcass weight, kg	64.5	66.4	66.9	64.5	1.10	<0.1
DFI, g	1757	1888	1867	1795	55.8	<0.1
ADG, g	705	780	746	735	23.6	<0.1
FCR	2.50	2.42	2.51	2.45	0.040	NS
P <sub>2</sub> fat depth, mm	11.1	12.0	11.3	11.1	0.42	NS
Lean meat, %	62.1	61.3	61.9	62.1	0.34	NS

**Conclusions** Housing finishing entire male pigs in all-male groups led to an increase in injuries when initial within-group weight variation was low, and led to increased levels of mounting, which is likely to have a negative effect on welfare. This suggests that entire male pigs should be housed in mixed-sex groups. Reducing the within-group weight variation at the start of the finishing period led to reduced within-group variation in slaughter and carcass weight. This is beneficial in terms of leading to a more efficient use of finishing accommodation, and reducing variability in the final product. However, in mixed-sex groups this practice led to slight numerical reductions in performance parameters, and increases in injury scores.

**Acknowledgements** The authors gratefully acknowledge funding from Teagasc Walsh fellowship.

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## Effect of piglet birth weight on serum triglyceride levels at weaning and at slaughter

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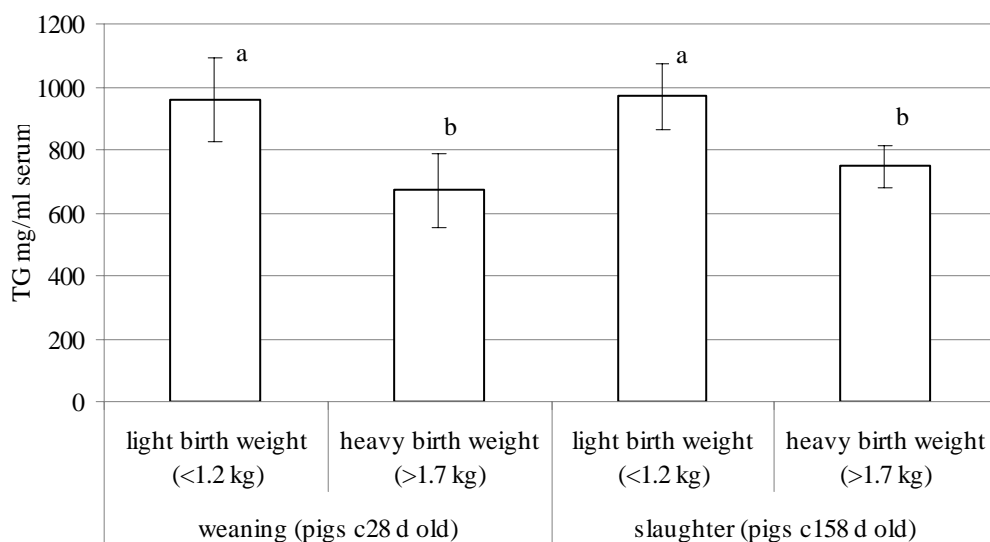
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**Introduction** In pigs, birth weight is associated with health status in later life. The heavier the piglet is at birth the more likely it is to survive. During critical growth periods *in utero*, an adverse foetal environment can lead to permanent changes in the metabolism of the offspring (foetal programming). Light birth weight piglets tend to have higher levels of free fatty acids in their blood at weaning (Lösel *et al.* 2009) and have higher levels of carcass fat at slaughter than heavy birth weight piglets (Rehfeldt and Kuhn 2006). The aim of this study was to determine the influence of back fat levels in gilts and nutrition during gestation on serum triglyceride (TG) levels in offspring at weaning and slaughter. The hypothesis was that lighter pigs at birth would have higher levels of serum triglycerides due to unfavourable foetal programming *in utero*.

**Materials and methods** Fourteen gilts (Landrace x Large White) were used in this study with either; low back fat depth (P2:~12 mm) or high back fat depth (P2:~17 mm) at service. At d 25 of gestation gilts were randomly allocated to a gestation diet (6.19 g/kg lysine, 13.0 MJ DE/kg) at the following levels: 1.8 kg, 2.5 kg or 3.5 kg until d 90 of gestation. Offspring from gilts were selected at weaning based on their birth weight. Two of the lightest (<1.2 kg at birth) and two of the heaviest piglets (>1.7 kg at birth) were selected from each litter. One light and one heavy piglet were sacrificed at weaning and blood samples were collected. The remaining light and heavy piglets from each litter were individually fed and followed through to slaughter at c. 130 days post weaning (c.100 kg) when blood samples were collected. Blood was allowed to clot at room temperature for a few hours before serum was separated by centrifugation at 2500 x g for 20 min at 15°C. Serum was analysed for TG levels using an enzymatic-colorimetric determination kit (Sentinel Diagnostics). Data was analysed using the mixed models procedure of SAS with effects for growth stage (weaning or slaughter), birth weight (light or heavy), feeding level (1.8 kg, 2.5 kg and 3.5 kg), sow back fat level (high or low) and their interactions. Sow was included as a random effect.

**Results** Mean serum TG levels of light and heavy birth weight pigs were similar at both weaning and slaughter ( $P>0.05$ ). Light birth weight piglets had higher serum TG levels than their heavy littermates when measured both at weaning and at slaughter ( $P<0.01$ ; Figure 1). There was no birth weight  $\times$  growth stage interaction for serum TG level. Serum TG level was influenced by gilt (dam) ( $P<0.05$ ).

**Conclusions** These preliminary results indicate that light birth weight littermates have higher serum TG levels than heavy littermates at weaning and this difference persists to 100 kg. This life long effect of birth weight on serum TG levels may



**Figure 2** Effect of birth weight and growth stage on offspring serum TG levels. <sup>a, b</sup> Columns within the graph that do not share a common letter are significantly different ( $P<0.05$ ).

be part of a foetal programming effect. Future work will look at the underlying mechanisms for the effects observed.

**Acknowledgements** This research is funded by Teagasc under the National Development Plan. C. Amdi's PhD is funded by Teagasc under its Walsh Fellowship Scheme.

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## Effect of supplementing late gestation sows with *Saccharomyces cerevisiae* on piglet growth performance

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**Introduction** Increasing concerns over antibiotic resistance led to a 2006 EU ban on the routine use of sub-therapeutic antibiotics as growth promoting agents and rising interest in alternative growth promoters. Probiotics have been reported to have numerous beneficial effects on growth including improved weight gains and feed:gain ratios as well as reduced morbidity and mortality (Bertin *et al.*, 1997a; Bertin *et al.*, 1997b; Alexopoulos *et al.*, 2004). However some studies report no effect (Estienne *et al.*, 2005), and others show adverse effects (Ratcliffe *et al.*, 1986). Close (2000) notes that when results are averaged across several studies, the effect of probiotics on pig growth appears to be an improvement in growth and in feed efficiency, but that the results are highly variable. The present study examined the effect of supplementing late gestation sows with a commercial preparation of *Saccharomyces cerevisiae* on suckling piglets' growth performance pre-weaning.

**Materials and methods** 28 sows in a commercial system were blocked according to parity and randomly assigned to one of two treatments: (i) no extra supplement (C; n=14) or (ii) probiotic supplement (T; n=14). Sows in the treatment group received the probiotic (Levucell SB Sow) for 3 weeks prior to farrowing. Measurements of piglet body weight in kilograms, crown-to-rump length (C2R), limb length (LL), heart girth (HG) and abdominal circumference (AC) were recorded weekly from farrowing until weaning. Weaning weights were also recorded. Data were analyzed using GLM ANOVA in Minitab version 15.0. Sow was included as a random factor in the model to account for fluctuations in litter size. Age at weaning varied between litters and was therefore used as a covariate in subsequent statistical analysis.

**Results** At day 1 there were no statistical differences between treatment groups (Table 1). By day 7 treated piglets were lighter, and had smaller C2R and LL measurements ( $P<0.05$ ). At day 14 treated piglets were smaller for all measurements, except weight. LL was again lower in treated piglets at day 21. At weaning there was no statistical difference in weight between the groups.

**Table 1** Effect of treatment on piglet weight, crown to rump length, limb length, heart girth and abdominal circumference

	Weight		C2R		LL		HG		AC	
	C	T	C	T	C	T	C	T	C	T
Day 1	2.03± 0.05	1.93± 0.05	30.68	30.11	18.28	17.95	26.48	26.67	21.39	21.35
Day 7	3.57± 0.10 <sup>a</sup>	3.28± 0.11 <sup>a</sup>	36.28 <sup>b</sup>	34.95 <sup>b</sup>	22.02 <sup>c</sup>	21.21 <sup>c</sup>	33.61	32.89	26.94	26.63
Day 14	5.41± 0.16	5.10± 0.17	41.86 <sup>a</sup>	40.81 <sup>a</sup>	25.29 <sup>b</sup>	24.55 <sup>b</sup>	38.65 <sup>c</sup>	37.23 <sup>c</sup>	32.02 <sup>d</sup>	30.70 <sup>d</sup>
Day 21	6.83± 0.20	6.64± 0.24	45.67	44.71	25.29 <sup>a</sup>	24.55 <sup>a</sup>	41.01	40.77	34.38	33.50
Weaning	8.15± 0.23	7.36± 0.22								

Values are presented as means, with weights as means ± SEM. Values within a row with shared superscripts <sup>a b c d</sup> are statistically different at a 95% confidence interval.

**Conclusions** In contrast to much of the published literature (Bertin *et al.*, 1997a; Bertin *et al.*, 1997b), dietary supplementation of *S. cerevisiae* to late gestation sows did not improve piglet growth performance at weaning, and may adversely affect growth in the first weeks of life. A number of factors may have affected the efficacy of the probiotics, including supplementation length and the cleanliness of the production environment. The differences in sizes but not in weight suggest a possible alteration in body composition in the probiotically treated group with treated piglets being leaner than those in the control group. Further work is currently being completed to see if there are any treatment effects at slaughter.

**Acknowledgements** The study was funded by Biotol. The pigs were housed at Sparsholt College, Hampshire; Technical assistance from J. Garrett

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## The impact of chronic environmental stressors on the social behaviour of growing pigs, *Sus scrofa*

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**Introduction** The effects of common and current environmental stressors on the social behaviour of farm animals are poorly understood and have never before been tested in controlled conditions. Here, we report the results of a longitudinal, multi-factorial experiment designed specifically to examine the individual, additive or interactive effects of elevated ammonia, noise and dim light levels on the social behaviour of growing pigs under strictly controlled environmental conditions.

**Material and methods** Two batches of 126 4-week-old hybrid gilts (50% Pietrain, 25% white Duroc, 12.5% Large White, 12.5% Landrace) were obtained at weaning. Eight rooms were allocated to eight experimental treatments in a 2<sup>3</sup> multi-factorial design. Each treatment comprised elevated or low levels of light intensity (nominally 200 vs. 40 lux), atmospheric ammonia concentration (nominally < 5 ppm vs. 20 ppm) and broadband noise, (nominal level < 60 dB(A) vs. 80 dB(A)). Social behaviour was measured in terms of the nature, frequency and duration of both initiated and respondent behaviours for four weeks following mixing of the groups according to an established ethogram (Jensen 1980). This was achieved by using a mixture of scan and continuous sampling. General activity patterns, group cohesion and social discrimination were also examined as a function of the environmental treatments. Frequency, nature and bout-duration were analysed using a mixture of general and generalised linear mixed models. The models were constructed with room\* batch entered as a random factor to control for inter-room variation. The fixed effects were ammonia, light and noise, as well as all two- and three-way interactions between these factors. In models where we were interested in change over time, week was entered as a covariate. Wald-type adjusted *F* statistics were used to test null hypotheses and least squares (LS) means output from the models was used to examine interactions.

**Results** Elevated concentrations of atmospheric ammonia (~20 ppm) and dim light intensity (~40 lux) had the most significant effects, particularly on the nature of social interactions, with pigs under these conditions showing more aggression in the early stages of the experiment. In addition, pigs exposed to a high level of mechanical noise representative of artificial ventilation (~80 dB (A)) were less submissive to aggressive acts, while pigs in ~20 ppm ammonia showed more reciprocated aggression when in coincident dim light (<5 lux).

**Table 1** Summary of Main Effects for Ammonia, Noise and Light Conditions

	Ammonia			Light			Noise		
	High (SEM)	Low (SEM)	<i>P</i>	High (SEM)	Low (SEM)	<i>P</i>	High (SEM)	Low (SEM)	<i>P</i>
Aggressive interactions (prop total acts)	0.09 (.01)	0.08 (.01)	< .01	0.08 (.01)	0.10 (.01)	< .01	0.08 (.04)	0.08 (.04)	> .05
Submissiveness (prop of responses to aggression)	0.40 (.04)	0.45 (.04)	> .05	0.41 (.04)	0.45 (.04)	> .05	0.52 (.03)	0.36 (.03)	< .05

**Conclusion** In conclusion, there is now evidence that commonly experienced concentrations of ammonia ( $\geq 20$  ppm) and dim light intensities ( $\leq 40$  lux) can affect aggression in pigs. Aggression in pigs is signalled with odour and visual cues. Group-mate recognition can be olfactory, and odour cues may be masked by the ammonia, affecting perception and modulating the formation of stability in the group. The findings reported here may have implications for the welfare of farmed pigs in the UK. Ventilation systems should be designed further to minimise the aerial ammonia concentrations to avoid potential exacerbation of aggressive acts early in the development of the pigs. In addition, it seems that dim lighting may increase aggressive acts, again, early in the development of the pigs, and the industry should take account of this when designing facilities in the future.

**Acknowledgments** This work was funded by the BBSRC

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## Effects of physical form of total mixed rations on feed intake and eating rate in lactating dairy cattle

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**Introduction** Nowadays total mixed ration (TMR) is commonly provided to lactating cows rather than separate feeding of concentrate and forage (DeVries *et al.*, 2007). However, complete ingestion of feeds in proportion to dietary concentration is not taken due to sorting undertaken by cow. When diets are closely formulated to minimum recommendations, sorting could reduce intake of long particles and NDF intake which in turn results in decreasing chewing activity, rumen pH, and milk fat test. It seems that no relevant data are available on sorting activity of lactating dairy cattle fed a TMR. Cows have shown that they preferentially sort for the grain component of a TMR and discriminate against the longer forage components (Leonardi and Armentano, 2003). As pointed out by Osafo *et al.*, 1997, sorting by dairy cattle could also be affected by physical processing. The main objective of the present research was to determine effects of different physical forms of total mixed ration on feed intake and eating rate in lactating dairy cows.

**Material and methods** Three ruminal cannulated primiparous Brown Swiss cows with average days in milk 130, body weight 560 kg and daily milk yield 20 kg were randomly assigned to a 3\*3 Latin square. Three 21d periods were considered in the experiment. During each period (including 14d for adaptation and 7d for record collection) the animals were offered one of three TMRs. Ingredients and chemical composition were the same over the experimental diets (NE<sub>l</sub> 7.10 MJ/kg, CP 160 g/kg and NDF 365 g/kg). the diets were 1. TMR with mash concentrate 2. TMR with pellet concentrate and 3. Cubed TMR. The cows were fed at hours 08:00 and 20:00 *ad libitum*. Feed intake were measured daily during the each 7-d period of the collection. Orts from individual cows were collected daily for calculation of DMI and NDFI (NDF intake). On days 5 and 6 of each period, feed bunk contents of each animal were weighed at 0, 2, 4, 8, and 12 h after a.m. feeding to determine DMI, and NDFI.

**Result** There was no significant difference among the different treatments regarding DMI and NDFI. Cows fed with cube TMR showed higher DMI and NDFI compared to other treatments (Table 1). Intake of DM at 2, 4, 8 and 12 h postfeeding are presented in Table 2.

**Table 1** Effects of TMR physical form on DMI and NDFI

Case	Diets Mash	Pellet	Cube	SEM
DMI <sub>kg/day</sub>	19.31	18.45	19.99	0.412
NDFI <sub>kg/day</sub>	6.47	6.02	6.91	0.181

**Table 2** Effects of TMR physical form on DMI at 2, 4, 8, and 12 h post-feeding

Hour	Diets Mash	Pellet	Cube	SEM
2	5.03 <sup>a</sup>	7.82 <sup>b</sup>	5.03 <sup>a</sup>	0.326
4	6.75 <sup>a</sup>	9.16 <sup>b</sup>	7.15 <sup>a</sup>	0.329
8	10.15	10.87	9.87	0.329
12	12.09	12.36	12.71	0.326

Means within the same row with differing superscripts are significantly different  $P < 0.05$ .

**Conclusion** It is concluded that cubed rations may increase DMI of lactating dairy cattle revealing the fact that increasing particle size of feed may increase sorting behaviour of the animal. Using pellet TMR could increase feed intake at early feeding hours.

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## Effects of physical forms of total mixed rations on ruminal pH and chewing activity of lactating cow

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**Introduction** Various chemical and physical factors such as NDF concentration and particle size affect rumen fermentation and as a result influence milk production and composition (Leonardi and Armentano, 2003). It is commonly believed that feeding forage with short particle size decrease chewing activity and salivary buffer secretion leading to lowering rumen pH and milk fat percentage (Mertens, 1997). Nocek and Braund (1985) suggested that feeding TMR is the optimal way to provide the balance of nutrients (including protein and structural and non-structural carbohydrates) that ruminants need to maintain a stable and efficient microbial population. In particular, the *ad libitum* feeding of TMR results in a ruminal steady state condition conducive to continuous rumen function and digesta flow (Nocek et. al, 1986). The objective of this research was to determine effects of different physical forms of total mixed ration (TMR) on eating behaviour of lactating dairy cows.

**Material and methods** Three ruminal cannulated primiparous Brown Swiss cows with average days in milk 130, body weight 560 kg and daily milk yield 20 kg were randomly assigned to a 3\*3 Latin square. Three 21d periods were considered in the experiment. During each period (including 14d for adaptation and 7d for record collection) the animals were offered one of three TMRs. Ingredients and chemical composition were the same over the experimental diets (NE<sub>i</sub> 7.10 MJ/kg, CP 160 g/kg and NDF 365 g/kg). The diets were 1. TMR with mash concentrate 2. TMR with pellet concentrate and 3. Cubed TMR. The cows were fed at hours 08:00 and 20:00 *ad libitum*. Total chewing activities (eating + rumination) were measured d 18 each period. On d 21 of each period, ruminal for determine pH were taken at 0.0, 2, 4, 6, 9 and 12 h after a.m. feeding.

**Result** Effects of TMR physical form on rumen pH and eating and ruminating activities are presented in Tables 1 and 2 respectively.

**Table 1** Effects of TMR physical form on ruminal pH of lactating dairy cow

Hour	Diets			SEM
	Mash	Pellet	Cube	
0	6.56	6.54	6.26	0.048
2	6.21	6.14	6.02	0.048
4	5.94	5.98	5.76	0.048
6	6.06	6.10	5.85	0.048
9	6.23	6.26	5.98	0.048
12	6.38	6.54	6.28	0.048

**Table 2** Effects of TMR physical form on chewing activities of lactating dairy cow.

case		Diets			SEM
		Mash	Pellet	Cube	
Eating	Time <sub>min/d</sub>	335 <sup>a</sup>	356.67 <sup>a</sup>	285 <sup>b</sup>	10.184
	DMI <sub>min/kg</sub>	17.3 <sup>a</sup>	19.4 <sup>b</sup>	14.26 <sup>c</sup>	0.134
	NDFI <sub>min/kg</sub>	51.57 <sup>a</sup>	59.41 <sup>b</sup>	41.28 <sup>c</sup>	0.353
	Time <sub>min/d</sub>	458.33 <sup>a</sup>	443.33 <sup>a</sup>	316.67 <sup>b</sup>	28.50
Rumination	DMI <sub>min/kg</sub>	23.75 <sup>a</sup>	24.3 <sup>a</sup>	15.85 <sup>b</sup>	0.954
	NDFI <sub>min/kg</sub>	70.88 <sup>a</sup>	73.58 <sup>a</sup>	45.81 <sup>b</sup>	2.796

Means within the same row with differing superscripts are significantly different P<0.05

**Conclusion** Based upon the findings of the present research it can be concluded that feeding cubed rations decrease chewing activity and salivary secretion and decrease rumen pH due to shorter particle size as compared to other forms of TMR applied.

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## Associations between age at first calving, liveweight at first calving and milk production in Holstein-Friesian dairy cows

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**Introduction** Current recommendations are that Holstein-Friesian dairy heifers should calve at 2 years of age at a target liveweight (LW) of 550 kg. There is, however, some evidence to suggest that the effects of a younger age at first calving (AFC) on milk performance can be offset by a heavier LW at calving (Dobos *et al.*, 2001). Nonetheless, most studies only report the associations between either AFC (Berry and Cromie, 2009) or LW at calving (Cowan *et al.*, 1974) and milk performance. The objective of this study was to quantify the association between both AFC and LW at first calving and first lactation milk production in spring-calving Irish Holstein-Friesian dairy cows.

**Materials and methods** Milk performance data from 485 spring-calving, Holstein-Friesian dairy heifers, calving for the first time between 20 and 28 months of age, over a five year period (2003 to 2008) were obtained from three research herds at Moorepark Dairy Production Research Center. LW at first calving was assumed to be the first recorded LW between days 1 to 14 post-calving. Predicted transmitting ability (PTA) for carcass weight for each animal was obtained from the May 2009 national domestic genetic evaluation run. PTA for carcass weight is based on the carcass weight of male animals aged between 300 and 1,200 days at slaughter and female animals aged between 300 and 875 days at slaughter. A multiple regression model was developed with LW at first calving as the dependant variable and both PTA for carcass weight and days post calving to first LW recording included as independent variables. The residuals from the model were added to the intercept to generate an adjusted LW at first calving which was used in all subsequent analyses. Logistic regression was used to quantify the association between both AFC and LW at first calving and survival to second lactation; animals culled because of poor genetic merit or surplus to requirements were excluded from this analysis. A fixed effects linear model was used to quantify the associations between both AFC and LW at first calving and first parity milk performance; lactation yield was based on the actual lactation length for each animal and not standardised to 305-days. Fixed effects adjusted for in the model were experimental treatment and year of calving. AFC and LW at first calving were included in all models as continuous variables and non-linear associations with survival and performance were also investigated.

**Results** Mean (standard deviation) AFC and LW at first calving among the 485 animals was 726 days (29.7 days) and 546 kg (45.6 kg), respectively. The correlation between AFC and LW at first calving was 0.29; however, no collinearity existed between the two variables. AFC was not associated with survival to second lactation but LW at first calving was non-linearly associated with survival to second lactation ( $\text{Logit}\{P=\text{survived}|LW\} = 0.0035 \cdot LW - 0.0001 \cdot LW^2$ ). AFC was non-linearly associated with all traits with the exception of milk fat percent. Yield was positively associated with AFC to an AFC between 677 and 704 days after which a negative association existed. No non-linear associations were observed between LW at first calving and performance and LW was not associated with milk dry matter content. Increased LW was associated with increased yield. With the exception of milk fat and milk protein percent, LW explained more of the variation in performance than AFC.

**Table 1** Linear and quadratic regression coefficients of milk production on AFC and LW at first calving as well as partial coefficient of determination ( $R^2$ ).

	Intercept <sup>1</sup> (se)	Age at first calving (days)			LW at first calving (kg)	
		Linear (se)	Quadratic (se)	$R^2$	Linear (se)	$R^2$
AFC (days)	725 (1.7)				0.22 (0.028)***	0.10
LW at first calving (kg)	549 (2.6)	0.53 (0.07)***		0.11		
Milk yield (kg)	5020 (40.8)	89.6 (37.5)*	-0.066 (0.026)*	0.02	4.6 (0.8)***	0.06
Fat yield (kg)	201.8 (1.7)	5.35 (1.57)***	-0.004 (0.001)***	0.04	0.18 (0.03)***	0.06
Protein yield (kg)	170.2 (1.4)	4.85 (1.25)***	-0.004 (0.001)***	0.05	0.15 (0.03)***	0.06
Milk solids yield (kg)	372.0 (2.9)	10.20 (2.70)***	-0.007 (0.002)***	0.05	0.33 (0.05)***	0.06
Fat percent (%*1000)	4.11 (0.02)	1.51 (0.68)*		0.01	-0.189 (0.430) <sup>NS</sup>	0.00
Protein percent (%*1000)	3.45 (0.01)	31.11 (10.21)**	-0.022 (0.007)**	0.03	-0.007 (0.204) <sup>NS</sup>	0.00

<sup>1</sup> Intercept represents the average across all treatments and years and the average adjusted liveweight of 546 kg and average AFC of 726 days. \*\*\*  $P < 0.001$ ; \*\*  $P < 0.01$ ; \*  $P < 0.05$ ; NS non-significance

**Conclusions** Both AFC and LW at first calving were associated with milk, fat and protein yield but only AFC was associated with milk dry matter content; most of the associations involving AFC were non-linear. The results indicate that optimum AFC is between 22 and 23 months of age, in addition it is clear that animals also need to attain their respective pre-calving target weight to ensure that production performance is maximised.

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## Effect of dairy management, season and breed on protein composition of retail whole milk

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**Introduction** Milk proteins are important in human nutrition since they provide the human body with essential amino acids, not only in high amounts but also in almost the ideal proportions for neonates. Milk proteins contribute 95% of total milk nitrogen and belong to two main categories; caseins and whey proteins. Caseins include  $\alpha$ -casein ( $\alpha$ CN),  $\beta$ -casein ( $\beta$ CN),  $\gamma$ -casein ( $\gamma$ CN) and  $\kappa$ -casein ( $\kappa$ CN) whereas whey proteins include  $\alpha$ -lactalbumin ( $\alpha$ La),  $\beta$ -lactoglobulin ( $\beta$ Lg), and its variants  $\beta$ LgA and  $\beta$ LgB, bovine serum albumin (BSA) and immunoglobulins (Ig) (Givens & Shingfield, 2004). Milk bioactive peptides are amino acid sequences within the milk proteins which are released after the digestion of either whey proteins or caseins. Peptides from casein (which include caseinophosphopeptides and other miscellaneous peptides) and whey proteins show antimicrobial, antihypertensive, antithrombotic, immunomodulating, and opioid properties (Clare & Swaisgood, 2000). Individual milk whey proteins have also been extensively associated with positive impacts in human health, demonstrating antimicrobial, antiviral, anticarcinogenic, immunomodulatory and other metabolic functions (Madureira *et al.* 2007). Factors like genetics, nutrition and animal health can strongly influence milk protein composition (DePeters & Cant, 1992). This study aimed to investigate the protein composition of different brands of retail milk, as they are differentiated by bottle labelling, and examine if the differences reported in the past between breeds or between different nutritional strategies are seen at the retail level.

**Materials and methods** In this survey, 28 different sources of commercial full-fat milk, characterised by brand, management system and animal breed, as identified by the bottle label were collected from retail outlets in the North East of England; 9 brands represented organic milk and 5 brands represented milk from Jersey & Guernsey cows (JG) the remainder were conventional milks from non specified breeds (NS). Milk purchase was carried out over two years on 4 sampling dates: 2 during winter (January 2007/2008) and 2 during summer (August 2006/2007). Separation of individual proteins was performed on high performance liquid chromatography (HPLC), using a Reversed-Phase C4 analytical column 250x4.6mm, 300A pore diameter and 5 $\mu$ m particle size. Protein contents of NS milk were compared with i) organic milk, and ii) JG milk. Analysis of variance (ANOVA) using linear mixed effects model (LME) was used to analyze results in R statistical environment using “season” (winter or summer) and “management system” (conventional or organic) or “animal breed” (JG, NS) as fixed factors and milk ID as a random factor.

**Results** Overall protein content of milk was not significantly influenced by either management or season although, as expected, Jersey milk was higher in total protein than NS milk (3.75 v 3.17 g/100g milk,  $p < 0.05$ ). As shown by tables 1 and 2 organic management did not significantly affect milk protein composition. A seasonal effect was significant for milk  $\beta$ CN and  $\kappa$ CN concentrations, which were higher in summer milk, and for  $\beta$ Lg variants and total whey protein concentrations, which were lower in summer than in winter milk. JG milk was associated with significantly higher concentrations of protein, casein and individual caseins compared with NS milk while animal breed did not significantly affect the concentrations of whey proteins in milk.

**Table 1** Differences in relative proportions (%) of total protein and caseins in organic, summer and JG milk compared with conventional, winter and NS milk

	Organic	P-value	Summer	P-value	JG	P-value
$\alpha$ CN	-0.6%	ns	-2.2%	ns	+21.1%	***
$\beta$ CN	-3.1%	ns	+9.2%	**	+12.6%	**
$\kappa$ CN	-0.7%	ns	+6.6%	*	+38.9%	***
Caseins	-1.7%	ns	+4.6%	ns	+21.8%	***
Protein	-1.3%	ns	+2.4%	ns	+19.2%	***

**Table 2** Differences in relative proportions (%) of whey proteins in organic, summer and JG milk compared with conventional, winter and NS milk

	Organic	P-value	Summer	P-value	JG	P-value
$\alpha$ La	-1.5%	ns	+3.6%	†	+5.6%	ns
BSA	+1.8%	ns	+4.4%	ns	-19.3%	†
$\beta$ LgA	+3.0%	ns	-19.0%	***	+6.9%	ns
$\beta$ LgB	+0.4%	ns	-8.1%	***	+5.2%	ns
Whey	+0.9%	ns	-8.8%	***	+4.7%	ns

Significances were declared at \*\*\*:  $P < 0.001$ , \*\*:  $P < 0.01$ , \*:  $P < 0.05$ , †:  $0.05 < P < 0.10$ , ns:  $P > 0.10$

**Conclusions** The different production practices used in organic and conventional systems in the UK were insufficient to produce any differences in milk protein composition of retail whole milk. The increase in milk casein in summer milk was found at the expense of whey protein content, thus showing the same total protein content as winter milk. Similar results for the seasonal effect were taken from both datasets examined. In contrast, the strong effect of animal breed in milk protein composition at individual animal level found in other studies was confirmed in retail milk with JG milk showing higher concentrations of protein and caseins.

**Acknowledgements** The authors gratefully acknowledge financial support from the European Community under the 6th Framework Programme Integrated Project “QualityLowInputFood”, FP6-FOOD-CT-2003-506358, the Greek State Scholarship Foundation and the Yorkshire Agricultural Society.

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## Fatty acid composition of retail whole milk from Jersey and Guernsey cows over a two year study

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**Introduction** The relatively high saturated fatty acid (SFA) content in milk fat has been linked with negative effects on human health. However, some monounsaturated fatty acids (MUFA), such as *cis*-9 C18:1 (oleic acid, OA) and *trans*-11 C18:1 (vaccenic acid, VA) and polyunsaturated fatty acids (PUFA) from the n-3 and n-6 series and *cis*-9*trans*-11 conjugated linoleic acid (CLA) found in milk, have been linked to beneficial effects on human health (Haug *et al.* 2007). C18:3n-3 (ALA) and C18:2n-6 (LA) are the main n-3 and n-6 fatty acids (FA) in milk, respectively. In other studies, milk collected from Jersey cows showed differences in the FA profile compared with milk from Holstein cows (Carroll, 2006) which are almost exclusively used in the UK. Seasonal effects, comparing summer diets with high intakes of fresh grass, with winter diets based on silages and concentrates, have been found to increase the concentrations of ALA, VA, CLA and PUFA in milk (Chilliard *et al.*, 2000). Milk collected in this survey was derived from conventional farms, in order to avoid the effects of organic management on milk FA profile, previously shown in retail milk (Stergiadis, 2009). The aim of this study was to investigate the FA composition of retail whole milk labelled as “Jersey” or “Jersey & Guernsey” during summer and winter periods to establish whether the differences reported in previous studies at the individual cow level were also seen in commercial milk.

**Materials and methods** In this survey, 16 different sources of commercial full-fat milk, characterised by brand and animal breed, were collected from retail outlets in the North East of England. 4 brands were labelled as “Jersey” or “Jersey and Guernsey” milk (JG) while the other 12 sources indicated no specification about the animal breed used (NS). Milk purchase was carried out over two years on 4 sampling dates: 2 during winter (January 2007/2008) and 2 during summer (August 2006/2007). Analysis of FA methyl esters was performed with a Gas Chromatography system (Shimadzu, GC-2014, Japan) using a Varian CP-SIL 88 fused silica capillary column (100m x 0.25mmID x 0.2µm film thickness). Peaks were identified using a 39 FAME and CLA standards. Analysis of variance (ANOVA) using linear mixed effects model was used to analyze results in R statistical environment using “year” (1<sup>st</sup> or 2<sup>nd</sup>), “season” (winter or summer) and “breed” (JG or NS) as fixed factors and milk ID as random factor.

**Results** Table 1 shows that there was no effect of breed on the individual PUFA concentrations in milk, while JG milk had lower concentrations of OA than NS milk. In contrast, the seasonal influence was high and summer milk was associated with higher concentrations of OA, VA, CLA and ALA compared with winter milk. Table 2 shows that JG milk had higher concentrations of SFA, lower concentrations of MUFA and tended to show lower concentrations of PUFA than NS milk. In addition, summer milk had higher concentrations of MUFA, PUFA and n-3 FA and lower concentrations of SFA than winter milk. Milk LA concentrations were not affected by either season or animal breed. Fat content of milk expressed as g fat/100g milk was; 4.80% for JG milk, 3.49% for NS milk, 3.73% for summer milk and 3.92% for winter milk with the differences between JG vs NS and summer vs winter being statistically significant.

**Table 1** Differences in relative proportions (%) of individual fatty acids in JG and summer milk compared with NS and winter milk

	JG	P-value	Summer	P-value
OA	-10.4%	**	+14.2%	***
VA	+14.3%	ns	+109.8%	***
CLA	-7.4%	ns	+70.3%	***
ALA	-1.0%	ns	+38.2%	***
LA	-8.5%	ns	-2.8%	ns

**Table 2** Differences in relative proportions (%) of fatty acid groups in JG and summer milk compared with NS and winter milk

	JG	P-value	Summer	P-value
SFA	+3.8%	**	-5.8%	***
MUFA	-9.5%	**	+16.2%	***
PUFA	-7.6%	†	+13.4%	***
n-3	+0.4%	ns	+31.9%	***
n-6	-9.8%	ns	-2.2%	ns

Significances were declared at \*\*\*: P<0.001, \*\*: P<0.01, †: 0.05<P<0.10, ns: P>0.10

**Conclusions** Retail milk without breed specification showed a slightly better fatty acid profile than retail milk from Jersey & Guernsey cows. The relatively high content of SFA shown in other studies for Jersey milk has been confirmed for milk at the consumer level although the beneficial PUFA concentrations were not different between the different sources of milk. The concentrations of the FA groups and individual FA that have previously been associated with positive effects in human health were higher in summer milk compared with winter milk. This also raises the importance for improvement of the FA profile of winter milk, possibly by the nutritional management of cows.

**Acknowledgements** The authors gratefully acknowledge financial support from the European Community under the 6th Framework Programme Integrated Project “QualityLowInputFood”, FP6-FOOD-CT-2003-506358, the Greek State Scholarship Foundation and the Yorkshire Agricultural Society.

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## Effect of incremental dietary addition of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on milk production and composition of dairy cows

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**Introduction** The incremental effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi; MetaSmart® Dry, Adisseo, France), a methionine analogue, on production of lactating dairy cows have to our knowledge not been reported. Feeding HMBi to dairy cows receiving a maize silage based diet increased milk protein concentration, but not milk yield (Phipps *et al.*, 2007). The failure to establish a significant milk yield response was attributed to relatively short treatment periods, which may have limited the expression of a milk yield response. In contrast, a significant milk yield response was observed in a continuous design lactation trial, but the response took 2 months to develop (St-Pierre and Sylvester, 2005). The objective of the present study was to determine the incremental effects of HMBi on feed intake and milk yield and composition of dairy cows over a 16-week treatment period.

**Materials and methods** Following a 1-week covariate period beginning in the 3rd week of lactation, sixty Holstein cows were allocated to one of four treatments (15 cows per treatment) in a randomized block design study. Treatments were based on an expected dry matter (DM) intake (DMI) of 24 kg/d and were 0, 25, 50, or 75 g HMBi/d. Cows were assigned to treatments in blocks based on parity and covariate period milk yield and all animals received a total mixed ration (TMR) provided fresh on a daily basis that contained (%) on a DM basis: grass silage (12.5), maize silage (37.5), and maize grain-based concentrates (50.0). Measured concentrations of crude protein, starch, and neutral detergent fibre for the TMRs fed averaged 171, 214, and 304 g/kg DM, respectively. Milk yield was measured daily and milk composition was measured weekly. Data were analyzed as repeated measurements using mixed models procedures and a model testing fixed effects of treatment, week, parity, and their interactions and random effects of cow using preliminary measurements as a covariate. Orthogonal contrasts were used to partition the main effect of HMBi amount into linear, quadratic, and cubic effects.

**Results** Feeding increasing amounts of HMBi had a quadratic positive effect on DMI, with the greatest DMI at 50 g/d of HMBi (Table 1). Milk yield was not affected by HMBi, although there was a numerical increase in energy corrected milk yield with HMBi supplementation (Table 1). Feeding HMBi increased milk protein concentration (Table 1), with the maximal response at 75 g/d. Feeding HMBi also linearly increased milk protein yield, whilst linearly decreasing milk urea concentration (Table 1). A linear increase was observed in both milk fat content and milk fat yield as HMBi intake increased (Table 1).

**Table 1** Incremental effects of HMBi on DMI and milk production of lactating dairy cows.

	HMBi, g/d				s.e.	P< <sup>1</sup>		
	0	25	50	75		Linear	Quad	Cubic
DMI, kg/d	22.6	23.4	24.2	22.8	0.42	0.413	0.010	0.236
Milk yield, kg/d	38.2	38.7	39.1	38.1	0.87	0.969	0.372	0.740
Energy corrected milk, kg/d	38.4	38.7	40.0	39.5	0.81	0.169	0.651	0.445
Protein, %	3.00	2.99	3.01	3.17	0.041	0.005	0.037	0.563
Fat, %	3.68	3.63	3.81	3.86	0.095	0.087	0.582	0.396
Urea, mg/L	267	256	248	224	5.9	0.001	0.259	0.431
Protein, g/d	1137	1156	1180	1189	23.9	0.078	0.821	0.837
Fat, g/d	1397	1394	1478	1463	38.0	0.091	0.862	0.274

<sup>1</sup>Probability for linear, quadratic (Quad) or cubic effects of HMBi.

**Conclusions** Feeding HMBi had positive effects on DMI, milk protein and fat concentrations, and milk protein and fat yields. Effects of HMBi on DMI may be due to effects of HMBi on rumen fermentation and thus rate of fibre degradation, or metabolic effects of improved amino acid balance. The increase in milk fat concentration has been observed previously (St-Pierre and Sylvester, 2005) and may reflect changes in rumen fermentation. The maximal effect on DMI was observed at 50 g/d. The lack of an effect of 75 g HMBi/d on DMI may be due to negative effects on rumen fermentation, or an imbalance of methionine and lysine supply. In this regard, the estimated ratio of metabolizable methionine to lysine was 0.26, 0.31, 0.36, and 0.41 for the 0, 25, 50, and 75 g/d HMBi, respectively. There was a linear positive effect on milk protein and fat yields, which was reflected by a linear reduction in milk urea concentration that suggests an improvement in the utilization of absorbed amino acids. However, HMBi had no significant effect on milk yield, despite the increase in DMI observed. Results of the present study suggest that for the basal TMR fed, the optimal feeding rate of HMBi was between 25 and 75 g/d, but there was no indication of adverse effects when HMBi was fed at 75 g/d.

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## The effect of forage source and supplementary rumen protected methionine on nitrogen balance in autumn calved dairy cows offered a low crude protein diet

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**Introduction** The apparent inability of the dairy cow to efficiently convert feed nitrogen (N) into useable milk N results in large quantities of ingested N being excreted and lost to the environment as  $\text{NH}_3$  and  $\text{N}_2\text{O}$ , the latter a potent green house gas. Feeding low crude protein (CP) diets has been shown to reduce excreted N (Mulligan *et al.*, 2002) however it has been suggested that for such diets the essential amino acid methionine may become limiting to production (Write *et al.*, 1998). Alternative forages to grass silage (GS) such as maize silage (MS) are becoming increasingly popular in the diet of dairy cows (Burke *et al.*, 2007). MS has also been shown to improve the efficiency of N utilization relative to GS. The purpose of this experiment was to determine the effects of (i) forage source (GS vs. MS) and (ii) supplemental rumen protected methionine on N partitioning and performance of lactating dairy cows.

**Materials and methods** 4 primiparous and 4 multiparous cows were offered 1 of 4 dietary treatments in a 2\*2 factorial, Latin square design. Cows were 120 days in milk at the beginning of the experiment. The diets were grass silage (GS), grass silage with methionine (GSM), maize silage (MS) and maize silage with methionine (MSM). Diets were formulated such that the forage component consisted of 81% grass silage and 19% maize silage on a DM basis or 83% maize silage and 17% grass silage on a DM basis, with or without supplementary rumen protected methionine (Metasmar<sup>®</sup>). The concentrate component consisted of rolled barley, beet pulp, soybean meal and a mineral/ vitamin premix, the forage to concentrate ratios were 52:48 and 63:37 for the grass silage based and the maize silage based diets respectively. All diets were designed to meet the UFL (18.71UFL day<sup>-1</sup>), PDI (1950g day<sup>-1</sup>, PDIN 1959g, PDIE 1950g, PDIA 897g) and lysine (7.1% of PDI) requirements for production (30kg day<sup>-1</sup>, 4.1% fat, 3.3% protein). GS and MS were calculated to be deficient in methionine (1.78% of PDI) whereas GSM and MSM met the requirements (2.3% of PDI) of the animal. Diets were offered daily as a total mixed ration. Total faecal and urine collections allowing N-balance calculations were performed for the last 5 days of a 10 day period; animals were housed in metabolic stalls to facilitate separate collection of excreta and milked in-situ. Milk yield, feed refusals, urine, faeces and feed offered were weighed and sampled each morning for analysis of N. Data were analysed using proc GLM of the SAS.

**Results** There was no significant difference found for either effect of forage source, supplementary rumen protected methionine or interactions between both on milk production or the partitioning of N between milk and excreta by the dairy cows on this experiment. Urea N levels in bodily fluids (UUN, MUN and BUN) were significantly reduced in cows offered grass silage based forages; there was no significant effect of methionine supplementation on these parameters.

**Table 1** Effect of forage source and supplementary methionine on N partitioning in autumn calving dairy cows

	Forage Source			N	Methionine		Significance	
	Grass Silage	Maize Silage	LSM±sem		Y	LSM±sem	Forage Source	Methionine
Milk Yield	20.23	20.29	0.666	20.17	20.36	0.666	0.9528	0.8447
DMI	19.13	20.23	0.716	19.71	19.65	0.717	0.285	0.995
N intake	0.422	0.460	0.0128	0.447	0.435	0.0128	0.0426	0.5371
Faecal N	0.186	0.201	0.0100	0.193	0.194	0.0101	0.3125	0.9554
Urine N	0.123	0.131	0.0055	0.131	0.123	0.0056	0.3018	0.3599
Milk N	0.103	0.105	0.0038	0.103	0.106	0.0038	0.6148	0.5891
ENU	24.57	23.33	0.740	23.22	24.68	0.7404	0.2453	0.1774
UUN	88.79	123.32	3.6853	108.29	103.82	3.6852	0.0001	0.3989
MUN	2.243	2.537	0.0908	2.365	2.415	0.0908	0.0304	0.7033
BUN	1.719	2.337	0.1156	2.0381	2.0170	0.1155	0.0009	0.8984

Key; DMI (dry matter intake), N (nitrogen), ENU (efficiency of nitrogen utilization % (milk N / N intake \*100)) UUN (urinary urea nitrogen), MUN (milk urea nitrogen), BUN (blood urea nitrogen). All values are in kg day<sup>-1</sup> with exception of UUN, MUN and BUN which are in mmol l<sup>-1</sup>

**Conclusion** For cows used in this experiment at the production levels presented above there is no significant advantage to be gained in milk yield or milk N yield through the supplementation of low crude protein diets with ruminally protected methionine. The observed differences between forage types on urea levels in the bodily fluids and in particular urine may have environmental implications as there is less urea being excreted and therefore less potential for  $\text{NH}_3$  loss from dairy systems. Further more a reduction in urea syntheses also results in more energy being available for productive purposes in the dairy cow

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries and Food, Ireland

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## Effect of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on rumen degradability of fibre in lactating dairy cows measured *in situ*

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**Introduction** Previous studies have reported that feeding 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi) to lactating dairy cows increases milk fat concentration, suggesting an effect on fermentation in the rumen (St-Pierre and Sylvester, 2005). In this regard it has been hypothesized that fibre utilization may be improved (Noftsker *et al.*, 2005). The objective of our study was to measure incremental effects of feeding HMBi to lactating dairy cows on rumen degradability of neutral detergent fibre (NDF) in specific feeds, measured using the *in situ* procedure, and rumen concentrations of volatile fatty acids (VFA) and ammonia (NH<sub>3</sub>).

**Materials and methods** Three rumen fistulated Holstein-Friesian cows in midlactation were used in a 3 x 4 incomplete Latin Square design experiment with 4 treatments and 3 week periods. Treatments were 0, 25, 50, or 75 g HMBi/d. The basal diet was a total mixed ration (TMR) provided twice daily that contained on a dry matter (DM) basis: 12.5% grass silage (GS), 37.5% maize silage (MS), and 50% maize grain-based concentrates. Degradability of NDF was measured on standardized samples of GS, MS and dried distillers grains (maize) with solubles (DDGS) using *in situ* rumen incubation of duplicate samples of each feedstuff in nylon bags over 48 (DDGS) to 72 (GS, MS) h during the last week of each period. Degradability profiles were calculated using nonlinear procedures and corrected for estimated water soluble and particle losses (Hvelplund and Weisbjerg, 2000). Rumen VFA concentrations and pH were measured in 5 samples taken just before and over 4 h after both feedings on day 19. Milk yield and composition and DM intake (DMI) were measured daily during the last week of each period. Data were analyzed using mixed models procedures and a model testing fixed effects diet and period and random effects of cow. Rumen pH and VFA concentrations were analyzed as repeated measures over time. Orthogonal contrasts were used to partition the main effect of HMBi amount into linear, quadratic (Quad), and cubic effects.

**Results** Feeding HMBi had no effect on DMI, milk yield, milk composition (data not shown) or MS NDF degradation (Table 1). Feeding increasing amounts of HMBi tended to linearly decrease potential degradability of NDF in GS, but rate of GS NDF degradation tended to be greater when 50 g/d of HMBi was fed (cubic, Table 1). For DDGS, feeding HMBi linearly increased rate of NDF degradation, but linearly decreased potential degradability (Table 1). Feeding increasing amounts of HMBi decreased rumen concentrations of NH<sub>3</sub> (quadratic, Table 1) and increased total VFA concentration (cubic, Table 1) due to increases in concentrations of all the VFA measured (data not shown).

**Table 1** Incremental effects of HMBi on DMI, milk yield, grass silage (GS), maize silage (MS) or dried distillers grains with solubles (DDGS) NDF degradability, and rumen NH<sub>3</sub> and VFA concentrations in lactating dairy cows.

	HMBi, g/d				s.e.	P<		
	0	25	50	75		Linear	Quad	Cubic
DMI, kg/d	21.31	21.61	22.00	22.42	1.004	0.220	0.915	0.985
Milk yield, kg/d	23.73	23.35	22.61	23.35	3.777	0.399	0.285	0.416
GS NDF degradability, %	41.6	42.1	39.1	39.0	1.52	0.066	0.715	0.200
Rate of GS NDF degradation, %/h	0.041	0.036	0.051	0.032	0.006	0.623	0.256	0.084
MS NDF degradability, %	54.1	55.2	52.0	53.4	2.46	0.615	0.943	0.410
Rate of MS NDF degradation, %/h	0.031	0.028	0.034	0.031	0.005	0.804	0.954	0.331
DDGS NDF degradability, %	86.2	80.4	76.7	77.4	1.64	0.028	0.145	0.772
Rate of DDGS NDF degradation, %/h	0.039	0.049	0.051	0.053	0.005	0.036	0.250	0.630
Rumen NH <sub>3</sub> , mg/L	184.1	165.2	156.5	165.0	7.6	0.110	0.001	0.093
Total VFA, mM	112.0	130.0	125.1	137.8	6.2	0.001	0.174	0.015

**Conclusions** Feeding increasing amounts of HMBi had positive effects on rumen VFA concentrations, and a negative effect on rumen NH<sub>3</sub> concentration, suggesting improvements in rumen fermentation. The increases in rumen VFA concentration were associated with increases in the rate of degradation of NDF from DDGS, and a tendency for an increase in degradation rate of NDF from GS when 50g/d of HMBi was fed. However, the potential degradability of NDF was reduced (DDGS), or tended to be reduced (GS), by HMBi. Effects of HMBi on rumen fermentation may have been due to effects of HMBi on microbial fermentation or populations.

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## Effect of phosphorus level and inulin inclusion in a wheat based finisher pig diet on nitrogen, phosphorus and calcium metabolism and intestinal microflora

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**Introduction** The formulation of commercial grower finisher pig diets supplies excess dietary phosphorus (P) through the high inclusion levels of cereals which generally consist of indigestible P in the form of phytate P. As a result incomplete digestion of P is largely responsible for unnecessary P excretion. Feeding reduced P diets supplemented with non digestible oligosaccharides (NDO) have been shown to promote mineral absorption in the large intestine of both humans and rats. Research data representative of NDO application in pigs are limited. Lopez *et al.* (2000) suggested that enhanced fermentation in the colon due to NDO feeding, such as inulin, apparently promotes better hydrolysis of phytate and, thus, enhanced colon P and calcium (Ca) absorption in small mammals. Inulin is classified as dietary fibre resistant to complete enzymatic degradation in the small intestine which is selectively fermented by *Bifidobacteria* and *Lactobacilli* spp. (Roberfroid *et al.*, 1998). The objective of this experiment is to investigate the interaction between P and inulin level on mineral metabolism and intestinal microflora in a low and high P wheat based diet.

**Materials and methods** The experiment was designed as a 2x2 factorial comprising four dietary treatments. The experimental treatments were as follows: (1) 4g/P kg, (2) 4 g/P kg + 20 g/inulin kg (3) 6 g/P kg, (4) 6 g/P kg + 20 g/inulin kg. The inulin used was a mixture of short and long-chain inulin (Synergy 1, Orafit). Sixteen finishing boars with a similar initial body-weight (50.7kg  $\pm$  4) were assigned to one of four dietary treatments (n=4). After a two week dietary adaption period pigs were transferred to metabolism crates for a 7 day apparent total tract nutrient digestibility study (n=4). Pigs remained on their respective diets until slaughter. Immediately post-slaughter, digesta samples (approximately 10g  $\pm$  1g) were aseptically recovered from the proximal colon in sterile conditions. Populations of *Lactobacillus* spp. and *Bifidobacteria* were selectively isolated and enumerated according to the method as described by previous authors (O'Connell *et al.*, 2005). Typical colonies of each bacterium were counted, log transformed and presented per gram of digesta. Experimental data were analysed as a 2x2 factorial using the GLM procedure of the SAS Institute (1985). The statistical model investigated the main effects of dietary P concentration, inulin inclusion and the associated two-way interaction.

### Results

There was no effect of Inulin or P level supplementation on P, Ca or N digestibility or proximal colon bacterial populations (Table 1).

**Table 1**

Treatment	g P/kg			Inulin			Significance <sup>a</sup>	
	4.0	6.0	s.e.	no	yes	s.e.	P	Inulin
Digestibility Coefficient								
Dry matter	0.903	0.901	0.005	0.899	0.905	0.005	ns	ns
Phosphorus	0.569	0.546	0.015	0.542	0.572	0.014	ns	ns
Calcium	0.649	0.563	0.029	0.590	0.622	0.028	ns	ns
Nitrogen	0.871	0.886	0.013	0.868	0.889	0.012	ns	ns
Proximal Colon bacterial populations								
<i>Lactobacillus</i> spp.	7.330	7.372	0.188	7.429	7.273	0.188	ns	ns
<i>Bifidobacteria</i> .	6.372	6.746	0.280	6.822	6.296	0.280	ns	ns

<sup>a</sup>. In the absence of an interaction main effects are presented.

**Discussion and Conclusions** In this experiment inulin was included at 2% which is in line with previous studies. The inclusion of inulin had no effect on P, Ca or N digestibility in finisher pigs which is in agreement with Houdijk *et al.* (1999). There was no effect of inulin supplementation on proximal colon bacterial populations of *bifidobacteria* and *lactobacilli* spp. This was surprising because the main site of inulin fermentation has been previously reported to be in the colon where it promotes the growth of bacterial populations. However, Yasuda *et al.* (2007) demonstrated that 96% of inulin fed to growing pigs was degraded before reaching the proximal colon when included at 4%, with the caecum displaying the highest inulin-degrading enzyme activity. In conclusion dietary inulin was probably totally degraded in the stomach and small intestine therefore diminishing any possible effect of inulin in the proximal colon of the finisher pig.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food.

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## Mitigation of phosphorus and nitrogen from pigs manure fed diets balanced according to ideal protein concept, supplemented with phytase

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**Introduction** The use of phytase enzyme in diets for pigs can contribute to the decrease use of nitrogen (N) and inorganic phosphorus (P) reducing the environmental impact caused by the excretion of these minerals. Phytase is an enzyme that catalyses the hydrolysis of orthophosphate groups from phytate molecules. Pigs lack this enzyme. Phosphorus losses from animal manures contribute to eutrophication in areas where animal feeding operations are geographically concentrated. Dietary modification with phytase is one strategy to reduce P inputs into animals, and thus P losses from manures. However, the full environmental impacts of dietary modification treatments are not fully understood. The aim of the experiment was to evaluate diets with phytase enzyme and based on the ideal protein for reducing the excretions of phosphorus and nitrogen.

**Material and methods** Twenty crossbred barrows (26.8 kg) were use in a randomized block design experiment, with five treatments and four replicates. The diets were formulated with corn, soybean meal, synthetic aminoacids and supplemented with quantum phytase of increasing levels, containing 14 % CP and 13.60 MJ DE/kg. The animals were kept in metabolic cages for a ten day adaptation period and seven days in total for collection of faeces and urine. Blood samples were taken for five days. On the first day of the collection period, each animal was injected intravenously with 7.4 MBq <sup>32</sup>P. At the end of the experimental period the animals were slaughtered and tissues of the muscle, heart, liver, kidneys and bones were collected for further studies.

**Results** There was a quadratic relationship of phytase levels with both P faeces and bioavailability. Figueirêdo *et al.* (2000) also observed a reduction in the P excretion in pigs fed diets supplemented with phytase enzyme. Urinary excretions showed a positive linear effect with phytase levels and P and N urinary. The use of phytase improved nutritional value of diets for nonruminant and also gave benefits to the environment, reducing P concentration in the faeces. It is necessary to create restrictive laws in Brazil for the use of manure in the soil, as well as rigorous control of P supplementation to avoid environmental pollution (Palhares, 2009).

**Table 1** Effect of the phytase increasing levels about the P and N metabolism parameter in pigs fed with diets supplement phytase enzyme.

	Phytase enzyme (FTU/kg diet)					CV <sup>c</sup>
	0	250	500	750	1000	
Feed intake (g/ kgLW /d)	41.88	41.92	41.85	41.39	41.77	10.81
P intake (mg/ kgLW /d)	146.60	146.71	146.48	144.87	146.21	10.81
P faeces (mg/ kgLW /d ) <sup>a</sup>	80.69	68.05	59.34	61.74	59.25	17.77
P endogenous (mg/ kgLW /d)	7.52	7.30	9.22	8.53	7.54	29.73
P urinary (%) <sup>b</sup>	0.17	0.22	1.55	5.63	5.78	58.07
Bioavailability (%) <sup>a</sup>	49.62	57.91	65.93	63.53	65.20	12.17
P retention (mg/ kgLW /d )	65.69	78.44	85.59	77.49	81.86	20.56
P plasma (mg/100ml)	8.31	8.10	8.66	8.54	8.83	5.58
N intake (mg/ kgLW /d)	942.93	943.98	942.18	931.82	940.44	10.81
N faeces (%)	47.15	52.42	47.18	53.08	49.37	17.52
N Apparent absorption (%)	52.85	47.58	52.82	46.92	50.63	17.41
N urinary (%) <sup>b</sup>	12.08	15.37	18.78	16.82	17.80	27.38
N retention (%)	75.70	65.95	63.13	62.93	64.45	10.31

<sup>a</sup> Quadratic effect (P<0.05), <sup>b</sup> Linear effect (P<0.08), <sup>c</sup> Coefficient of variation.

**Conclusions** The level of 500 FTU/kg of diet is indicated for diets for pigs based on soybean meal and corn, formulated in agreement with the concept of ideal protein.

**Acknowledgements** This experiment is part of projects supported by FAPESP (06/57574-5). The authors gratefully acknowledged the help of Nutron and ABVista Feed Ingredients Company.

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## Lameness and limb lesions in replacement gilts on a commercial farm

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**Introduction** In a 1998 survey removals due to lameness in 1<sup>st</sup> parity gilts were over 30% and surpassed culling for reproductive failure in this parity class (Boyle *et al.*, 1998). Evidence from other countries indicates that culling rates for lameness are still unacceptably high in the early parities. Anecdotal evidence indicates that housing and management of replacement gilts is sub-standard on many farms and this may predispose them to lameness. The objective of this study was to determine the prevalence of lameness and limb lesions in replacement gilts on entry to the breeding herd and at service on a commercial farm.

**Materials and methods** Over six months 112 gilts were inspected in the gilt accommodation within 3 days of entry (ENTR) to a 1000 sow herd and within one week of service (SERV). P2 back-fat thickness was determined by ultrasound and flank to flank dimensions were measured. Injuries caused by fighting at 12 locations on the body were scored from 0 to 5 according to severity. The locomotion ability of each animal was scored according to Main *et al.* (2000) where score 1 = shortened stride; 2 = uneven posture/limp; 3 = reluctance to bear weight on one limb; 4 = limb elevated and 5 = non-ambulatory. Wounds and bursitis on the limbs were also recorded. All gilts in four pens of between 25 and 30 animals were inspected. They were fed a wet diet twice daily from two long troughs in a concrete slatted area and had free access to a large unbedded, solid concrete area for lying. Data were analysed using SAS by the Chi-square or Mann-Whitney test as appropriate.

**Results** ENTR data were collected from 112 gilts and SERV data were available for 89 of these. However some gilts were transferred to gestation stalls immediately after service which precluded locomotion scoring. The mean (+SD) back-fat depth at ENTR was 9.9mm (1.86) and at SERV was 16.8mm (2.99). The corresponding figures for flank to flank dimensions were 79.3mm (3.04) and 91.7mm (7.72). There was a reduction in the fight lesion score between ENTR and SERV [median (minimum-maximum)] [19 (1-36) vs. 4 (0-34);  $P < 0.001$ ] reflecting establishment of the dominance hierarchy following re-mixing of gilts on arrival. Nevertheless while 100% of gilts were affected by fight lesions at ENTR, 91% of gilts were still affected by fight lesions at the SERV inspection which could reflect the competitive feeding arrangement. This could also explain the high proportion (31.9%) of gilts with locomotion scores  $>1$  at the SERV inspection (Table 1) as fighting on slats is a major risk factor for lameness. Although only two gilts received locomotion scores of 3 and none received scores greater than 3, the scale of the increase between ENTR and SERV ( $P < 0.001$ ) in the proportion of gilts with scores of 1 and 2 is a welfare concern. While it is not possible to determine whether low scores are associated with pain they reflect abnormal weight bearing and are thus likely to be associated with a biological cost because of the increased strain placed on the locomotion system (KilBride *et al.*, 2009). As bursitis is chronic in nature the high proportion of animals affected by this lesion to the fore and particularly the hind limbs (Table 1) on entry to the herd suggests that they were kept on unbedded or minimally bedded solid or slatted concrete at the breeding unit.

**Table 1** Percentage [number affected/number inspected] of gilts affected by different locomotion scores, at least one bursa to the limbs and at least one wound anywhere on the body at each inspection

	Entry (ENTR)	Service (SERV)	P
Locomotion Score			
1	8.9 [10/112]	39.1 [27/69]	0.001
$>1$	7.1 [8/112]	31.9 [22/69]	0.001
Forelimb Bursa	8.9 [10/112]	9.0 [8/89]	n.s.
Hind Limb Bursa	40.2 [45/112]	42.7 [38/89]	n.s.
Wounds	10.7 [12/112]	9.0 [8/89]	n.s.

**Conclusions** KilBride *et al.* (2009) found an association between bursitis and other limb lesions and abnormal locomotion although it is not clear whether this is because limb lesions cause discomfort or because lame pigs spend more time lying and this increases the risk of limb lesions developing. In any case, the high prevalence of gilts with lesions to the limbs and showing abnormal locomotion helps to explain why so many animals are culled for lameness in the early parities. These findings indicate that measures to reduce culling for lameness in breeding stock should be directed towards the young replacement animals. These measures should include minimising aggression associated with re-mixing and feeding but most importantly protecting gilts feet and limbs from the concrete floor either by the use of bedding or cushioned flooring such as slat mats. The advent of group housing in 2013 means that the adaptation capabilities of gilts entering the breeding herd will be more severely challenged making it even more important that they are sound at the start of their productive lives.

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## Influence of regrouping regime on lying behaviour parameters in weaned pigs

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**Introduction** It is evident that increasing the number of unfamiliar pigs per group has an adverse effect on welfare and productivity during the post regrouping period (Arey and Franklin 1995, Stooky and Gonyou 1998, Samarakone and Gonyou 2009). The aim of the present study was to assess the effect of number of litters per group on lying behaviour parameters in weaned pigs. This was to investigate the usefulness of these parameters as potential welfare indicators, and also to determine the effect of regrouping strategy at weaning on subsequent littermate contact.

**Materials and methods** One hundred and sixty Large White x Landrace pigs were allocated to one of four treatments at weaning at 4 weeks of age ( $9.05 \pm 0.96$  kg). Treatments were as follows: (1) group formed from 1 litter, (2) group formed from 2 litters, (3) group formed from 3 litters and (4) group formed from 4 litters. All pigs were housed in groups of eight animals that were balanced for gender (within litter where possible) and body weight. The pigs were housed on slatted floors with access to an enrichment device, and were fed on an *ad-libitum* basis. Pigs were individually marked on their backs and were video recorded (in 72 hour time-lapse mode) during 2 x 24 hour periods during the first week of the study, and then during 1 x 24 hour period each week until the pigs were 10 weeks of age. Each 24 hour recording was scanned at 15 minute intervals between 12.30 and 18.30 hours and the following factors were recorded for each pig: (1) whether a pig was lying or standing/sitting, (2) whether the pig was lying in contact with one or more other pigs, and (3) whether the animal was lying in contact with one or more littermates. A pig was defined as lying in contact with another pig if their bodies were in physical contact, but not if the only source of contact was the head, rear or limbs. The effect of treatment on the parameters measured was analysed by REML Variance Components Analysis. Treatment 1 was excluded from analysis of the parameter "lying in contact with littermates" as all animals in this treatment were littermates. The proportion of observations where pigs would be expected to lie in contact with littermates if these lying partners were chosen randomly was calculated for each treatment ('random proportion'). This value was then subtracted from the actual proportion of observations where pigs were observed to be lying in contact with littermates ("actual-random"). This parameter was included in analysis to determine if treatment influenced the motivation of pigs to lie next to littermates.

**Results** Treatment effects are presented in Table 1. Treatment did not have a significant effect on the average proportion of scans where pigs were observed lying ( $P > 0.05$ ). Increasing the number of littermates per group tended to increase lying in contact with other pigs but this did not reach statistical significance ( $P < 0.09$ ). As expected, as the number of littermates per group increased, the proportion of scans where pigs were lying in contact with littermates increased ( $P < 0.001$ ). However, when adjusted for group litter composition, there was still a trend for pigs to spend more time lying in contact with littermates when there were increased littermates in the group ( $P < 0.06$ ).

**Table 1** Effect of number of litters per group on the average proportion of scans where pigs showed different lying parameters

	Treatment				S.E.D.	P
	1 litter	2 litters	3 litters	4 litters		
Lying	0.62	0.57	0.60	0.57	0.041	NS
Lying in contact with another pig	0.38	0.32	0.27	0.24	0.060	<0.09
Lying in contact with littermate/s (actual proportion)	-	0.59 <sup>c</sup>	0.34 <sup>b</sup>	0.21 <sup>a</sup>	0.036	<0.001
Lying in contact with littermate/s (actual-random proportion)	-	0.16	0.10	0.07	0.036	<0.06

**Conclusions** Earlier data from this trial showed increased levels of aggression-related injury and reduced productivity as number of litters per group increased (O'Connell, 2008). The fact that pigs also showed reduced time lying in contact with other pigs as number of litters per group increased is further evidence of reduced welfare. The time spent lying did not differ significantly between treatments, which may suggest this parameter is not a good welfare indicator, or that it was not recorded over a sufficient timeframe. As expected, pigs housed with more of their littermates spent more time lying in contact with them. However, when lying behaviour was corrected for proportion of littermates in the group, animals with more littermates in the group still appeared to spend more time in contact with them. This suggests that pigs housed in groups formed from fewer litters maintain better sibling relationships.

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## An investigation of the relationship between pig weight and subsequent variation in weight

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**Introduction** Strategies to reduce the variable growth rate and hence variable weight of pigs at slaughter are continuously being investigated (e.g. O'Connell *et al.*, 2005). However, key to designing strategies to reduce variability is the need to understand where variability arises. The objective of this study was to identify which pigs, based on their wean and 10 week weight, within a normal population of pigs, contribute most to end weight variability.

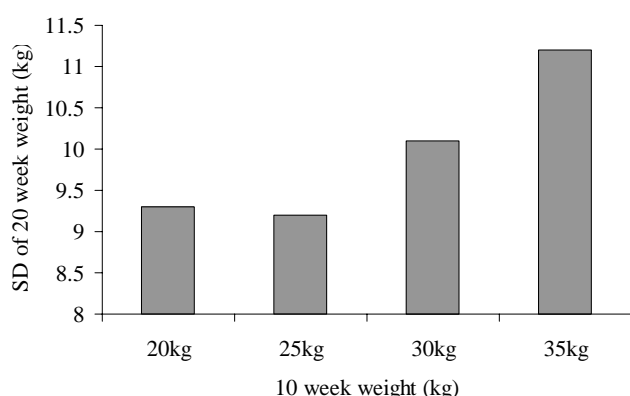
**Materials and methods** All pigs (Landrace x Large White) were born and reared on the research herd at AFBI Hillsborough between 1997 and 2009. All pigs were weaned at the same age (28 days  $\pm$  2 days). Two datasets were built from data attained from 22 experiments where pigs were penned in groups of 10 or 20. One dataset contained the weaning and 10 week weight of 12,000 pigs, and the second the 10 and 20 week weight of 4,950 pigs. Regression analysis were performed to investigate the relationships between wean and 10 week weight and between 10 week and 20 week weight. The datasets were subdivided into weight categories. At weaning these categories represented 6kg (102 pigs), 7kg (493 pigs), 8kg (1152 pigs), 9kg (1614 pigs), 10kg (1482 pigs), 11kg (810 pigs), 12kg (324 pigs) or 13kg (102 pigs) with a tolerance of  $\pm$ 0.2kg. At 10 weeks of age, the categories represented 20kg (84 pigs), 25kg (678 pigs), 30kg (1224 pigs) and 35kg (408 pigs) with a tolerance of  $\pm$ 1kg. Within each weight category of each dataset pigs were randomly assigned to one of six groups which represented a 'replicate'. The average, standard deviation (SD) and coefficient of variation (CV) of 10 and 20 week weight of each replicate respective of dataset was then determined. Analysis of variance (Genstat Version 10) was used to test the effect of start weight on end weight variation.

**Results** A significant ( $P < 0.001$ ) but weak ( $R^2 = 0.383$ ) linear relationship ( $y = 2.11x + 10.9$ ) was found between wean and 10 week weight. Likewise the relationship between 10 and 20 week weight was also significant ( $P < 0.001$ ) but weak ( $R^2 = 0.274$ ) and linear ( $y = 1.53x + 40.7$ ). The 10 week weight of pigs increased as their wean weight increased (Table 1). The CV for 10 week weight was highest for pigs with a weaning weight of 6kg and tended to decrease as pigs got heavier (Table 1). However, the SD of 10 week weight was highest for pigs with a weaning weight of 12kg and 13kg. As 10 week weight increased, so did 20 week weight ( $P < 0.001$ ). However, 10 week weight had no significant effect on the CV of 20 week weight, although the standard deviation of 20 week weight from pigs weighing 35kg at 10 weeks of age was significantly higher (11.2) ( $P < 0.01$ , LSD 1.16) than that of pigs in the other weight categories (Figure 1).

**Table 3** Effect of wean weight on the average, SD and CV of ten week weight

	6.0kg	7.0kg	8.0kg	9.0kg	10.0kg	11.0kg	12.0kg	13.0kg	LSD	P
Average (kg)	24.4 <sup>a</sup>	26.18 <sup>b</sup>	27.9 <sup>c</sup>	29.67 <sup>d</sup>	31.18 <sup>e</sup>	33.08 <sup>f</sup>	34.95 <sup>g</sup>	37.88 <sup>h</sup>	0.151	<.001
SD	3.70 <sup>a</sup>	3.82 <sup>a</sup>	3.79 <sup>a</sup>	3.52 <sup>a</sup>	3.74 <sup>a</sup>	3.93 <sup>ab</sup>	4.64 <sup>c</sup>	4.18 <sup>b</sup>	0.30	<.001
CV	0.152 <sup>a</sup>	0.146 <sup>ab</sup>	0.136 <sup>b</sup>	0.119 <sup>c</sup>	0.120 <sup>c</sup>	0.120 <sup>c</sup>	0.133 <sup>b</sup>	0.110 <sup>c</sup>	0.010	<.001

<sup>a, b, c</sup> numbers with the same superscript are not significantly different ( $P > 0.05$ )



**Figure 1** The effect of 10 week weight on the standard deviation of 20 week weight

**Conclusion** Although the relationships between wean and 10 week weight and between 10 and 20 week weight were significant, the  $R^2$  of the best fit line was very low indicating a large degree of variability. The study also suggests that the 10 week weight of pigs with low weaning weights (6-8kg) is more variable than of those with higher weaning weights. A similar trend is not present when relating 10 and 20 week weight although pigs with a high 10 week weight (35kg) were found to have a larger spread of weights, as indicated by a higher SD at 20 weeks compared with pigs weighing 20, 25 or 30 kg at 10 weeks of age. This study would suggest that there is a greater potential to manipulate the 10 week weight of light weight pigs at weaning than that of heavy weight pigs since their variable weight at 10 weeks was higher than that of heavy weight pigs.

**Acknowledgements** The authors gratefully acknowledge funding from Devenish Nutrition Ltd.

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## Fusarium mycotoxins in UK straw from the 2008 harvest – Implications for pigs on straw bedding

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**Introduction** Straw based production systems are common in the UK compared to other countries. The effects of mycotoxins in cereal feed on livestock performance are relatively well documented with pigs being particularly sensitive to mycotoxicosis. The European Commission has recently set guideline limits for fusarium mycotoxins in animal feedstuffs (Anon, 2006). Guidance limits for feedstuffs for young pigs is 900 ppb deoxynivalenol and 100 ppb zearalenone. A previous study in 2006 identified straw as a potential source of fusarium mycotoxins for livestock on straw bedding. The cereal harvest in 2008 was the wettest for many years in the UK. This resulted in severe losses in grain quality and the highest recorded levels of fusarium mycotoxins in UK wheat and barley grain samples. This project was conducted to evaluate the corresponding levels of fusarium mycotoxins in straw from the 2008 harvest.

**Material and methods** A total of 58 samples of wheat and barley straw were collected from pig farms across England and Scotland. All straw samples were from the 2008 harvest and collected in July 2009. Samples (ca. 500 g) were collected from several points in the interior of a cut bale. Each sample was dried to ca. 12% moisture content and milled in a hammer mill with a 1mm screen. Zearalenone (ZON) and deoxynivalenol (DON) were analysed using Ridascreen DON and Zearalenone ELISA test kits (R-Biopharm Rhone), which based on the modified extraction procedure had limits of quantification for DON and ZON of 75 and 7 parts per billion (ppb) respectively. Mycotoxin data were normalised using a log transformation and wheat and barley mycotoxin levels compared by unbalanced ANOVA using Genstat software (version 12).

**Results** The concentrations of fusarium mycotoxins in wheat and barley straw (Table 1) were higher than those found in corresponding wheat and barley grain samples from the same harvest (HGCA, 2009). The concentrations were also higher than those found in a previous study of wheat straw in 2005 (White *et al.* 2007), particularly for ZON. There was significantly ( $P < 0.001$ ) higher DON and ZON in wheat compared to barley straw. High concentrations of fusarium mycotoxins, however, existed in both barley and wheat samples with both cereals having maximum DON and ZON concentrations of ca. 2000 and 4000 ppb respectively. Five barley samples (17%) exceeded the ZON guidance limit for piglet feed (100 ppb) and two samples (7%) exceeded the DON guidance limit for pig feed (900 ppb). Twelve wheat samples (44%) exceeded the ZON guidance limit for piglet feed (100 ppb) and 13 samples (48%) exceeded the DON guidance limit for pig feed (900 ppb).

**Table 1** Mean DON and ZON concentrations from UK barley and wheat straw and grain from various studies

Year	Cereal	Product	DON (ppb)	ZON (ppb)	Reference
2005	Wheat	Straw	460	23	White <i>et al.</i> 2007
2008	Barley	Grain	32	36	HGCA 2009
2008	Wheat	Grain	584	121	HGCA 2009
2008	Barley	Straw	166	12	This study
2008	Wheat	Straw	983	499	This study

**Conclusions** UK cereal straw, in particular wheat straw, can contain high levels of fusarium mycotoxins. There are limited data on the rate of consumption of bedding straw, however one study calculated weaned pigs consumed about 1.6 kg per day. Based on the levels of fusarium mycotoxins found in straw, then this could be a significant proportion of the mycotoxin load consumed by pigs and contribute to sub-clinical (reduced weight gain) and clinical mycotoxicosis. There have been several cases of ZON mycotoxicosis reported within the pig industry since the 2008 harvest. Zearalenone mimics oestrogen resulting in hyperoestrogenism. Symptoms reported include swollen vulva in newborn piglets, reduced litter numbers and increased numbers of weak and/or deformed piglets at birth. Results from this study would indicate that mycotoxins within bedding straw could contribute to mycotoxicosis. Farmers using a straw bedding system should therefore consider straw as a component of the diet and as such it should be tested as part of any veterinary investigation of mycotoxicosis.

**Acknowledgements** This project was funded by Alltech (UK) Ltd. We also thank the commercial producers that kindly supplied samples and Terry McArdle (Alltech) and Allan Ward (QMS) who coordinated sampling in England and Scotland respectively.

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## Enhanced utilization of palm kernel cake as a feed resource for growing-finishing pigs using exogenous enzyme

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**Introduction** The scarcity of conventional feeds has hindered the growth and development of the livestock industry in Nigeria. The general shortage of energy and protein feeds appear to be more severe for non-ruminants that depend to a great extent on compounded feeds, especially pigs, which are bulk feeders. Palm kernel cake (a by-product of cottage Oil Palm - *Elaeis guineensis* processing industries in Nigeria) has since become an important feed ingredient. In view of severe constraint of the present high cost of feeding conventional energy and protein feedstuffs to pigs, increased utilization of PKC is inevitable. This has made a substantial contribution towards better and more economic feeding of non-ruminants. However, fibrousness is a feature of most locally available agro-industrial by-products and wastes which may limit their use and many enzymes have been found to be beneficial when added to non-ruminant animal diets containing carbohydrate or protein sources containing high levels of non-starch polysaccharides (Acamovic, 2001). Allzyme® Vegpro 5X is a combination of naturally-occurring enzymes - Protease, Cellulase, Pentosanase (Xylanase),  $\alpha$ -Galactosidase and Amylase (Allzyme, 2008) routinely used in poultry and pig feeds to improve the nutritive value of plant-based feeds (Campbell and Bedford, 1992). It was therefore the aim of this study to investigate the utilization of palm kernel cake-based diet supplemented with or without exogenous enzyme, Allzyme® Vegpro 5X by growing-finishing pigs.

**Materials and methods** Forty-five (45) crossbred grower pigs of 39.10±1.04 kg body weight were randomly allocated based on body weight, sex and litter origin in a completely randomised design to five experimental diets containing two levels of PKC: (1) 20% maize+3% FM+7% GNC+20% PKC-based control diet, (2) 20% maize+10% GNC +20% PKC-Enz, (3) 20% maize+10% GNC +20% PKC+Enz (Allzyme® Vegpro 5X), (4) 0% maize+10% GNC +40% PKC-Enz and (5) 0% maize+10% GNC +40% PKC+Enz based diet. The PKC contained 15.9% CP, 11.9% EE, 8.3% CF and 50% carbohydrate (by difference). Each treatment was replicated thrice in a completely randomized design, with 3pigs/pen representing a replicate for each of the five treatments. The diets were formulated to contain about 17% crude protein and the pigs were housed in concrete floored pens containing feeding and watering troughs for the 56-day duration of the study. Proximate compositions of test ingredients and feed samples were done according to the methods of A.O.A.C. (1990). Two pigs were randomly selected from each replicate at the end of the feeding trial and sacrificed to evaluate some carcass traits, internal organs and external offals. All the data obtained were subjected to analysis of variance and where statistical significance were observed, the means were compared using the Duncan's Multiple Range (DMR) test. The SAS Computer software package (1991) was used for all statistical analyses.

**Results** The feed intake of the pigs was comparable ( $P>0.05$ ) across the groups but for the 20%Mz + 20% PKC group without FM and enzyme supplementation, which was lower. This resulted in comparable gains across the groups but for the 40% PKC-based diet group with enzyme supplementation (Table 1). Highest net benefit of ₦2,199.70 was recorded for pigs fed with 40% PKC without enzyme, followed by pigs fed 40% PKC with enzyme. The Marginal Rate of Return of 234.44% indicated that farmers stand to gain additional net benefit of ₦234.44 for every additional ₦100 incurred as a result of changing from 40% PKC+enzyme diet for the pigs.

**Table 1** Performance, carcass traits, internal organs and partial budget analysis of growing-finishing pigs fed experimental diets

Parameters	Control	20%PKC	20%PKC+Enz	40%PKC	40%PKC+Enz	SEM
Daily Feed Intake (kg)	2.19 <sup>a</sup>	2.01 <sup>b</sup>	2.15 <sup>a</sup>	2.11 <sup>ab</sup>	2.23 <sup>a</sup>	0.02
Daily weight gain (kg)	0.65 <sup>a</sup>	0.61 <sup>ab</sup>	0.57 <sup>ab</sup>	0.54 <sup>b</sup>	0.56 <sup>ab</sup>	0.02
Feed cost/gain (₦)	289.54 <sup>a</sup>	217.98 <sup>ab</sup>	275.93 <sup>ab</sup>	214.74 <sup>ab</sup>	208.57 <sup>b</sup>	11.50
Dressing (%)	70.40	65.20	65.00	63.40	72.70	1.79
Back-fat thickness (cm)	1.18	1.43	1.31	1.16	1.07	0.07
Liver (%)	2.24 <sup>a</sup>	1.79 <sup>ab</sup>	1.96 <sup>ab</sup>	1.77 <sup>b</sup>	1.81 <sup>ab</sup>	0.07
Value of weight gain @ ₦330/kg	7,140.00	6,090.00	5,610.00	5,790.00	5,460.00	-
Net Benefit (₦)	1,455.64	1,885.88	1,016.74	2,199.70	1,968.37	-

a,b: Means along the same row having different superscript differ significantly ( $P<0.05$ ). \$1= ₦150 (Nigerian Naira)

**Conclusion** It could be concluded that the pigs were able to tolerate up to 20% PKC without enzyme supplementation while the enzyme enhanced the utilization of a higher level of 40% PKC. Feeding of growing-finishing pig with 40% PKC is more cost effective and worthy of adoption by farmers in tropical environments where PKC is found in abundance.

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# Effect of forage species, ryegrass water soluble carbohydrate concentration, and red clover polyphenol oxidase activity on *in vitro* rumen efficiency of nitrogen utilization

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**Introduction** A relatively small proportion of the nitrogen (N) consumed by the ruminant is transferred into meat (5-15%) or milk (15-30%), and most of the rest is excreted, with potential impacts on water, soil and air quality. Many fresh forage diets used for dairy cows present an imbalance between the rapidly available N and the slowly available energy in the rumen, which can limit microbial protein synthesis and increase excretion losses. In this experiment two strategies to improve the dietary energy/protein balance and their effects on microbial synthesis were studied. The first was to use perennial ryegrass (*Lolium perenne*) bred to express high water soluble carbohydrate (HWS) concentrations. The second was to reduce and/or delay protein degradation in the rumen, to improve the energy/N synchronisation, by employing the activity of the enzyme polyphenol oxidase (PPO) in red clover (*Trifolium pratense*; RC). The latter promotes the formation of protease resistant cross-linked protein complexes (Lee *et al.*, 2004).

**Material and methods** Two ryegrass varieties with high (AberMagic<sup>®</sup>; HWS) or control (Premium; LWS) WSC concentrations were used. Two types of RC were used: wild type (PPO+) or knockout (PPO-) strains, with normal and undetectable PPO activities respectively. The experiment was carried out in sixteen continuous culture rumen simulation technique (Rusitec) systems inoculated with fresh rumen contents from cows, using 4 vessels (700 ml nominal volume) per treatment. Grass was harvested daily during May 2009 and used fresh to avoid WSC degradation. Red clover material was grown in pots and was frozen to collect sufficient material. It was defrosted before use, which also helped to activate the PPO enzyme. All plants were chopped into 5 cm lengths, placed in nylon bags (50 g fresh material per bag) and remained in the fermenter vessels for 48h. The liquid dilution rate was maintained at 3.65%/h by continuous infusion of artificial saliva. After 9 days adaptation 2.3 mg <sup>15</sup>N/d was continuously infused to label the microbial protein. Days 10 to 12 of the experiment were used to determine the diet degradability, gas production and rumen fermentation, while effluent was collected during days 13 and 14 and mixed with their correspondent bag residue to reconstitute the digesta flow. Finally, on day 15 total (TB), liquid- (LAB) and solid-associated bacteria (SAB) were isolated (Carro and Miller, 1999) to determine microbial synthesis. Data were analysed by ANOVA blocking by machine and using three orthogonal contrasts to separately determine the effect of forage, WSC and PPO: C1 (grass vs. red clover), C2 (HWS vs. LWS) and C3 (PPO+ vs. PPO-).

**Table 1** Effect of WSC and PPO content on rumen fermentation and microbial synthesis

**Results** Ryegrass led to higher VFA concentrations, while RC increased pH, ammonia concentrations and degradabilities of OM and N. Red clover also increased the flow of total N and non-ammonia-N (NAN) compared with ryegrass, even when normalized for N supply. In agreement with that, RC also increased microbial N flow and synthesis efficiency in comparison with grass, regardless of the microbial extraction used. Estimates of SAB synthesis were higher than those estimated using TB and LAB. No differences between treatments were observed in either gas production or methane emissions. Within ryegrass treatments, WSC promoted an increase in ammonia and fibre degradability but did not affect microbial synthesis. PPO activity neither decreased the N degradability nor improved the N utilization by this system.

**Conclusion** RC promoted a more efficient use of dietary energy and N than ryegrass; however no significant effects of WSC content or PPO activity were observed under our conditions.

	Ryegrass		Red clover		SED	Significance		
	HWSC	LWSC	PPO+	PPO-	<i>n</i> =4	C1	C2	C3
Rumen fermentation								
pH	6.76	6.75	6.88	6.87	0.035	***	NS	NS
VFA (mM)	48	51	42	41	4.2	*	NS	NS
N-NH <sub>3</sub> (mg/dl)	6.6	5.8	9.3	9.6	0.33	***	*	NS
Gas production (ml/h)								
Total	84	70	67	65	8.6	†	NS	NS
Methane	0.74	0.71	0.63	0.57	0.371	NS	NS	NS
Degradability (%)								
OM	63	59	73	73	2.1	***	†	NS
N	75	72	85	83	2.0	***	NS	NS
NDF	38	29	36	16	4.2	NS	*	*
ADF	28	17	21	22	4.2	NS	*	NS
Post-ruminal flow (g/d)								
OM	9.2	9.9	9.5	10.2	0.99	NS	NS	*
N	0.16	0.15	0.29	0.32	0.015	***	NS	NS
NAN	0.11	0.10	0.20	0.21	0.008	***	NS	NS
NAN/N intaked	0.56	0.52	0.58	0.62	0.027	*	NS	†
Microbial N flow (g/d)								
TB	0.06	0.06	0.10	0.10	0.005	***	NS	NS
LAB	0.08	0.07	0.09	0.10	0.005	***	NS	†
SAB	0.13	0.12	0.18	0.19	0.010	***	NS	NS
Synthesis efficiency (g MN/kg OMADR)								
TB	10	10	19	20	0.9	***	NS	NS
LAB	12	12	18	19	1.1	***	NS	NS
SAB	20	20	35	38	2.2	***	NS	NS

NS P>0.1; † P<0.1; \* P<0.05; \*\* P<0.01; \*\*\* P<0.001

**Acknowledgement** This experiment was funded by the Commission of the European Communities FP7, KBB-2007-1.

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## Effects of forage species, grass water soluble carbohydrates and red clover polyphenol oxidase activity on the *in vitro* rumen microbial ecosystem

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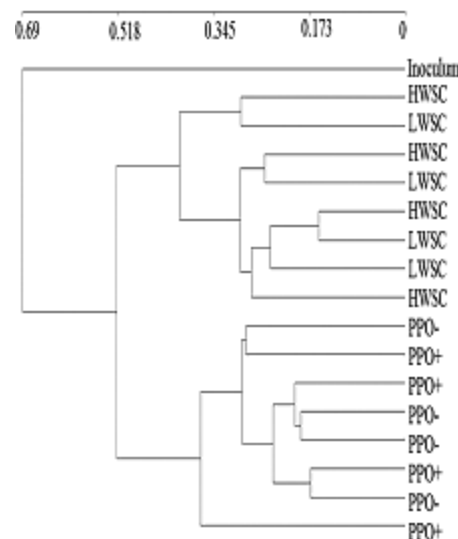
**Introduction** Novel varieties of perennial ryegrass with high water soluble carbohydrate (WSC) concentrations have been bred to increase energy availability in the rumen. In red clover (RC) the activity of polyphenol oxidase (PPO) can help protect protein in the rumen by decreasing or delaying proteolysis. Both factors (WSC and PPO) offer potential to improve the synchronization or balance between energy and nitrogen availability for rumen microorganisms and consequently to optimise rumen microbial synthesis. However, it remains unclear if these effects can be attributed to differences in diet composition or to changes in the rumen microbial ecosystem. The objective of this *in vitro* experiment was to study how the rumen microbiota was affected by diet WSC content and PPO activity.

**Material and methods** Two ryegrass varieties, bred to express high and normal (control) WSC concentrations (HWSC vs. LWSC), and two strains of RC, wild type with normal PPO activity (PPO+) and a PPO knockout strain with undetectable PPO activity (PPO-), were used. The experiment was carried out using sixteen continuous culture rumen simulation technique (Rusitec) systems allocating 4 vessels per treatment that were inoculated with fresh rumen contents. Ryegrass was cut from outdoor plots daily (at approximately 09:00h) and RC had been previously grown in pots in growth chambers to collect sufficient material; it was then defrosted to help to activate the PPO enzyme. Each day new plant material (50 g fresh matter/d) was added in one of two nylon bags in each Rusitec vessel, where it remained for 48h. Artificial saliva was infused continuously to obtain a liquid dilution rate of 3.65%/h. After 9 days of adaptation the fermentation vessels were sampled by aspiration for 3 consecutive days at 2, 4, 8 and 24h after 'feeding'. DNA was extracted from lyophilised samples and the bacterial biodiversity was analysed by the terminal restriction fragment polymorphism (TRFLP) procedure using 4 different restriction enzymes (HAE3, RSA1, MSP1 and HHA1). A dendrogram was created using the Bray-Curtis average linkage clustering method. Absolute bacterial and protozoal DNA concentrations were determined by quantitative PCR (qPCR), while the relative abundance of different species was estimated using the  $\Delta\Delta C_T$  method. Samples from 2 and 4h (<4h) or from 8 and 24h after feeding (>8h) were considered representative of early or later rumen fermentation respectively. Data were analyzed by ANOVA with blocking by machine and using three orthogonal contrasts to separately determine the effect of forage (C1; grass vs. RC), WSC content (C2; HWSC vs. LWSC) and PPO activity (C3; PPO+ vs. PPO-).

**Results** Forage species had a significant effect on rumen bacterial biodiversity and clustering, with RC promoting a higher diversity than ryegrass. No differences were attributed to WSC content or PPO activity. Quantitative PCR agreed with the TRFLP results, with significant forage effects on the concentrations of most of the microorganisms studied. Within the ryegrasses, WSC concentration had no effect on the rumen ecosystem, and RC PPO activity only increased bacterial concentrations in the later fermentation times, possibly because of a higher proportion of slowly available N in PPO+ diets.

**Table 1** Abundance of different microbial groups determined by qPCR

		Ryegrass		Red clover		SED	Significance		
		HWSC	LWSC	PPO+	PPO-	n=4	C1	C2	C3
Abundance ( $\mu\text{g/g DM}$ )									
Bacterial	<4h	808	829	610	558	64.5	***	NS	NS
	>8h	531	516	561	436	42.9	NS	NS	*
Protozoa	<4h	9.0	8.3	7.3	10.2	3.40	NS	NS	NS
	>8h	10.1	7.2	10.2	10.3	3.32	NS	NS	NS
Relative abundance (%)									
<i>R. albus</i>	<4h	0.22	0.24	$9 \times 10^{-3}$	0.01	0.04	***	NS	NS
	>8h	0.19	0.19	$7 \times 10^{-3}$	$7 \times 10^{-3}$	0.02	***	NS	NS
<i>F. succinogenes</i>	<4h	2.69	2.96	$1 \times 10^{-3}$	$1 \times 10^{-3}$	0.36	***	NS	NS
	>8h	2.02	1.91	$8 \times 10^{-6}$	$4 \times 10^{-6}$	0.28	***	NS	NS
<i>B. fibrisolvens</i>	<4h	2.17	2.33	1.94	1.87	0.31	NS	NS	NS
	>8h	2.02	2.01	1.66	1.67	0.35	NS	NS	NS
<i>S. ruminantium</i>	<4h	9.76	9.90	$1 \times 10^{-6}$	$1 \times 10^{-5}$	1.87	***	NS	NS
	>8h	6.22	6.52	$2 \times 10^{-7}$	$2 \times 10^{-8}$	1.18	***	NS	NS
Anaerobic fungi	<4h	1.22	1.27	$3 \times 10^{-3}$	$4 \times 10^{-3}$	0.20	***	NS	NS
	>8h	0.80	0.79	$4 \times 10^{-4}$	$1 \times 10^{-3}$	0.17	***	NS	NS
Biodiversity (TRFs/enz.)		55.0	55.4	59.4	60.3	2.27	**	NS	NS



**Figure 1** Effect of diet on bacterial biodiversity determined by TRFLP.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; Contrasts, effects of: C1 = type of forage; C2 = WSC concentration; C3 = PPO activity.

**Conclusion** Type of forage (ryegrass vs. RC) led to significant differences in the rumen microbial population. No effects of WSC concentration, and only minor effects of PPO activity, were observed under our experimental conditions.

**Acknowledgement** This experiment has been funded by the Commission of the European Communities FP7, KBB-2007-1.

## ***In vitro* rumen simulated metabolism (RUSITEC) of freshly cut or wilted grasses with contrasting polyphenol oxidase activities: the effect on rumen parameters, lipolysis and biohydrogenation**

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**Introduction** Polyphenol oxidase (PPO) in red clover has been shown to reduce both proteolysis and lipolysis in the silo and rumen. Lee *et al.* (2006) showed *in vitro* that grass PPO resulted in a reduction in plant mediated proteolysis and lipolysis. However, it is yet to be determined whether grass PPO has any effect on proteolysis and lipolysis in the presence of rumen micro-organisms. For PPO activity to occur, cell damage (mixing of enzyme and substrate) and aeration (oxidation) are required. These two criteria are easily met during silage making but during grazing the anaerobic nature of the rumen provides only a small window of opportunity during mastication for PPO activation. Therefore this study investigated the need to mix enzyme and substrate in the field by wilting and chopping versus cut grass in two species cocksfoot (*Dactylis glomerata*; high PPO) and tall fescue (*Festuca arundinacea*; low PPO).

**Materials and methods** A 16 vessel RUSITEC as described by Czerkawski and Breckenridge (1977) was used with four treatment combinations: cocksfoot wilted (C<sub>w</sub>); cocksfoot fresh (C<sub>f</sub>); tall fescue wilted (TF<sub>w</sub>) and tall fescue fresh (TF<sub>f</sub>). Rumen liquor was collected from 4 fistulated dairy cows maintained on permanent pasture. The wilted treatments (C<sub>w</sub> and TF<sub>w</sub>) were cut the previous day and left on the laboratory bench for 24 h. The fresh treatments (C<sub>f</sub> and TF<sub>f</sub>) were collected daily on ice. Once transferred to the laboratory all treatments were passed through a garden shredder, a sample taken for chemical analysis and ca. 10 g DM of each grass weighed into 4 Dacron bags for the relevant vessels. This was repeated daily with the bags remaining in the vessels for 48 h. The experiment ran for 12 days with sampling of effluent for rumen parameters on days 10 and 11. At the end of d 12 the grass residue and effluent from the vessels were collected and analysed for N and fatty acids. Lipolysis was calculated as the proportional loss of membrane glycerol-based lipid between the forage and 24 h residue. Biohydrogenation was calculated as the retention of C18 polyunsaturated fatty acids (PUFA) during incubation per unit PUFA supply. All analysis was performed as a general analysis of variance with species x wilt as the fixed effects (Genstat, Release 11.1).

**Results** PPO was significantly higher in C<sub>f</sub> than the other treatments; C<sub>w</sub> was higher than both TF treatments, with no difference between TF<sub>w</sub> and TF<sub>f</sub>. The level of bound phenol (product of oxidation reaction) was higher for C<sub>w</sub> and TF<sub>w</sub> than C<sub>f</sub> and TF<sub>f</sub>, and for C than TF. As an average across the day ammonia-N was lower in C than TF, despite the lower N concentration of the respective grasses, and in fresh as opposed to wilted grass. There was a trend for lipolysis to be lower in C than TF, and for both wilted treatments to be lower than the fresh. There was no difference in lipolysis between the species during wilting 0.14 and 0.11 for C and TF, respectively. Biohydrogenation and total VFA were not different between treatments.

**Table 1** Chemical composition of grasses and the rumen parameters, lipolysis and biohydrogenation in RUSITEC

	Cocksfoot		Tall Fescue		S.e.d	P	F/W	Sp x F/W
	Fresh	Wilted	Fresh	Wilted				
PPO (μkatal (μmol/s))	15.7	8.06	1.26	1.57	0.534	***	*	***
Bound Phenol (mg/g DM)	1.81	2.11	0.21	0.52	0.279	***	*	NS
Grass N (g/kg DM)	27.1	27.4	25.3	25.5	0.71	**	NS	NS
Total VFA (mmol/l)	36.1	38.6	36.8	37.9	4.84	NS	NS	NS
Ammonia-N (μg/ml)	77.6	82.3	82.9	94.1	6.21	**	**	NS
Lipolysis (g/g membrane lipid)	0.83	0.80	0.87	0.82	0.023	†	*	NS
C18:2 Biohydrogenation (mg/g C18:2 input)	33.7	27.1	25.2	27.6	3.58	NS	NS	NS
C18:3 Biohydrogenation (mg/g C18:3 input)	7.70	7.25	6.05	9.16	2.505	NS	NS	NS

Sp, species effect; F/W, wilting effect; Sp x F/W, interaction; †P<0.1; \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; NS P>0.1.

**Conclusions** As expected C had higher PPO activity and subsequent bound phenol concentrations than TF which may have resulted in a lower rumen ammonia N in C despite its greater N content. The greater level of ammonia N in wilted compared to fresh was possibly due to levels of plant mediated proteolysis during wilting. The effect of PPO in reducing proteolysis could also help explain the smaller effect on C as opposed to TF when the grasses were wilted. There was a trend for lower lipolysis in C than TF during incubation. Wilting resulted in a lower lipolysis than the fresh grasses during incubation which may have been due to the lower initial level of membrane lipid due to lipolysis during wilting. There was no effect on the flow of C18 PUFA in a simulated rumen environment. Differences in grasses other than just PPO activity such as lipase activity and digestibility may have confounding effects between grasses thus diminishing the protective effect of PPO.

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## Effects of timing and duration of fish oil supplementation of pregnant ewes on maternal and offspring performance up to weaning

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**Introduction** Long chain polyunsaturated fatty acids (PUFA) of the n-6 and n-3 series are essential for development of the nervous and immune systems in growing foetuses. Pregnant ewe rations are often undersupplied with these fatty acids, and there is evidence of improved neonatal vigour (Capper *et al.*, 2006) and reduced lamb mortality (Annett *et al.*, 2009) when diets are supplemented with long chain PUFA (fish oil) during the period of rapid foetal growth in late pregnancy. However, fish oil supplementation prior to lambing has also been shown to reduce colostrum production (Annett *et al.*, 2008) which could offset the developmental benefits for lamb survival within extensive, easier-care sheep systems. Foetal brain development reaches its peak between 6-10 weeks pre-lambing (Turley *et al.*, 1996); therefore fish oil supplementation earlier in pregnancy could provide a suitable compromise, as suggested by Pickard *et al.* (2008). The objectives of the current study were to investigate the effects of fish oil supplementation at different stages of pregnancy and for different periods of time on ewe and lamb performance.

**Materials and methods** One hundred and twenty six multiparous ewes (live weight  $75 \pm 11.1$  kg; body condition score  $2.9 \pm 0.5$ ) of mixed breeds (Lleyen X, Belclare X and Texel X) were housed on day 84 following a synchronised mating to Suffolk, Texel, Highlander and Primera sires. Half the ewes were individually housed while the remainder were housed according to litter size in groups of 3-4. At housing, ewes were allocated to one of six treatments ( $n = 21$ ) balanced for condition score, ewe breed, sire breed and litter size. All ewes were offered precision chop grass silage plus protein balancer (228 g CP/kg DM), the quantities being adjusted weekly depending on stage of pregnancy and litter size. In addition, ewes were offered 20 g/d crude herring oil (United Fish Industries Ltd, Killybegs, Ireland), using milled barley as a carrier, which commenced 3, 6 or 9 weeks pre-lambing and continued for a period of either 3 or 6 weeks, as follows: 9/3 (T1); 6/3 (T2); 3/3 (T3); 9/6 (T4), 6/6 (T5) or 0/0 (Control, T6). A protected fat supplement (Maxfat-CS®, Trouw Nutrition, Belfast) was offered to ewes not receiving fish oil to ensure diets were iso-lipidic. Silage was offered daily at 0930 h while supplements were offered in two equal size feeds at 0930 h and 1600 h. Intakes of silage and supplement were recorded daily. Ewe live weight and body condition scores were measured fortnightly pre-lambing, within 24 h lambing, 6 weeks post-lambing and at weaning. Lambs were tagged and weighed at birth, 6 weeks of age and at weaning. Lambing difficulty was scored on a four-point scale where 1 = no assistance and 4 = manual delivery with difficulties. Lamb viability was scored on a 3-point scale (1 = up & sucked; 3 = helped to suck). Data were analysed as 6 treatments using Residual Maximum Likelihood (REML) analysis with contrasts used to make factorial comparisons. Covariates were included for ewe breed, sire breed, litter size, lamb age and sex, where appropriate. Lambing difficulty data were analysed using regression analysis.

**Results** There were no significant treatment interactions so only the main effects are presented. Neither fish oil supplementation *per se*, stage of gestation when supplementation commenced or the duration of the supplementation period had any significant effects on silage dry-matter intake, lamb mortality, lamb viability, the incidence of lambing difficulties or lamb growth rate up to weaning. However, commencing fish oil supplementation at 9 weeks rather than 6 weeks pre-lambing led to a 0.5 kg increase in mean lamb birth weight, regardless of the duration of the supplementation period.

**Table 1** Effects of fish oil supplementation strategy on ewe and lamb performance

Treatment	9/3	6/3	3/3	9/6	6/6	0/0	s.e.d	F	S	D
Silage DM intake (kg/d)	0.93	0.91	0.92	0.93	0.90	0.92	0.032	NS	NS	NS
Prop. lambs born dead	0.07	0.07	0.08	0.05	0.07	0.03	0.058	NS	NS	NS
Mean lamb birth wt (kg)	4.7	4.3	4.8	4.8	4.3	4.5	0.23	NS	*	NS
Lamb viability score	1.02	1.00	1.00	1.00	1.00	1.03	0.023	NS	NS	NS
Prop. ewes assisted	0.62	0.72	0.75	0.71	0.68	0.59	0.104	NS	NS	NS
Prop. lambs died birth to wean	0.07	0.12	0.13	0.05	0.14	0.06	0.077	NS	NS	NS
Birth to weaning LWG (g/d)	251	265	275	267	276	264	12.3	NS	NS	NS

D, duration of supplementation; DM, dry-matter; F, fish oil supplementation; LWG, live weight gain; S, start time of supplementation; Treatments are denoted in the format A/B where A = Start of fish oil supplementation (weeks pre-lambing) and B = Duration of supplementation period (weeks)

**Conclusion** The results of this study suggest that supplementing ewes with crude fish oil during mid and late pregnancy has limited benefits for lamb viability and weaned lamb output, irrespective of the timing or duration of the supplementation period.

**Acknowledgement** Funding from the Department of Agriculture and Rural Development is gratefully acknowledged.

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## Compensatory growth in suckler beef cattle production systems on two commercial farms in Scotland

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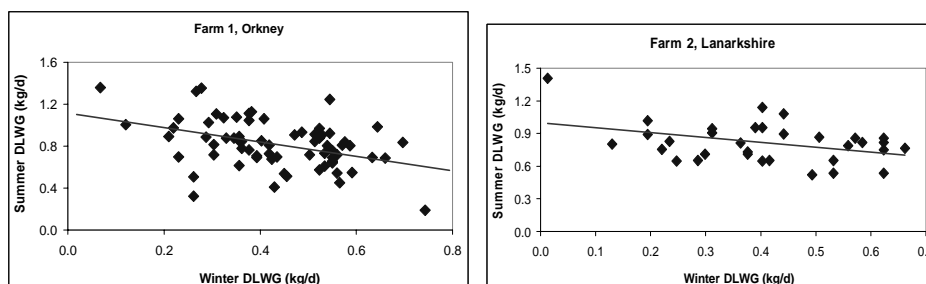
**Introduction** Compensatory growth can be described simply as the ability of animals to “compensate” for a period of growth restriction (usually during winter) with a subsequent period of enhanced growth (usually during the subsequent summer) such that overall growth over the entire period is substantially the same. When practiced successfully, this feeding strategy can reduce the costs of beef production by minimising winter feeding costs within appropriate feeding systems. The objective of this on-farm study was to examine compensatory growth in weaned steer and heifer suckled calves on two commercial farms in Scotland.

**Materials and methods** Two studies were carried out on 2 commercial farms in Scotland to study compensatory growth within spring calving suckler beef production systems. On one commercial suckler beef farm in Orkney, 69 weaned suckled calves of various breed types were used whilst on a similar suckler beef farm in Lanarkshire, 34 weaned suckled calves (all Limousin crossbreds) were used. On both farms, homebred weaned steer and heifer suckled calves were split into two balanced groups on the basis of breed type, sex and liveweight (LW) at weaning and offered one of two 1<sup>st</sup> winter diets designed to grow calves at either a high (HIGH) or low (LOW) rate of winter gain according to a 2 x 2 (calf sex x winter diet) factorial, continuous design experiment. Winter diets were based primarily on grass silage (although some wholecrop wheat was fed on farm 2) and a barley based concentrate was used at either a high or low level to achieve the divergent winter growth rates (Table 1). All steer and heifer calves on each farm were then subsequently grazed throughout the following summer on the same grazing and animal growth rates determined throughout by difference between initial (turnout) and final (housing) LWs. On the Orkney farm, good quality grazing was available throughout the summer whilst on the farm in Lanarkshire poorer quality rough grazing was available during the 1<sup>st</sup> half of the summer and silage aftermaths were available during late summer. Cattle LWs and daily liveweight gains (DLWG) were statistically analysed for each farm separately using the REML procedure in Genstat 8. The relationship between winter and following summer DLWG was determined using linear regression analysis.

**Results** Both steers and heifers responded similarly ( $P>0.05$ ) to the divergent winter diets so only average animal performance figures for each diet on each farm are given in Table 1. Whilst full compensatory growth was achieved during the summer months on Farm 1 such that LW at the end of the summer was almost identical, only partial compensatory growth was achieved on Farm 2, probably due to the quality of early summer grazing available. Financial calculations also showed that this compensatory growth resulted in £12 - £44 per head extra margin depending on the extent to which compensatory growth was realised in any given situation. The summer vs winter rates of daily liveweight gain (DLWG) on both farms are plotted in Figure 1 confirming the significant ( $P<0.05$ ) relationships between low winter and high summer growth rates.

**Table 1** Winter diets offered during the 1<sup>st</sup> winter period, along with LWs and DLWGs of animals throughout the study

Fresh weight intake		(Farm 1 – Orkney)				(Farm 2 – Lanarkshire)			
		HIGH	LOW	s.e.d.	Sig	HIGH	LOW	s.e.d.	Sig
Grass silage	(kg/h/d)	23	23			17	19		
Wholecrop wheat	“	-	-			1.5	1.75		
Barley based concentrate	“	2.2	0.7			2.0	-		
LW @ weaning	(kg)	299	300	11.2		308	308	10.7	
LW @ turnout	“	393	377	12.2		386	353	13.0	*
LW @ housing	“	522	525	11.4		483	462	12.5	
DLWG winter period	(kg/day)	0.49	0.36	0.024	***	0.51	0.29	0.042	***
DLWG summer period	“	0.76	0.87	0.040	***	0.77	0.86	0.061	



**Figure 1** Summer vs winter DLWG (Compensatory growth) in suckler bred animals on two farms in Scotland

**Conclusion** The results indicate that compensatory growth can be exploited to beneficial effect on commercial suckler farms under appropriate circumstances and that the quality of summer grass available may be a key factor in the nature of the response.

**Acknowledgements** QMS provided financial support for this work and we also grateful to D Baillie and E Sinclair for the provision of farm facilities throughout this study.

## Effect of restricted access time to pasture in spring on the performance of autumn- and spring-calving beef suckler cows and their progeny

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**Introduction:** Extending the grazing season via earlier turnout to pasture in spring can lead to greater profitability in grass-based suckler beef systems by reducing slurry handling and feed costs (Crosson *et al.*, 2009). This is not easily achievable under adverse weather and soil conditions. O'Riordan *et al.* (1996) showed that allowing yearling steers restricted access time to grazed pasture daily was an alternative strategy, that may be applicable in poor grazing circumstances. Two experiments were carried out to determine the effect of early turnout to pasture in spring via restricted access time daily on the performance of lactating autumn- (Experiment 1) and spring- (Experiment 2) calving suckler cows and their calves.

**Materials and methods:** In Experiment 1, thirty two lactating autumn-calving suckler cows were blocked by genotype, weight, calf gender and calving date and from within block, randomly allocated to one of two dietary treatments: (i) grass silage ad libitum plus 2 kg of concentrate daily or (ii) 0.2 total grass silage dry matter intake plus 6 hours access to pasture daily. Calves remained indoors and were offered grass silage ad libitum and had twice-daily access to their dams for suckling. The dietary treatments lasted from 3<sup>rd</sup> March until 1<sup>st</sup> April. Similarly, in Experiment 2, twenty four lactating spring-calving suckler cows were allocated to one of two treatments: i) grass silage ad libitum or ii) 0.2 of total grass silage dry matter intake plus 6 hours access to pasture daily. Calves remained indoors and had twice-daily suckling access. The duration of the dietary treatments was from 26<sup>th</sup> March until 16<sup>th</sup> April. At the end of experiments all animals were turned out to pasture until weaning. Cow live weight, body condition score (BCS), ultrasonic fat and muscle depth, and milk yield (weigh-suckle-weigh procedure – Exp. 1 only) and, calf live weight was measured. To facilitate adjustments in gut fill to the grass diet, cows were weighed 14 and 7 days following turnout to pasture in Experiments 1 and 2, respectively. Data were statistically analysed using ANOVA.

**Results:** In Experiment 1, live weight gain to post-turnout was greater ( $P < 0.01$ ), BCS gain to turnout was lower ( $P < 0.05$ ) and milk yield was higher ( $P < 0.05$ ) in cows turned out early than those turned out late (Table 1). During the dietary experimental period, calves of autumn-calved cows turned out early had significantly higher average daily gain (ADG) but ADG to weaning did not differ ( $P > 0.05$ ) between treatments. In Experiment 2, cow performance was similar ( $P > 0.05$ ) between treatments. Calves of spring-calved cows turned out early had higher ( $P < 0.05$ ) ADG to turnout, but subsequent ADG did not differ ( $P > 0.05$ ) between treatments.

**Table 1** Performance of autumn- and spring-calving cows and growth of their calves

		Exp. 1: Autumn-calving				Exp. 2: Spring-calving				
		Early	Late	SEM	Sig.	Early	Late	SEM	Sig.	
Cow:	Initial live weight (kg)	623	632	5.2	NS	560	564	17.4	NS	
	Change to turnout	-36.6	-30.5	3.52	NS	-3.2	-3.8	5.19	NS	
	Change to post-turnout	18.7	1.0	3.47	**	4.1	-7.0	4.97	NS	
	Change to weaning	33.6	19.9	12.39	NS	39.5	7.0	17.14	NS	
	Initial body condition score (0-5)	3.1	3.1	0.05	NS	2.0	2.1	0.05	NS	
	Change to turnout	0.0	0.2	0.07	*	0.1	0.1	0.03	NS	
	Change to weaning	-0.6	-0.6	0.06	NS	0.1	0.0	0.06	NS	
	Ultrasonic measurement (mm):									
	Fat - 13 <sup>th</sup> rib	Initial	2.1	1.9	0.22	NS	1.6	1.5	0.31	NS
		Change to turnout	-0.1	0.3	0.29	NS	0.1	0.5	0.18	NS
	Fat - 3 <sup>rd</sup> lumbar	Initial	1.9	1.8	0.22	NS	1.4	1.7	0.37	NS
		Change to turnout	-0.2	0.2	0.32	NS	0.3	0.1	0.16	NS
	Fat - rump	Initial	5.2	4.9	0.32	NS	3.0	2.4	0.65	NS
		Change to turnout	1.7	2.8	0.54	NS	0.3	0.7	0.32	NS
Muscle - 3 <sup>rd</sup> lumbar	Initial	58.9	63.4	1.55	NS	58.1	58.0	2.06	NS	
	Change to turnout	7.7	1.3	2.28	NS	-3.3	-7.0	2.06	NS	
	Milk yield (kg/day)	9.4	7.5	0.48	*	-	-	-	-	
Calf :	Initial weight (kg)	200	199	4.22	NS	62	65	2.2	NS	
	ADG to turnout (g)	983	849	36.3	*	1138	898	66.0	*	
	ADG to post-turnout (g)	1136	923	33.8	***	847	822	54.3	NS	
	ADG to weaning (g)	1163	1122	36.3	NS	934	979	38.4	NS	
	ADG post-turnout to weaning (g)	1175	1205	47.7	NS	950	1005	40.8	NS	

**Conclusion** Results suggested that allowing beef suckler cows restricted access time to grazed pasture daily is a strategy to permit early-spring grazing.

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## The effect of oil Palm Petiole in total mixed diet on the consumption and digestibility in Bali cows (*Bos Sondaecus*)

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**Introduction** Oil palm petiole (OPP), a by product from oil palm plantation, is the stalk of the oil palm frond (OPF) without the leaflets and the outer layer. The possibility of using OPF and OPP as feed has been successfully tested with ruminants in Malaysia and Indonesia. However OPP was a possible alternative for OPF in feeding Bali cows (*Bos sondaecus*) since Bali cows were observed to reject fresh OPF. In term of palatability, Afdal *et al* (2009) reported that pellets of OPP were very much liked by Bali cows which could consume around 429 g/kg in total ration compared with other kinds of OPP processing like fresh chopped OPP and OPP meal. There is however no information on the effect of OPP on the productivity of Bali cows. The aims of this study were therefore to investigate the effects of OPP in the mixed ration on the feed consumption, feed digestibility, daily gain and feed efficiency of Bali cows.

**Materials and methods** Four Bali cows, liveweight  $113 \pm 2.30$  kg were used in this experiment. Animals were fed in DM basis according to the procedure of Darlis *et al.*, (2001) with diet A of 100 % field grass (FG), diet B of 50 % FG and 50 % pelleted OPP (POPP), diet C of 25 % FG and 75 % POPP and diet D of 33.3 % A, 33.3 % B and 33.3% C. Animals were fed once a day at 08.00h and had free access to water. The diet chemical composition can be seen at Table 1. The design of this experiment was a Latin Square (4x4) with the length of each period being 3 weeks with a two weeks of adaptation period and one week of data collection. Animals were weighted at the beginning and end of the data collection period. Diet consumption and faeces collection was done every morning and 10 % sample of faeces was taken for chemical analysis. Feed and faeces samples were dried at 60 °C and analysed according to procedure AOAC (1984). Values measured included feed consumption, the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) together with daily gain and feed consumption efficiency. ANOVA followed by Duncan test were applied for statistical analysis

**Table 1** Feed chemical composition and ration consumption of experimental cows

Treatment	Chemical composition (g/kg)					Consumption (kg/d)				
	DM	OM	CP	ADF	NDF	DM	OM	CP	NDF	ADF
A	898.2	908.0	122.8	320.1	558.4	9.54 <sup>a</sup>	8.65 <sup>a</sup>	1.14 <sup>c</sup>	5.31 <sup>a</sup>	3.05 <sup>a</sup>
B	905.8	925.5	97.0	356.9	568.9	7.14 <sup>cb</sup>	6.54 <sup>b</sup>	0.96 <sup>b</sup>	4.03 <sup>b</sup>	2.42 <sup>a</sup>
C	909.5	928.3	84.0	375.2	574.2	6.66 <sup>c</sup>	6.14 <sup>b</sup>	0.91 <sup>a</sup>	3.79 <sup>b</sup>	2.38 <sup>a</sup>
D	895.5	911.4	100.3	347.2	561.5	7.28 <sup>b</sup>	6.66 <sup>b</sup>	0.87 <sup>a</sup>	4.10 <sup>b</sup>	2.45 <sup>a</sup>

Different superscripts within the same column shows significantly difference ( $P < 0.05$ ).

**Results** Consumption of DM, OM and NDF was significantly ( $P < 0.05$ ) higher on treatment A than on B, C and D except for CP in which it was significantly ( $P < 0.05$ ) higher on treatment C than on treatment A, B and D (Table 1). Results showed that treatment affected the digestibility of DM, OM, NDF and ADF experimental cows (Table 2). There was significantly ( $P < 0.05$ ) different higher digestibility of treatment A than treatment B but there were not different with treatment C and D. The digestibility of CP was significantly lower on treatment A than B, C and D.

**Table 2** The digestibility of nutrients of experimental cows

Treatment	Digestibility (%)				
	DM	OM	CP	NDF	ADF
A	90.60 <sup>a</sup>	91.20 <sup>a</sup>	92.72 <sup>b</sup>	89.69 <sup>a</sup>	87.61 <sup>a</sup>
B	85.47 <sup>b</sup>	85.97 <sup>b</sup>	96.67 <sup>a</sup>	82.84 <sup>b</sup>	80.43 <sup>b</sup>
C	87.34 <sup>ab</sup>	87.87 <sup>ab</sup>	97.99 <sup>a</sup>	85.27 <sup>ab</sup>	84.06 <sup>ab</sup>
D	88.39 <sup>ab</sup>	89.07 <sup>ab</sup>	96.32 <sup>a</sup>	86.72 <sup>ab</sup>	84.84 <sup>ab</sup>

Different superscripts within the same column shows significantly difference ( $P < 0.05$ ).

**Conclusion** It can be concluded that OPP could be applied as ruminant feed as it provided better daily gain and feed efficiency on the Bali cow than field grass.

**Acknowledgement** The authors appreciatively acknowledge funding from DP<sub>2</sub>M project 2008, Directorate of High Education, Department of National Education of Republic of Indonesia

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## Effect of phosphorus supplementation of ammoniated rice straw on rumen fermentability, synthesised microbial protein and degradability *in vitro*

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**Introduction** Nutritive value of some straws and other by-product feeds can be improved simply by adding urea and minerals as such as P. These bacteria are needed for pregastric digestion of fibre in cattle. Replication and growth of the bacteria are dependent upon a P supply (Petterson, 2002). P supplementation is important for rumen fermentation and growth of rumen microbia, therefore, a study was conducted to examine the effects of P addition on *in vitro* fermentability, synthesised microbial protein and degradability of ammoniated rice straw..

**Material and methods** The experimental diet composed of 50% ammoniated rice and 50% concentrate, and this diet was used as a control diet (A). The rice straw was previously treated with 4% urea. The crude protein of the diet was 10.16%. P2O5 was used as a P source and added to the diet 0.2, 0.4 and 0.6% on dry matter, respectively. *In vitro* fermentability and degradability of nutrients were determined following the first stage of the Tilley and Terry procedure (1969). Ruminal fluid was obtained from a cannulated steer. Fermentation tubes contained of 10 ml of ruminal fluid and 40 ml of McDougall buffer solution. Samples were incubated in duplicate in 100 ml polyethylene tubes in 39°C in a shaken water bath for 48 h. Two fermentation tubes that did not contain diets were also incubated and used as blanks. Sample was taken from each fermentor for bacterial counting. Fermentation was terminated at 48 h by injecting the tubes with 1 ml of HgCl<sub>2</sub>. Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Tubes with residue were dried at 60°C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, OM and N by using standard procedures (AOAC, 1984), the NDF, ADF, and cellulose of residues were determined by Goering and Van Soest (1970) procedures. Supernatants were used in order to determine NH<sub>3</sub> concentration (microdiffusion Conway method), total VFA concentration (Gas chromatography) and rumen fluid pH. Total and cellulolytic bacterial population was determined by methods described in Suryahadi (1990), cellulase enzyme activity and the amount of synthesised microbial protein was, determined by methods described in Widyastuti (2004) and (Gopar, 1981) respectively. A completely randomized design was used as experimental design consisting of four treatments. Data were analyzed by ANOVA using the GLM procedure (Steel and Torrie, 1981). Differences between the control treatment and P supplementation treatment were analyzed by Duncan multiple range test (DMRT) (Steel and Torrie, 1981).

**Result** Table shows results of P supplementation effects on bacterial population and other variables of rumen fermentability. Effects of treatments were significant (P<0.05) for ammonia and total VFA concentrations. Data on *in vitro* degradability of ammoniated RS are presented in the table and show that the addition of P at different level affected all degradability variables (P<0.05).

**Table 1** Effect of phosphorus supplementation on total and cellulolytic bacterial population and fermentation in the rumen and *in vitro* degradability

Variables	P Supplementation (%DM)			
	0	0.2	0.4	0.6
Synthesised microbial protein (%/g)	0.19	0.18	0.21	0.13
N-NH <sub>3</sub> (mM)	11.09 <sup>a</sup>	10.02 <sup>b</sup>	9.25 <sup>c</sup>	8.80 <sup>d</sup>
Total VFA (mM)	88.75 <sup>c</sup>	98.12 <sup>b</sup>	106.87 <sup>a</sup>	111.87 <sup>a</sup>
Dry matter degradability (%)	52.91 <sup>c</sup>	54.85 <sup>bc</sup>	57.66 <sup>a</sup>	60.79 <sup>a</sup>
Organic matter degradability (%)	54.69 <sup>c</sup>	58.43 <sup>b</sup>	60.18 <sup>a</sup>	62.69 <sup>a</sup>
NDF degradability (%)	39.31 <sup>b</sup>	41.58 <sup>b</sup>	43.94 <sup>a</sup>	50.91 <sup>a</sup>
ADF degradability (%)	27.99 <sup>c</sup>	32.78 <sup>bc</sup>	37.59 <sup>a</sup>	40.30 <sup>a</sup>
Cellulose degradability (%)	29.47 <sup>b</sup>	33.04 <sup>b</sup>	38.74 <sup>a</sup>	41.61 <sup>a</sup>

Means within rows with the same superscript letter are significantly different at P<0.05

**Conclusion** The effects occurred through a reduction in ammonia concentration, increase in total bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. In terms of the synthesis of microbial protein, most effective level of P supplementation occurred at a supplementation rate of 0.4% of dry matter. Further study is required to determine effects of supplementation in an *in vivo* experiment.

**Acknowledgment** This work was supported by an Insentif Riset Dasar Programme at Ministry of Research and Technology, Republic of Indonesia in 2007.

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## An evaluation of the effects of extended grazing pasture with ewe lambs on sward botanical composition

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**Introduction** Previous studies at this centre have shown that extended (deferred, winter) grazing ewes in mid (Keady *et al* 2007, Keady and Hanrahan 2009b) late (Keady *et al* 2007) or throughout (Keady *et al* 2007, Keady and Hanrahan 2009a) pregnancy increased lamb birth and weaning weights relative to progeny from ewes which were housed unshorn. However Keady *et al* (2009) concluded that whilst year round grazing provided a viable system for sheep production, stocking rate had to be dramatically reduced relative to systems in which ewes were housed and offered conserved forage during the winter feeding period. During extended grazing, ewes graze pasture with heavy sward cover, at set herbage allowances which are normally allocated either daily or twice weekly. Furthermore extended grazing occurs during the winter months which are normally associated with high rainfall. Consequently pasture becomes soiled and poaching can occur. There is circumstantial evidence that extended grazing has a negative impact on subsequent sward botanical composition. The aim of the current study was to evaluate the effects of extended grazing on subsequent sward composition.

**Materials and methods** A sward which had been harvested for silage in early September received fertiliser N at 34 kg/ha for extended grazing between 12-18 December, 16-22 January and 20-26 February. One hundred and two ewe lambs (40.9 kg) were allocated to two grazing herbage DM allowances of 0.75 and 1.75 kg DM/hd daily (0.75G and 1.75G). During extended grazing fresh herbage was allocated daily. Within each herbage allowance area a 0.5 m x 1 m plot was mowed to 3 cm in early December (A) and a second 0.5 m x 1 m plot was mowed on the day of extended grazing (B). These plots were protected by mesh cages during grazing. Herbage yield and vascular plant botanical composition was determined for treatments A, B, 0.75G and 1.75G at 25 points (20 cm intervals) using a point quadrat for 19 plots per treatment, between 23 and 27 April. The data were analysed, using SAS, within blocks considered random and with fixed effects for defoliation treatment, grazing date and interactions.

**Results** The sward herbage dry matter mass at grazing was 2407 kg/ha. The species identified were *Lolium perenne* (54.5%), *Dactylis glomerata* (20.2%), *Phleum pratensis* (10.9%), *Holcus lanatus* (10.7%), *Alopecurus pratensis* (1%), *Cerastium fontanum* (1%) *Taracum officinale* (1%) and *Trifolium repens* (0.5%) *Poa annua* (0.1%) and *Rumex obtusifolius* (0.1%). The effects of defoliation treatment, grazing date and herbage allowance on sward botanical composition is presented in Table 1. Delaying defoliation either by clipping or grazing tended to decrease the content of *L. perenne*. Delaying grazing from mid December to mid January tended to increase ( $P=0.06$ ) *P. pratensis* content. Increasing herbage allowance at grazing increased *H. lanatus* and decreased *P. pratensis*. Removing herbage by grazing at the low herbage allowance rather than clipping (BvG) decreased *H. lanatus*.

**Table 1** Effects of defoliation treatment on grazing date on herbage composition (%) and yield

	Defoliation treatment (DT)				Defoliation date (D)			sig		Contrasts		
	Mowing		Grazing(G) allowance		Dec	Jan	Feb	DT	D	AvB	0.75 v 1.75	BvG
	Early Dec (A)	At grazing (B)	0.75	1.75								
<i>L. perenne</i>	71	61	62	57	67	62	59	$P=0.11$	NS	$P=0.06$	NS	NS
<i>D. glomerata</i>	16	18	21	27	18	21	22	NS	NS	NS	NS	NS
<i>P. pratensis</i>	9	9	16	6	6	11	12	*	$P=0.06$	NS	**	NS
<i>H. lanatus</i>	6	10	2	9	8	5	6	*	NS	NS	*	$P=0.07$
DM Yield (kg/ha)	3655	3349	3573	4071	3842	3517	3626	**	NS	$P=0.08$	$P=0.07$	

**Conclusion** Delaying defoliation either by clipping or grazing decreased the content of *L. perenne* primarily due to the effect of herbage mass rather than poaching during grazing. Consequently extended grazing has a negative impact on sward composition and subsequently reducing the reseeding interval.

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## Polymorphisms in the growth hormone and insulin-like growth factor 1 genes are associated with milk production, somatic cell count, and survival in Holstein-Friesian dairy cattle

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**Introduction** Milk production, udder health, fertility and survival represent a large proportion of the emphasis within international dairy cattle breeding objectives. In this study, we used a candidate gene approach to detect novel single nucleotide polymorphisms (SNPs) associated with performance. The growth hormone (*GH*) and insulin like growth factor 1 (*IGF1*) genes were chosen because of their known role in milk production and reproduction (Velazquez, 2008). The objective of this study was to discover novel SNPs in these genes and to quantify their association with production, somatic cell count, calving interval and survival in dairy cattle.

**Materials and methods** A panel of 22 cattle (four Belgian Blue crossbreds, four Charolais, four Simmental, four Aberdeen Angus crossbreds and six Holstein-Friesians) was selected for SNP discovery. Regions of the *GH* and *IGF1* genes encompassing both promoter and regulatory flanking sequences were PCR-amplified and sequenced. Sequence validation and *de novo* polymorphism detection was carried out using a combination of software packages including BLAST, ClustalW and Chromas. Discovered SNPs, as well as previously published SNPs, were then genotyped using iPLEX-Mass technologies (Sequenom Inc.) across 848 HF sires with progeny in Ireland. The association between each SNP and performance was quantified using weighted mixed models in ASREML (Gilmour *et al.*, 2009) with genotyped individual included as a random effect, and average expected relationships among individuals accounted for. Year of birth (divided into five yearly intervals) and percent Holstein of the individual sire were included as fixed effects in the model. In all instances the dependent variable was daughter yield deviation for milk yield, fat yield and percent, protein yield and percent, and somatic cell score (SCS) and de-regressed PTA for calving interval and functional survival, weighted by their respective reliability less the parental contribution. Genotype was included in the model as a continuous variable. A multiple regression model was developed for each gene separately by backward elimination of non-significant ( $P > 0.05$ ) SNPs.

**Results** Sequence analysis of ~ 13 kb across these two genes identified; 44 *de novo* SNPs in the *GH* gene and nine *de novo* in the *IGF1* gene. Significant associations were evident between both novel and previously published SNPs in both genes with performance (Table 1). For example: in the *GH* gene, a C to T substitution at *GHr17*, was associated with increased milk fat and protein composition of 0.03% and 0.02%, respectively; and in the *IGF1* gene for example, a substitution of the C allele with a T allele at *IGF1r10* was associated with increased functional survival of 0.79 %. The *IGF1* SNP *rs2901285* has previously being associated with carcass traits in cattle; however, this study has also shown its association to milk fat composition, additionally four novel SNPs were identified across both genes with associations to milk production traits.

**Table 1** Associations (standard errors in parenthesis) between SNPs in the *GH* and *IGF1* genes and performance

Gene	SNP <sup>a</sup>	dbSNP	Allele	Fat yield (kg)	Protein yield (kg)	Fat % (*100)	Protein % (*100)	SCS (log <sub>e</sub> *100)	Survival (%*100)
GH	<i>GHi36</i>	<i>rs41923523</i>	C → T					2.03 (0.62)	-29.54
	<i>GHi24</i>	<i>de novo</i>	C → T					1.54 (0.64)	
	<i>GHe5</i>	<i>rs41923484</i>	C → G		-0.85 (0.42)				
	<i>GHr17</i>	<i>rs41923483</i>	C → T			3.35	1.54		
	<i>GHr19</i>	<i>rs41923481</i>	C → T	-0.98					
IGF1	<i>Igfl13</i>	<i>de novo</i>	A → G	-2.17	-1.31 (0.61)			-2.90 (1.30)	
	<i>Igfl14</i>	<i>rs29012855</i>	A → G			-3.07			
	<i>Igfl16</i>	<i>de novo</i>	A → G	-2.16					
	<i>Igfl1r10</i>	<i>de novo</i>	C → T						78.56

<sup>a</sup> :*IgflrX* = SNP X located downstream i.e. 3' of the *IGF1* gene, *GHi/eY* = SNP Y located within an intron/exon of the *GH* gene.

**Conclusion** SNPs were identified in both genes that were associated with traits of economic importance substantiating previous research that reported a role of GH and IGF-1 in milk production. Further sequencing would be required to identify the any additional SNPs located in both the *IGF1* and *GH* genes, and functional genomic studies undertaken to determine causation.

**Acknowledgements** The authors acknowledge the Irish Cattle Breeding Federation, the Irish Dairy Research Trust, DAFF Food Institutional Research Measure and DAFF Research Stimulus Fund.

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## Functional characterization of the promoter region of bovine Neuropeptide Y (NPY) gene

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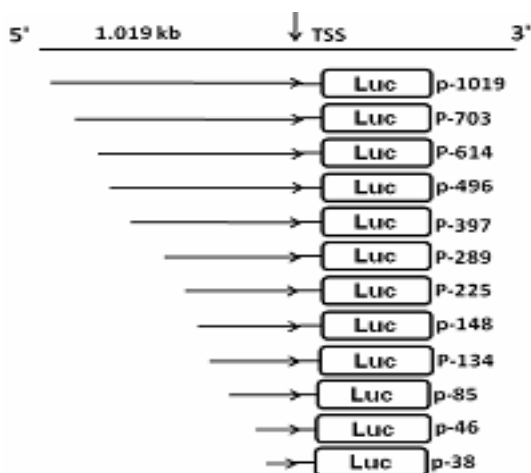
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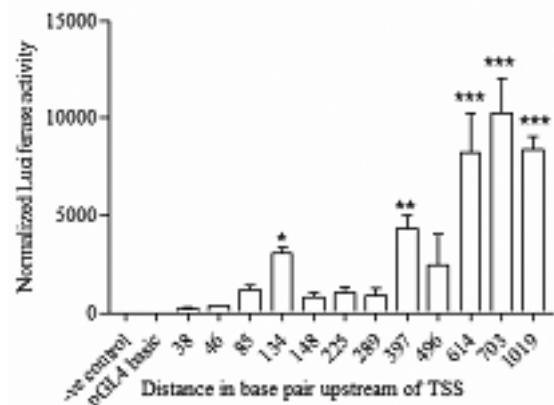
**Introduction** Neuropeptide is a mammalian neurotransmitter produced by the brain which plays a vital role in energy homeostasis of mammals, including the bovine (Kaiyala *et al.*, 1995). Neuropeptide Y (NPY) is a potent orexigenic agent that increases appetite and feed intake, and therefore is a potential candidate for enhancing feed energy utilization in cattle (White, 1993). Single nucleotide polymorphisms (SNPs) characterized in the promoter region of the bovine NPY gene (Bahar and Sweeney, 2009) have potential for genetic selection of animals with high feed energy efficiency. However, the molecular mechanisms underlying the regulation of bovine NPY gene expression by its promoter region is currently unknown. The objectives of this research were to characterize the transcriptional activities of the bovine NPY gene and identify the minimal promoter required for basal activity of NPY gene *in vitro*.

**Materials and methods** For identification of minimal promoter of the bovine NPY gene, -1019 kb to +1 (reference to the transcription start site) region of the bovine NPY gene (GeneBank: AY491054) was targeted. Promoter deletion constructs of 38-1019 nt length (Figure 1) were made in a firefly luciferase expression vector system (pGL 4.17, Promega Corp.). The *in vitro* promoter transcription efficiency of the promoter constructs were performed using a mouse 3T3-L1 host cells system. Firefly luciferase activity in the cell lysate was measured using a luminometer. Total protein concentration in the cell lysate was estimated using the BCA protein assay kit (Thermo Scientific). Firefly luciferase activity was normalised against the total protein. Data was analysed using the GraphPad prism 5 and are reported as the mean of the three independent assay  $\pm$  standard error of the mean.

**Results** The *in vitro* transcriptional activities of the different deletion constructs of the bovine NPY promoter are shown in Figure 2. It is evident from the promoter activity data that there was an increase in the promoter activity with increase in the promoter length upto -134 nt. However, there was a substantial decrease in the promoter activity for the promoter length -148 to -289 nt indicating the presence of suppressor elements in this region. Again, promoter activity increases upto -703 nt. Taken together, these results suggested that the sequence up to -134 nt are sufficient for basal promoter activity, however, for the maximal promoter activity the region upto -703 nt is essential.



**Figure 1** Schematic representation of the 12 bovine NPY promoter deletion constructs. The number (eg. p-1019) indicates the relative nucleotide position from the transcriptional start site (TSS; Position +1) and hence the construct size (eg. 1019n).



**Figure 2** Luciferase activity of the bovine NPY promoter deletion constructs. Luciferase activity was normalized against total protein. Data points shown are the means  $\pm$  SE.  $P < 0.05$  considered significant

**Conclusion** *In vitro* promoter activity of the deletion constructs ranging from 38 nt to 1019 nt of the bovine NPY promoter demonstrated that a promoter length up to -134 nt is sufficient for the basal promoter activity, while a length up to -703 nt is actually required for maximal promoter activity. The SNPs present in the various regions of the bovine NPY promoter especially those affecting the transcription factor binding sites and those in the enhancer and repressor regions have the potential to affect neuropeptide Y mediated feed energy utilization in cattle.

**Acknowledgements** This research was supported by a Teagasc Walsh Fellowship.

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## Associations between SNPs in the bovine GnRH receptor gene and breeding values for fertility traits in dairy cattle

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**Introduction** Subfertility is an increasingly important problem in dairy cattle. An unfavourable genetic correlation between fertility and milk yield (Royal *et al.*, 2002) means that losses in breeding values for the former accompany gains in the latter. We have investigated whether single nucleotide polymorphisms (SNPs) in the GnRH receptor (GnRHR) are associated with fertility in dairy cattle, using predicted transmitting abilities (PTAs) for fertility traits available through the UK fertility index (Wall *et al.*, 2003).

**Materials and methods** Using a panel of 54 sires, DNA was isolated from semen by phenol-chloroform extraction, and from whole blood using a modification of the Puregene DNA Purification Kit (Gentra) based on sequential precipitation of protein and DNA with a phenol/chloroform extraction step. The coding sequence of bovine GnRHR (NW\_001495209.1 | Bt6\_WGA699\_4) spanning 3 exons and a fragment of the GnRHR gene promoter region were amplified from 100 ng aliquots of genomic DNA in 35 PCR cycles with high fidelity DNA polymerase (Accuzyme Mix, Bioline). PCR products were purified and sequenced (Beckman CEQ8000 Sequencer). A total of 481 bulls of known parentage were subsequently genotyped by primer extension; however not all were genotyped at every locus. The 7 SNPs found in the GnRHR gene were analysed to determine the minimum number of segregating haplotypes (Schouten *et al.*, 2005). Genotype associations with PTAs for calving interval (CI), days to first service after calving (DFS), non-return rate at 56 days after first insemination (NR56), number of inseminations required to achieve conception (CINS), 305 day milk yield (305 MY), and an index of milk yield (Profit Index, PIN) were tested by REML in GENSTAT. PTAs for CI, DFS, NR56, CINS and PIN were calculated in 2004; values for CI, NR56 and 305 MY only were also derived in January 2009 (data available for 399 of the same bulls). The multivariate linear mixed model fitted was:  $y_{ijkl} = \mu_i + \alpha_{ij} + \beta_{ik} + \varepsilon_{ijkl}$ , where  $y_{ijkl}$  is the value of trait  $i$  for son  $l$  of sire  $j$  inheriting haplotype  $k$ ;  $\alpha_{ij}$  is the effect of sire  $j$  for trait  $i$ ;  $\beta_{ik}$  is the effect of haplotype  $k$  for trait  $i$ ; and  $\varepsilon_{ijkl}$  is the multivariate residual error. As PTAs were not selected for reliability, they were deregressed to account for variation in reliabilities of estimates.

**Results** Seven SNPs were identified in the GnRHR gene. Relative to the translation initiation codon these were at -331 (A>G), -108 (T>C), +206 (G>A), +260 (C>T), +341 (C>T), +383 (C>T) and +410 (C>T), the most frequent allele being given first. All the SNPs in exon 1 were silent. The SNPs at +206 and +383, and those at -108, +260, +341 and +410 formed two groups with complete linkage disequilibrium within groups but incomplete linkage disequilibrium between groups. Therefore, -331, -108 and +206 were selected as tag SNPs for haplotype and association analysis. The following 5 haplotypes were sufficient to explain the genotypes (maximum likelihood estimates of frequencies in parentheses): ATG (0.692), ATA (0.013), GCG (0.145), ACA (0.101) and ACG (0.048), SNPs being referred to by the most 5' position in each group (i.e. -331A>G, -108T>C and 206G>A), and identified by the nucleotides at each position (i.e. A-T-G). All loci were in Hardy-Weinberg equilibrium. The -108T>C alteration was associated with beneficial effects on fertility (Table 1) after accounting for effects of sire (fitted as a random effect), particularly with CI and DFS (reductions in PTAs of  $0.43 \pm 0.203$  and  $0.43 \pm 0.130$  days,  $p < 0.03$  and  $< 0.001$  respectively). The association with DFS (but not with CI) remained significant ( $p < 0.01$ ) when sire and PIN were both accounted for, suggesting that the association with fertility was not mediated solely through milk yield. The association with CI remained significant after deregression with ( $p = 0.01$ ) or without ( $p < 0.05$ ) accounting for 305 MY. There were no significant associations with other fertility traits, and no effects of the SNPs at positions -331A>G or +206G>A.

**Table 1** Estimated effects of the linked allelic substitutions ( $\alpha$ ) at -108/+260/+341/+410 on fertility breeding values.

Model	CI (days)			DFS (days)			NR56 (%)			CINS (number)		
Benefit:	Decrease			Decrease			Increase			Decrease		
	$\alpha$	s.e.	P	$\alpha$	s.e.	P	$\alpha$	s.e.	P	A	s.e.	P
Sire+SNP	-0.86	0.40	<0.03	-0.86	0.26	<0.001	-0.11	0.23	NS	-0.000	0.005	NS
Sire+PIN+SNP	-0.67	0.44	NS	-0.58	0.27	<0.01	-0.10	0.24	NS	-0.002	0.006	NS

Table shows analysis of genotypes of 406 bulls using REML (multivariate linear mixed model) with sires accounted for as a random effect. P indicates the significance of the Wald test. Note PTAs have been doubled to show effects on breeding values.

**Conclusions** Selection against the GnRHR ATG and ATA haplotypes will improve fertility.

**Acknowledgements** We thank Mike Coffey and Raphael Mrode for access to fertility PTAs. Sausan Ahmad received a scholarship from the Department of Education of Syria through the British Council. Supported by Defra Link grant LK0639.

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## Associations between leptin polymorphisms and performance traits in Holstein-Friesian dairy cattle

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**Introduction** Leptin modulates appetite, energy expenditure and the reproductive axis by signalling the status of body energy stores to the brain. Following parturition, cows enter negative energy balance (NEB), and mobilise body reserves in support of mammary milk synthesis. During early lactation NEB, adipose expression of leptin and circulating leptin concentrations are profoundly reduced. The reduction in leptin is likely involved in coordinating the neuroendocrine adaptations necessary to prioritise milk production over other physiological processes that are considered, temporarily at least, nonessential (e.g. reproduction, immune function). The aim of the present study was to quantify the associations between 9, novel and known, single nucleotide polymorphisms (SNPs) in the promoter and coding region of the leptin gene with performance traits in Holstein-Friesian dairy cattle in Ireland.

**Materials and methods** Genomic DNA was isolated from semen straws of 848 Holstein-Friesian sires with progeny in Ireland. For SNP discovery, ~3 kb of the regulatory region was amplified by polymerase chain reaction (PCR) from genomic DNA of 14 sires using six pairs of oligonucleotide primers designed based on the published sequence (GenBank Acc. No. AB070368). PCR amplicons were sequenced bi-directionally by Lark Technologies. Genotyping analysis was performed by Sequenom® using the iPLEX Gold assay on a MassARRAY® Platform. Associations between genotypes and performance were quantified using weighted mixed models with genotyped individual included as a random effect and average expected relationships among individuals accounted for; genotype was included as a continuous effect. The dependent variable was daughter yield deviation for milk production traits and de-regressed predicted transmitting ability for calving interval, functional survival, body condition score (BCS) and angularity. Weighting on the dependant variable was based on the reliability of the proof less the parental contribution. Multiple regression models were developed by backward elimination of non-significant ( $P > 0.05$ ) SNPs.

**Results** All SNPs were segregating in this sample population and none deviated ( $P \geq 0.05$ ) from Hardy-Weinberg equilibrium. Because of the strong linkage disequilibrium between phases among the SNPs LEP-1457, LEP-1609 and LEP-580, only LEP-1457 was retained for the association analysis. The T allele in LEP-2470 was associated ( $P < 0.05$ ) with reduced milk protein concentration and showed a tendency to associate with increased ( $P < 0.10$ ) milk yield (Table 1). The G allele of LEP-1239 was associated with reduced milk fat and protein concentration and tended to be associated with increase somatic cell score (SCS). LEP-963 demonstrated an association with milk fat %, milk protein % and a tendency to associate with milk yield. The T allele of Try7Phe, as well as being associated with protein yield (Table 1) was associated with reduced angularity (allelic substitution effect = -0.74 standard deviation units; SE = 0.23) and tended to be associated with BCS. However the minor allele frequency for TryPhe was 7% with no homozygous TT animals present in the data set. When a multiple regression model was developed the inclusion of the most significant SNP negated the inclusion of any other SNP indicating that the significance of several SNPs in the univariate analyses was an artefact of linkage with a possible causative mutation or mutations. There was no association between LEP-1457 and calving interval or survival.

**Table 1** Allele substitution effect (standard error in parenthesis) between seven SNPs and milk performance traits

SNP	Allele substitution	Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Fat (%*100)	percentProtein (%*100)	percentSCS (units*100)
LEP-2470	C → T	23.61 (14.29) <sup>†</sup>	0.05 (0.51)	0.20 (0.40)	-1.72 (1.08)	-1.14 (0.52)*	0.06 (0.96)
LEP-1457	A → G	-7.73 (9.16)	0.16 (0.32)	0.00 (0.25)	0.72 (0.69)	0.45 (0.33)	-0.97 (0.61)
LEP-1239	C → G	14.57 (9.87)	-0.38 (0.35)	0.03 (0.27)	-1.59 (0.73)*	-0.80 (0.35)*	1.06 (0.65) <sup>†</sup>
LEP-963	C → T	-16.29 (9.62) <sup>†</sup>	0.27 (0.34)	-0.15 (0.27)	1.54 (0.72)*	0.73 (0.35)*	-0.79 (0.64)
Tyr7Phe	A → T	-39.19 (22.70) <sup>†</sup>	-1.11 (0.80)	-1.25 (0.63)*	0.56 (1.70)	-0.07 (0.83)	-1.52 (1.53)
Arg25Cys	C → T	-13.32 (9.61)	0.36 (0.34)	-0.07 (0.27)	1.52 (0.72)*	0.71 (0.35)*	-1.00 (0.64)
Ala80Val	C → T	7.97 (10.55)	-0.12 (0.38)	0.21 (0.29)	-0.73 (0.79)	-0.09 (0.39)	1.05 (0.70)

Significance of difference from zero <sup>†</sup> =  $P < 0.10$ ; \* =  $P < 0.05$

**Conclusions** In summary, 3 SNPs in the promoter region and one SNP in the coding region significantly associated with milk production traits. All 3 of the promoter SNPs lie within putative transcription factor binding sites. In addition, Tyr7Phe associated with angularity. Previous reports have found associations between both leptin polymorphisms and milk production traits. Interestingly none of the SNPs, including LEP-1457, associated with calving interval in our data set even though previous work had found an association with LEP-1457 and first postpartum luteal activity (Leifers *et al.* 2005).

**Acknowledgements** The authors acknowledge the Irish Cattle Breeding Federation, the Irish Dairy Research Trust and DAFF Research Stimulus Fund. We thank Dr. David Magee for technical assistance.

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## Associations between single nucleotide polymorphisms in the leptin gene with body measurements and IGF-I in UK dairy heifers

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**Introduction** Appropriate growth of dairy heifers is important to the dairy industry as it affects their future fertility and longevity. Growth is controlled by a complex series of interactions involving the somatotrophic axis, including growth hormone (GH) and insulin like growth factor-I (IGF-I). Leptin, produced primarily by adipose tissue, plays a key role in energy homeostasis and has also been implicated in controlling bone growth through both direct and indirect actions (Gat-Yablonski and Phillip, 2008). Growth can be analysed by measuring size traits such as weight and height. Juvenile height is a useful indicator of skeletal development and is also related to adult stature. These traits can be affected by single nucleotide polymorphisms (SNPs) in the effective genes. This study investigated the associations between four SNPs with size traits and circulating IGF-I concentrations. The SNPs included were: UASMS1 and UASMS2 (both located in the leptin gene promoter region, Nkrumah *et al.*, 2005); Exon2FB (located in exon 2 of the leptin gene, Buchanan *et al.*, 2002) and BGHR (located on exon 8 of the bovine growth hormone receptor; Blott *et al.*, 2003).

**Materials and methods** Holstein-Friesian heifers (n = 449), born between August 2003 and October 2004 on 18 commercial UK dairy farms and 1 primarily research farm (with 3 groups) were recruited for this study. Each heifer was measured aged approximately 1 month ( $28 \pm 0.8$ d), 6 months ( $184 \pm 0.8$ d, pre-pubertal) and 15 months ( $452 \pm 3$ d, post pubertal at start of service period). Weight, height at withers (HT), crown rump length (CRL) and heart girth diameter were recorded. Blood samples were collected. Whole blood was spotted onto Whatman FTA cards (Whatman International Ltd, Maidstone, UK) for subsequent DNA extraction. Genotyping was performed by Orchid Cellmark (Abingdon, Oxford, UK). Plasma was harvested for measurement of IGF-I by OTEIA IGF-I plate kits (Immunodiagnostic Systems Ltd, Tyne and Wear, UK). Mixed model association analysis was used to determine any significant associations between SNP genotypes and the individual traits measured at 1, 6 and 15 months. Animals were grouped according to their herd (1 to 21), year (2003 or 2004) and season (1 = March to May, 2 = June to August, 3 = September to November and 4 = December to February) of birth. Age was fitted as a 4<sup>th</sup>-order polynomial. SNP was fitted as a fixed effect with 3 levels. All known pedigree information for the preceding 3 generations for each heifer was included (n=2251 animals) and a heifer permanent environmental effect was fitted to account for repeated measurements on each animal. Analyses were performed with ASREML v2.0.

**Results** The phenotypic measurements of the heifers at each age are summarized in Table 1 and the genotype frequencies are reported in Table 2. Allele frequencies of all SNPs were distributed according to the Hardy-Weinberg equilibrium expected values. UASMS1 and Exon2FB were in close linkage disequilibrium ( $r^2 = 0.98$ ).

**Table 1** Phenotypic measures, mean  $\pm$  SE (n = 449)

Age	1 month	6 months	15 months
Weight (kg)	$56 \pm 0.7$	$175 \pm 1.7$	$373 \pm 2.4$
HT (cm)	$80 \pm 0.2$	$104 \pm 0.3$	$126 \pm 0.3$
CRL (cm)	$94 \pm 0.4$	$135 \pm 0.5$	$169 \pm 0.5$
Girth (cm)	$89 \pm 0.4$	$131 \pm 0.4$	$174 \pm 0.5$
IGF-I (ng/ml)	$42 \pm 1.3$	$86 \pm 1.8$	$105 \pm 1.5$

**Table 2** SNP genotype frequencies

SNP	CC	CT	TT
UASMS1	0.17	0.48	0.35
UASMS2	0.74	0.24	0.02
Exon2FB	0.35	0.48	0.17
	TT	TA	AA
BGHR	0.65	0.31	0.04

The only significant associations of SNPs with the traits measured related to HT. UASMS1 had a significant association with HT ( $P < 0.05$ ) throughout the study period. Animals with a TT allele were on average 0.85cm and 1.10cm taller at 1 and 15 months respectively than CC or CT animals. Exon2FB and BGHR were also weakly associated with HT: Exon 2FB,  $CC > CT = TT$ ,  $P < 0.05$  at 15 months only; BGHR,  $TT = AT > AA$ ,  $P < 0.05$  at 6 months only. No associations were found with UASMS2.

**Conclusion** Leptin SNPs have previously reported associations with milk production, body condition score (BCS), fertility, energy balance and protein yield in adult dairy cows. For example, cows carrying the CT genotype of Exon2FB had a lower BCS in early lactation, whereas homozygous CC cows produced less milk (Chebel *et al.*, 2008). We show here that leptin SNPs are also associated with juvenile height measurements. The tallest heifers carried the T allele of UASMS1 and the C allele of Exon2FB. This provides further evidence that leptin SNPs are informative for marker assisted selection, as they relate to both growth and productivity.

**Acknowledgements** The authors gratefully acknowledge the support of Merial Animal Health Ltd and BBSRC.

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## Bovine lactoferrin promoter polymorphisms associate with reproductive performance and somatic cell count in Holstein-Friesian cattle in Ireland

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**Introduction** Lactoferrin plays an important role in the innate immune system, with well characterized anti-bacterial, anti-viral and immune modulatory properties. Lactoferrin gene expression is both constitutive and inducible and is species-, tissue- and cell-type specific. The objective of this study was to determine the allele and haplotype frequency of polymorphisms at positions -586, -190 and -28 of the bovine lactoferrin promoter in Holstein-Friesian sires and to quantify their association with performance.

**Materials and methods** Genomic DNA was isolated from semen straws of 848 Holstein-Friesian sires with progeny in Ireland. Genotyping analysis was performed by Sequenom® using the iPLEX Gold assay on a MassARRAY® Platform. Associations between genotypes and performance were quantified using weighted mixed models with genotyped individual included as a random effect and average expected relationships among individuals accounted for through a numerator relationship matrix; genotype was included as either a continuous or class fixed effect. The dependent variable was daughter yield deviation for milk production traits and de-regressed predicted transmitting ability for calving interval and functional survival.

**Results** The genotypes of Lf-586 and Lf-28 SNPs deviated ( $P<0.001$ ) from Hardy-Weinberg equilibrium, with a marked deficiency in the TT and CC genotype respectively. The minor allele frequency of Lf-586, Lf-190 and Lf-28 was 0.14, 0.21 and 0.26, respectively. None of the three SNPs investigated were associated with milk, fat or protein yield or milk fat and protein concentration, although when included as a class effect in the model, Lf-28 was associated ( $P<0.05$ ) with milk yield and protein yield. The strength of the association between the SNPs and the non-production traits differed by SNP and model fit (i.e. genotype included in the model as either a continuous variable or a class variable). The C to T allele substitution of the Lf-586 SNP associated with shorter calving interval ( $P=0.03$ ) but increased somatic cell score (SCS) ( $P=0.06$ ) (Table 1). Interestingly in a preliminary study, homozygous TT cows tended to have lower lactoferrin protein concentration in their milk over a lactation curve than their CC herd mates. In addition, transfection studies in mammary epithelial cells showed that a promoter variant which included the T allele had decreased transcriptional activity *in vitro* compared to a variant with the C allele. The G to A allele substitution of the Lf-190 SNP was associated with longer calving interval ( $P=0.01$ ) and reduced functional survival ( $P=0.02$ ) (Table 1). The CC genotype of Lf-28, which associated ( $P<0.10$ ) with superior functional survival, also associated ( $P=0.02$ ) with greater SCS. A multiple regression model that included all three SNPs indicated that the associations between both Lf-586 and Lf-190 and calving interval were additive. The strength of the association with calving interval for Lf-586 ( $b=-0.98$ ;  $SE=0.45$ ) and Lf-190 ( $b=0.68$ ;  $SE=0.31$ ) was similar in strength to the univariate analyses. Five haplotypes were reconstructed (posterior means of the frequency in parenthesis) in the sequence of Lf-586, Lf-190 and Lf-28: CGA (53%), CAA (21%), TGC (13.5%), CGC (12%) and TGA (0.5%). Linkage disequilibrium ( $r^2$ ) between Lf-586 and Lf-190, between Lf-586 and Lf-28 and between Lf-190 and Lf-28 was 0.04, 0.44, and 0.09 respectively. Association analysis indicated that sires with the  $T_{-586}G_{-190}C_{-28}$  haplotype had improved genetic merit for calving interval and survival compared to  $C_{-586}A_{-190}A_{-28}$  haplotype.

**Table 1** Association between performance variables (somatic cell score, calving interval and functional survival) and (a) replacing a C allele with a T allele in the Lf-586 SNP (standard error in parenthesis), and (b) replacing a G allele to an A allele in the Lf-190 SNP (standard error in parenthesis)

Trait	Lf-586	P-value	Lf-190	P-value
	C to T allele substitution		G to A allele substitution	
SCS ( $\log_e$ units*100)	1.64 (0.86)	0.06	0.22 (0.65)	0.75
Calving interval (days)	-0.72 (0.33)	0.03	0.68 (0.28)	0.01
Survival (%)	0.27 (0.18)	0.13	-0.39 (0.16)	0.02

**Conclusions** The C to T polymorphism at -586, which distorts a putative activating protein 2 (AP-2) binding site, was associated with shorter calving interval and higher SCS. The G to A polymorphism at -190, located in a putative selective promoter factor 1 (SP-1) transcription binding site, was also associated with longer calving interval and decreased functional survival and the association with calving interval was independent of the association with Lf-586. Based on the data we proposed a haplotype combination that associated with improved reproductive performance in the Holstein Friesian breed. We hypothesized that the observable phenotypic associations to lactoferrin promoter polymorphisms can potentially be explained by allele specific differences in constitutive or inducible levels of gene expression. The lack of a pleiotrophic effect of the promoter SNPs studied on both fertility and milk production traits strengthens the importance of these SNPs, or at least the lactoferrin promoter, in selection for improved fertility.

**Acknowledgements** The authors acknowledge the Irish Cattle Breeding Federation, the Irish Dairy Research Trust, DAFF Food Institutional Research Measure and DAFF Research Stimulus Fund. We thank Dr. David Magee for technical assistance.

## Economic response that can be achieved from including genomic information to the terminal sire index of beef cattle

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**Introduction** Genomic selection (GS) utilises information about the association of large numbers of SNP markers located throughout the genome with phenotypic information. This has become feasible due to the availability of large numbers of SNP markers and the development of the bovine SNP chip. Selection index methods can be utilized for deterministic modelling of the potential benefits of including genomic information in genetic improvement programmes. The aim of this study was to investigate the benefits of applying genomic selection to the terminal sire index of beef cattle considering training population size and different breeding structures.

**Materials and methods** Selection index theory was applied to investigate the response of the beef terminal sire index under conventional selection or GS given the structure of the UK beef industry. Breeding objectives of the terminal sire index incorporate carcass weight, carcass fat score, carcass conformation score, gestation length and calving difficulty. Currently recorded traits include birth weight, weight at 200 and 400 days, muscle score, muscle and fat depth, gestation length and calving difficulty. Parameters were obtained from UK beef genetic evaluations and information for the breeding goal traits were obtained from Amer *et al.* (1998). Selection strategies were derived from the structure of the beef industry which was calculated from UK beef genetic evaluations data (Table 1). The effect of different breeding structures was investigated. This included selection based on young sires, where no progeny information is available, and selection based on older sires. Genomic information was included in the selection index model based on the theory of Dekkers *et al.* (2007). Accuracies of GEBVs are predicted based on trait heritability, number of phenotyped animals in the training population, the number of QTL underlying the trait and the effective population size ( $N_e$ ) (Daetwyler *et al.* 2008; Goddard 2009). In this research the effect of different training population sizes (1000 to 5000) and the effect of  $N_e$  of 100 and 500 were investigate.

**Table 1** Selection strategies based on the structure of the UK beef industry.

	Proportion of candidates selected		Generation Interval	
	Sires	Dams	Sires	Dams
Young sires	0.06	0.53	3.00	6.25
Industry average	0.04	0.53	4.55	6.25

**Table 2** Economic response (£) of different breeding programmes based on conventional selection and genomic selection.

	Young sires		Industry average	
	No GS			
	3.43		3.61	
	$N_e100$	$N_e500$	$N_e100$	$N_e500$
GS1 <sup>a</sup>	3.89	3.57	3.90	3.70
GS2 <sup>a</sup>	4.15	3.68	4.08	3.77
GS3 <sup>a</sup>	4.33	3.78	4.20	3.83
GS4 <sup>a</sup>	4.45	3.87	4.30	3.89
GS5 <sup>a</sup>	4.55	3.94	4.38	3.93

<sup>a</sup>GS1 to GS5 refer to training population sizes of 1000, 2000, 3000, 4000 and 5000 animals, respectively

**Results** The results of selection with and without

GS are shown in Table 2. Under conventional selection, the economic response is similar between the two breeding structures, however the slightly higher response (5%) when selecting older sires is due to higher accuracy of breeding values due to the availability of phenotypic records on more relatives. Including genomic information increases the economic response to selection in both breeding structures, however the magnitude of the response in comparison to no GS is higher when selecting younger sires (up to 33% increase) in comparison to the industry average (up to 21% increase) where selection is based on approximately 40% young sires (generation interval < 3) and 60% of older sires. The size of the training population influences the economic response that can be achieved when including genomic information, where the highest response is achieved with a training population of 5000. However, this is constrained by the breeding program structure and  $N_e$ . Larger training population sizes had more impact when younger sires were selected. Furthermore, the rate of economic response was higher for  $N_e$  of 100 than 500.

**Conclusions** The results show that there is potential benefit that can be achieved from including genomic information in selection programmes in beef cattle. The benefit that can be achieved is highest when selecting younger sires compared to older sires. This research outlines the importance of the training population size and  $N_e$  as these constrain the potential benefit that can be achieved. GS is expected to be of particular benefit for traits which have low heritability and are difficult to measure or are only available late in the animals life or are sex limited. Therefore, GS may facilitate the inclusion of further traits, such as residual feed intake, in breeding programmes which are important to the efficiency of beef cattle.

**Acknowledgements** The authors gratefully acknowledge funding from the Scottish Government and DEFRA. We would also like to acknowledge Peter Amer (Abacus Biotech, New Zealand) for providing the selection index program.

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## Changes in the genetic variance of a simulated dairy cattle population in the presence of a major gene

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**Introduction** Intense selection for quantitative traits has recently led to the unexpected appearance of new genes with major effects. Some examples of major genes affecting dairy traits are kappa-casein gene which has a significant effect on milk yield and milk protein. When having genes with large effects on the trait, in addition to polygenes, the truncation selection of animals with best polygenic and major breeding values affects the whole variances of the trait in population. In this research our aim was to study the rate of changes in different genetic and phenotypic variances under different levels of major gene contribution having the same parameters which may be seen in dairy cattle populations.

**Materials and methods** Stochastic simulation was applied to generate populations with 20 replicates. Breeding values of major gene were simulated depending on allelic frequencies and genotype of each individual. Phenotypic value for each animal was made using a model consisting fixed effects, polygenic and major gene breeding values and residual random effect. The inclusion of fixed effects in the model was same as the method of Abdel-Azim and Freeman (2002). The total phenotypic value was partitioned to 40% due to fixed factors and 60% due to random factors (Kuhn *et al.*, 1994). Of the 40%, due to fixed components, the herd effect accounted for 22% and the year effect for 6%. Other fixed factors accounted for the remaining 12%. The trait of interest was milk yield in cows having the phenotypic variance of 1200,000 and mean of 6500 kg. The base population was consisted of 5000 females and 50 males with overlapping generations. Maximum of five parities was assumed for each dam. The simulation was applied for 20 years of selection with three different polygenic heritabilities of 0.1, 0.3 and 0.5 respectively. For the major gene, there were three different initial frequencies of favourable allele ( $p=0.1$ ,  $p=0.3$  and  $p=0.6$ ), three different modes of inheritance including co-dominance, partial and complete dominance ( $d=0$ ,  $d=0.5$  and  $d=1$  additive value) and three different values for the magnitude of additive effect ( $a=0.1$ ,  $a=0.5$  and  $a=1$  phenotypic standard deviation). So there exist 81 different combinations for our simulation. The contribution of major gene was calculated as the ratio of genetic variance caused by major gene to total additive genetic variance. For each level of contribution the four variances (total breeding values, major gene, polygenic and phenotypic variance) were computed. The rate of changes in these four variances was calculated as the slope of regression line when each variance was regressed over years of simulation. Then, these coefficients were plotted against levels of contribution for each of four variances. Again new linear coefficients were found for variances, showing the trend of increase or decrease in rate of their changes. The linear and quadratic regression coefficients of year on each variance were derived under each combination and tested using T-test procedure.

**Results** There were different levels of major gene contribution ranging from 0.36% to 84%. As it is shown in Table 1, as level of contribution increases, the rate of changes in major gene variance reduces significantly. Results show that the rate of changes in polygenic variance does not differ significantly with the changes in levels of major genes contribution. For the total additive variance, the rate of changes for different levels of contribution is much denser in comparison to major gene variance. Also it can be shown that the rate of changes in phenotypic variance follows the same pattern of total additive variance. The reduction in the rate of changes and the divergence happens in the same way that total additive variance did.

**Table 1** Linear regression coefficients of rate of variance changes on levels of major gene contribution

Variance	Standardized linear regression coefficients
Total breeding values	-0.571 *
Major gene	-0.319 *
Polygenic	-0.169 <sup>n.s</sup>
Phenotypic	-0.574 *

**Conclusion** In general, as the level of major gene contribution in total additive variance increases, the rate of changes in genetic and phenotypic variances decreases. This means that at the low levels of contribution we may expect fast changes in genetic variances, but by an increase in frequency of favourable allele, level of dominance and magnitude of additive effect which leads to increase of major gene variance, changes in variances occur more slowly. So when there is a major gene in a population of dairy cattle with overlapping generations, the breeder may expect to have rapid changes in variances at low levels of contribution and so choose the best policy of utilizing these favourable variations as a tool of selection in his herd. But with increase in frequency of favourable allele of major gene which tries to reach to fixation and also increase of gametic phase disequilibrium, this variation begins to reduce but not as fast as it happened in early years of selection.

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## Effect of sodium selenite on male Sprague-Dawley rats exposed to sublethal dose of cadmium chloride

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**Introduction** Cadmium (Cd) is a dangerous occupational and environmental toxin which can affect livestock and human health. It accumulates in liver and kidneys of Cd exposed animals and humans. Cadmium half-life is about 10 years, so the symptoms of cadmium intoxication may occur several years after the exposure. Selenium (Se) is generally recognized as an important antioxidant with numerous protective biological functions. The principal role of Se is associated with the control of lipid peroxidation because this trace element is a component of selenoenzymes contributing to the antioxidant system. In spite of intense research during recent years, the role of this microelement needs further elucidation. Therefore this study examined the role of Se as Sodium selenite in minimising the harmful effects of Cadmium chloride in male Sprague-Dawley rats.

**Materials and methods** Following approval by the Quaid-i-Azam University Islamabad, Pakistan's Ethics Committee, twenty male Sprague Dawley rats (28 days old) were housed at the animal unit of this University. The rats were acclimatized to their housing and feeding for two weeks before they were weighed and distributed into four groups of 5 rats each with similar initial body weight (BW) per group. These rat groups were randomly housed in steel cages (38x23x10cm) which were maintained in a room at 25±2°C with dark to light cycle of 14 to 10 hours. A same commercial diet and fresh water was available *ad libitum* to these rats throughout this study. The rats were injected with subcutaneous doses (1mg /kg BW) of normal saline (Control), Cadmium Chloride (Cd group), Sodium selenite (Se group) and Cadmium chloride + Sodium selenite (Cd-Se group) on alternate days for four weeks of this study. All rats were then weighed at weekly intervals for four weeks before their sacrifice on 29<sup>th</sup> day, to collect their blood, livers and kidneys. The livers and kidneys were weighed and these weights were expressed as g/100g final BW. Cd and Se contents in blood, liver and kidneys were determined by atomic absorption and haemoglobin (Hb) in blood was determined by a spectrophotometer. The data were then statistically analysed as a completely randomised design with repeated measures for body weight by using ANOVA in Minitab software. The analysis compared the effect of above mentioned treatments on weekly BW, tissues and metals in different tissues at P<0.05. Tukey's test was used to compare treatments means at P<0.05.

**Results** Table 1 shows significant differences between different treatments for mean weekly BW from week 2 onwards and different tissues of rats (P<0.05). While the initial mean BW of rats was similar, it was lower for Cd (>0.05) but greater (P<0.05) for Se than the control group for most weeks. The Cd-Se rats showed more comparable BW to the control group. The mean liver and kidney weights and Hb were significantly lower in the Cd (P<0.05) than the Control, Se and Cd-Se groups (P<0.05) but these did not differ between the Cd-Se and Control groups (P>0.05). Table 2 shows that the Cd contents were many folds higher in blood, kidneys and livers for the Cd than the control group whereas it was below the detection limit in Se group. While Cd content in livers and kidneys for the Cd-Se group was many fold higher than the control group it was lower than the Cd group.

**Table 1** Weekly (W) mean BW (g), liver and kidney weights (g/100g BW) and Hb (g/dL) of rats for different treatment groups

	Control group	Cd Control	Se group	Cd-Se group	SEM
Initial BW	86.2	86.6	86.2	83.5	2.81
W1	111.2 <sup>ab</sup>	105.8 <sup>a</sup>	122.2 <sup>b</sup>	112.3 <sup>ab</sup>	3.00
W2	146.6 <sup>a</sup>	136.2 <sup>a</sup>	166.2 <sup>b</sup>	147.8 <sup>a</sup>	3.70
W3	185.4 <sup>b</sup>	170.6 <sup>a</sup>	218.6 <sup>c</sup>	192.8 <sup>b</sup>	3.84 <sup>**</sup>
W4	217.6 <sup>b</sup>	199.8 <sup>a</sup>	258.0 <sup>d</sup>	226 <sup>c</sup>	3.83 <sup>***</sup>
Liver	4.73 <sup>bc</sup>	4.08 <sup>a</sup>	4.85 <sup>c</sup>	4.69 <sup>b</sup>	0.06 <sup>***</sup>
Kidney	0.87 <sup>b</sup>	0.70 <sup>a</sup>	0.80 <sup>b</sup>	0.80 <sup>b</sup>	0.02 <sup>*</sup>
Hb	12.34 <sup>b</sup>	10.38 <sup>a</sup>	14.06 <sup>c</sup>	12.35 <sup>b</sup>	0.16 <sup>***</sup>

(Means with similar letters in columns did not differ significantly; \*= $P<0.05$ ; \*\*= $P<0.01$ ; \*\*\*= $P<0.001$ )

**Table 2** Mean Cadmium (Cd) and Selenium (Se) contents (mg/kg) and SEM in different rat tissues for different treatment groups

Tissues	Cadmium Contents					Selenium Contents				
	Control	Cd	Se	Cd-Se	SEM	Control	Cd	Se	Cd-Se	SEM
Blood	0.03 <sup>a</sup>	0.07 <sup>b</sup>	ND	0.10 <sup>c</sup>	0.002 <sup>***</sup>	3.73	2.93	2.90	2.40	0.30
Kidney	0.02 <sup>b</sup>	3.50 <sup>a</sup>	ND	3.05 <sup>a</sup>	0.17 <sup>***</sup>	2.60 <sup>b</sup>	3.70 <sup>c</sup>	3.40 <sup>bc</sup>	1.40 <sup>a</sup>	0.32
Liver	0.02 <sup>b</sup>	4.17 <sup>a</sup>	ND	3.71 <sup>a</sup>	0.172 <sup>***</sup>	2.92 <sup>a</sup>	2.42 <sup>a</sup>	2.42 <sup>a</sup>	1.13 <sup>b</sup>	0.31

(Means with similar letters in columns did not differ significantly; \*\*\*= $P<0.001$ )

**Conclusion** This study indicates that selenium in the form of Sodium selenite may have a protective effect against cadmium chloride induced toxicity in male Sprague-Dawley rats.

**Acknowledgements** Farhat Jabeen thanks Islamic Development Bank Jeddah, Saudi Arabia for funding this post-doctoral research, Professor Dr. Samina Jalali for providing research facilities at Quaid-i-Azam University, Islamabad, Pakistan and Dr. Sarwat Jehan, QAU, Islamabad, Pakistan for providing Sprague Dawley rats. Riffat Gillani and Noshaba Memon are acknowledged for their help and cooperation during animal trial at QAU, Islamabad, Pakistan.

## Prevalence of tuberculosis in cattle in the highland regions of Cameroon assessed by the tuberculin skin test

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**Introduction** Tuberculosis (TB) is an important zoonosis caused by bacteria of the *Mycobacterium tuberculosis* complex. *M. bovis* is virulent for cattle but can infect humans and cause disease and pathology similar to *M. tuberculosis*, which is virulent for man (Biet *et al.* 2005). The epidemiology of bovine TB is largely unknown in Central Africa but its occurrence and implications on livestock production and human health are evident. Politico-economic constraints and lack of attention on zoonotic TB is drastically preventing the “test and slaughter” strategy which has proved very effective in the developed world. The prevalence of human TB in Cameroon is high and increasing rapidly with the spread of the HIV/AIDS infection. Indications of bovine TB occurrence in Cameroonian livestock are based on post mortem or meat inspection findings. The highlands of Cameroon (3°30'-12°30'N & 8°-16°E) including the Western highlands (WHC) bordered to the northeast by the Adamawa Plateau (ADP) contribute over 60% of the 6 million cattle in Cameroon. Cattle keeping is integral to the socio-economic, cultural and religious activities in these regions. Transhumance, semi-intensive and other traditional systems, where animal graze on sparse vegetations with limited veterinary care, are common and less frequently the intensive system. Herders trek long distances with their herds, sharing grazing and overcrowded night enclosures and watering points usually heavily contaminated with animal and human wastes. These conditions do provide opportunities for the emergence and transmission of bovine TB and no on-station or pre-movement TB tests are carried out on the animals. The aim of this study therefore, was to determine the prevalence of bovine TB based on tuberculin skin test of cattle under various husbandries in the wide geographic and cattle producing highland regions of Cameroon.

**Material and methods** During the period of March to September 2009, the responses to comparative intradermal tuberculin skin (SICCT) test of 2853 cattle in 7 administrative areas of the WHC and ADP agro-ecological regions of Cameroon were investigated. Intradermal avian (2500IU/ml) and bovine (3000IU/ml) tuberculin purified protein derivative (PPD) injections (0.1ml) were used (Kazwala *et al.* 2001). Cattle keeping communities and owners were identified, listed and subjected to the random-number generation method to select cattle herds for the study. Cluster sampling was also used for within herd selection such as preventing recently calved cows (within 2 months post partum) and calves (>6 months) from being tested. The animals used were reared traditionally with or without transhumance, as well as in semi-intensive and intensive systems. The cattle tested were the indigenous zebu, upgraded and exotic breeds. The purpose of the study was explained to the farmers and animals were tested after an informed consent was given by the owner. The ages and breeds of the animals were provided by the farmers, otherwise determined as described earlier (Blench 1999; Turton 1999). Skin responses to Bovine PPD and Avian PPD individually were also assessed.

**Results** The prevalence of bovine TB in the highlands of Cameroon based on the responses to the tuberculin skin tests is shown in Table 1. Significantly higher ( $P<0.05$ ) skin responses were recorded in the WHC zone, among females and in adult / old ( $\geq 4$  years) cattle reared under the semi-intensive management. There were more skin positive upgraded/exotic cattle ( $P<0.05$ ), but positive reactors were spread over the study locations with 57.14% (46.56 – 67.72%) of the herds infected and particularly ( $P<0.05$ ) in the WHC. A strong association (Pearson  $\chi^2=2853$ ;  $P<0.001$ ) between skin reactions to bovine PPD and avian PPD was noted. Overall, 6.98% of the animals responded positively to both avian and bovine PPDs while 2.52% of the animals reacted only to bovine PPD and 1.86% only to avian PPD.

**Conclusion** Bovine TB is prevalent in Cameroon and is posing serious risk to public health. Also, many opportunities exist for the emergence of zoonotic TB in the study regions and necessitate further investigation into the modes of transmission and the link between human and bovine TB through molecular techniques.

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**Table 1** Prevalence of bovine TB by skin test reactors (Percentage, %; Standard Error, SE)

Variable	No tested herds	No of PPD reactors (%; SE)	No of Bovine PPD reactors (%; SE)
All animals	2853 (84)	82 (2.87; 0.31)	121 (4.24; 0.38)
WHC	2126 (61)	68 (3.2; 0.38)	104 (4.89; 0.47)
ADP	727 (23)	14 (1.93; 0.51)	17 (2.34; 0.56)
Upgraded /Exotic	381	14 (3.67; 0.96)	27 (7.09; 1.31)
Guadali	1308	20 (1.53; 0.34)	46 (3.52; 0.51)
Namchi	33	0 (0; 0.00)	1 (3.03; 2.98)
Red Bororo	484	33 (6.82; 1.15)	31 (6.4; 1.11)
White Fulani	647	15 (2.32; 0.59)	16 (2.47; 0.61)
Female	2211	67 (3.03; 0.36)	96 (4.34; 0.43)
Male	642	15 (2.34; 0.60)	25 (3.89; 0.76)
Age $\leq 2$	613	22 (3.59; 0.75)	17 (2.77; 0.66)
2 < Age $\leq 4$	868	26 (3; 0.58)	32 (3.69; 0.64)
4 < Age $\leq 6$	681	18 (2.64; 0.61)	39 (5.73; 0.89)
Age > 6	691	16 (2.32; 0.57)	33 (4.78; 0.81)
Extensive	1725 (52)	55 (3.19; 0.42)	58 (3.36; 0.43)
Intensive	138 (4)	2 (1.45; 1.02)	6 (4.35; 1.74)
Semi-intensive	990 (28)	25 (2.53; 0.50)	57 (5.76; 0.74)
Beef herds	2409 (69)	74 (3.07; 0.35)	109 (4.52; 0.42)
Dairy herds	444 (15)	8 (1.8; 0.63)	12 (2.7; 0.77)
Herds $\leq 40$	1368 (57)	34 (2.49; 0.42)	68 (4.97; 0.59)
Herds > 40	1485 (27)	48 (3.23; 0.46)	53 (3.57; 0.48)

## Bovine tuberculosis: molecular evolution of *Mycobacterium bovis* in the British Isles

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**Introduction** Bovine tuberculosis (TB) is a chronic disease of animals caused by infection with the slow-growing, obligate intracellular bacterium *Mycobacterium bovis*. It is the most complex and difficult multi-species endemic disease currently facing government, the veterinary profession and the farming industry in the UK (Reynolds 2006). Despite sustained (and costly) implementation of eradication programmes since the 1950s, bovine TB has not been eradicated from either the UK or Ireland. Indeed, there has been a sustained and largely unexplained increase over the last 20 years in parts of the UK (Gilbert *et al.*, 2005).

In order to optimise control measures it is important to investigate the neutral and selective forces which have shaped the current epidemic and to investigate the influence of genetic variation in the pathogen (Smith *et al.*, 2006). Different classes of bacterial genetic marker, which inform on different evolutionary scales, have been identified recently in sequenced *M. tuberculosis* complex genomes (Gagneux and Small 2007). Deletions and SNPs are well suited to reconstructing the evolutionary history (phylogeny) of these clonal pathogens. This study was undertaken to investigate the molecular evolution of *Mycobacterium bovis* in the UK and Ireland. Knowledge of the evolutionary history and population structure of *M. bovis* in the British Isles could lead to the rational development of population-specific diagnostic tests, genotyping tools and vaccines.

**Materials and methods** Structured sampling of the *M. bovis* populations in Great Britain (GB), Northern Ireland (NI) and the Republic of Ireland (ROI) was undertaken (476 isolates in total: GB 126, NI 240, ROI 110). SNPs were genotyped by DNA sequencing and SNaPshot assays. Spoligotype- and VNTR-defined genotypes (Skuce *et al.*, 2005) were fitted to lineages along a linear phylogeny separating sequenced *M. bovis* BCG (France) and extant *M. bovis* (GB). We compared the phylogenies from GB, NI and ROI.

**Results** The backbone linear phylogenies of *M. bovis* isolates from GB, NI and ROI were very similar. The majority of extant GB, NI and ROI *M. bovis* isolates fitted close to the sequenced contemporary GB isolate, although some more ancient genotypes were evident, particularly in the ROI sample. GB, NI and ROI populations are now dominated by *M. bovis* of a particular spoligotype-defined lineage (the SB0140 clonal complex). However, each region appears to have developed its own unique genotypes within the SB0140 clonal complex.

**Conclusion** The populations of *M. bovis* in GB, NI and ROI are of the same major lineage and descend from a common ancestor. The *M. bovis* populations in each area could have been identical in the past (homogenised) and subsequently barriers to movement were established that allowed each population to evolve independently. These *M. bovis* populations continue to evolve over time and may not be the same as when eradication was nearly achieved in past decades. Traditional control methods, which were highly effective, may no longer be sufficient. In conjunction with cattle movement recording, the identification of region-specific *M. bovis* genotypes also informs outbreak investigations between and within these regions.

**Acknowledgements** The authors gratefully acknowledge funding from DARDNI (Project DARD0407) and DEFRA.

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## Dental abnormalities in horses

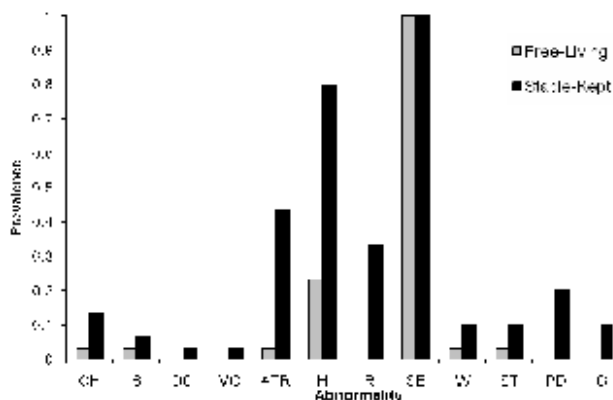
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**Introduction** The equine, free grazing at grass, will graze for up to 16 hours a day (Arnold, 1984), depending on seasonal daylight variations in a natural environment. When this time budget is compared to the eating times of modern domesticated and stabled equines, it is easily understood why domestication may adversely affect the essential attrition of horses' teeth. The specific aims of this study were to investigate and compare the prevalence of common abnormalities in the equine dentition between thoroughbred-type horses when stabled and when kept at grass. It is hypothesised that stable kept horses, fed a cereal based diet and therefore spending less time chewing, will have greater prevalence of dental abnormalities than free-living horses.

**Materials and methods** Two groups of 30 thoroughbred-type horses, aged 5-15 years, both mares and geldings, were used. Group one, 'free-living', were kept at a stock horse stud at Holbrook, New South Wales, Australia. These horses remained at pasture all year round with little human contact. Their diet comprised entirely of grassland. Group two, 'stable kept' were stabled in Gloucestershire, UK. They were stabled for the majority of the day on a bed of wood shavings with rubber matted floor and only removed from the stable for exercise once or twice a day. They were fed a diet consisting of a cereal based compound mix and a hay net twice a day. The two groups underwent a routine dental examination following British Equine Veterinary Association recommended procedures by the same person for both groups. A generic dental chart was completed, which monitored oral health and dental condition and recorded the occurrence of 12 recognised dental abnormalities. These abnormalities are recognised and defined by the British Association of Equine Dental Technicians (BAEDT, 2008) and include Sharp Edges (SE) and Accentuated Transverse Ridges of the occlusal surface of molar arcade (ATR) for example. A count of one was given for recognition of any and each abnormalities, regardless of severity. For example, a horse showing SE and ATR, was given a count of two. No horses had received any dental treatment for at least nine months prior to examination. The data were collected as part of a student's final year placement requirements, and were retrospectively analysed at the University. The difference in occurrence between groups was analysed using a Chi Squared test (Microsoft Office Excel 2003; Microsoft Corporation). The difference in total number of abnormalities per horse in each group was analysed using a Mann-Whitney Test (SPSS version 16; SPSS Inc.).



**Figure 1** The prevalence of each abnormality

**Results** There were clear numerical differences in the prevalence of all disorders except sharp edges (SE; see figure 1 which shows the prevalence of each abnormality, as a proportion of group size). All twelve abnormalities were observed in the stable kept group and the occurrence was greater than in free living horses, except in the case of SE ( $P=1$ ). Five of the twelve abnormalities were not observed in any free living horses. The difference in occurrence was confirmed to be significant in the case of ATR, Hooks (H) and Ramps (R) ( $P<0.001$ ). In total, significantly more dental abnormalities were observed in stable kept horses when compared to free living horses ( $p<0.001$ ).

**Discussion** The above observations are of importance as they add weight to the anecdotal argument that a modern equine lifestyle and diet, based on energy

dense cereals as opposed to forage, may adversely affect the dentition of the horse, by increasing the prevalence of dental abnormalities. This is particularly interesting when considered with the observation that there is no difference between the occurrence of sharp edges between free-living and stable-kept horses. Sharp edges are considered to be detrimental and would commonly be removed by an EDT during dental correction (Ralston et al., 2001). The result of our study potentially suggests that this particular abnormality is not induced solely by a lack of forage and may also suggest that further work is required to decide if routine removal of sharp edges is actually a necessity. This being said, there is clearly merit in preventing and removing such abnormalities that begin to cause pain and potentially lead to infection. A scoring system, that indicates the severity of an abnormality could be developed, incorporating a cut off point at which routine procedures are necessary. The stable kept horses were fed forage as part of their ration, so the effect of masticating this kind of material was not totally removed. However, when eating from a hay net, the teeth are not aligned in the same way in which they would be in a grazing horse and this may affect normal teeth attrition. It has also been suggested by Tell et al., (2008) that the use of a bridle can increase dental abnormalities, although in this particular study, ulceration was the focus.

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## Occurrence of Pulmonary Adenomatosis (Jaagsiekte) in sheep of animal research institute: the strategy of working with pulmonary adenomatosis in a flock of sheep

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**Introduction** OPA is a contagious, viral, neoplastic disease of the lungs of sheep (Palmarini *et al*, 1997)). It is a contagious tumour of sheep, a progressive respiratory disease, principally affecting adult animals. Two viruses, a herpes virus and a retrovirus (slow virus), have been associated with the disease, but only the latter has an aetiological role (Palmarini *et al*, 1999). Natural transmission seems to occur generally by the respiratory route. Close contact (e.g., at feeding troughs) may spread the virus (Palmarini *et al*, 1997). The tumours produce clinical signs when they become sufficiently large, or numerous enough to interfere with respiration. Affected sheep lose weight and show respiratory symptoms. Moist rales may be heard even without a stethoscope. Forced lowering of the head often causes frothy mucus to run from the nostrils. There is no specific treatment or vaccine available. The best is once the diagnosis confirmed and animals showing signs suggestive of pulmonary adenomatosis should be removed from the flock. A diagnostic test for the disease is by raising the hindquarters and lowering the head of affected sheep may cause frothy mucoid fluid to run from the nostrils. The objective of the present study was to monitor the cases of OPA in a sheep flock that consisted of 5 breeds and identify how long it will take to eradicate the disease from the flock.

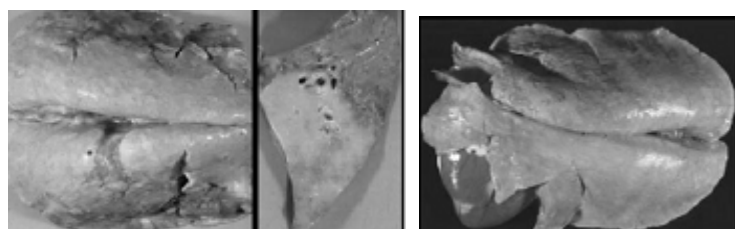
**Material and method** Every month the adult sheep were tested to find the suspected cases (for numbers of sheep see Table 1). Those which suffer from pulmonary distress such as difficult breathing, tachypnea, abdominal breathings, emaciated were examined thoroughly. The rear of affected sheep were lifted if excess fluid run from the nose, then it was diagnosed as an OPA case and slaughtered. In carcass inspection the lungs were examined for the lesions of disease and then sent to pathology lab for confirmation of the diagnosis. In post-mortem the lungs were enlarged (as much as triple their normal size) grey-yellow non collapsible (do not collapse when the thoracic cavity is opened) with rubbery consistency. In cross of lung parenchyma showing a meaty appearance mostly in ventral part of the lung. The mediastinal lymph nodes were enlarged as like a small tube. The lungs sink in water when submerged. The disease came under control after 4 years in this flock. In 5<sup>th</sup> year of this study no more cases was seen. The design was completely randomized with imbalanced replication and mean comparison was performed by Duncan's multiple comparison test.

**Results** As the data in Table 1 shows the merinos and its crosses have lowest susceptibility among imported and native breeds of sheep. Suffolk and kiosi breed with their crosses had highest susceptibility to the disease. These two breeds are high producer of meat and milk accordingly. There are not any significant differences between the susceptibility of native breeds.

**Table 1** Breeds, total no of sheep, no of sheep affected, and % of sheep affected

Breed of sheep	Total number of sheep examined in 5 years of study	Number affected during 5 year period	% of sheep affected
merinos and crosses	231	33	14.3 <sup>b</sup>
Kiosi and crosses	125	32	25.6 <sup>a</sup>
Suffolk and crosses	195	47	24.1 <sup>a</sup>
Mogani breed	33	7	21.2 <sup>ab</sup>
Shal breed	66	13	19.6 <sup>ab</sup>
Total	650	132	20.3

Values with different superscripts denote significance within column



## Conclusion

By selection of clinically positive cases and their slaughter, the disease appeared to be controlled after 4 years of the regime, whereas it disappeared in 5 years. Early diagnosis and slaughter at the early stages of the illness enables the carcass to be used for human consumption. If the disease is not diagnosed until the later stages, the sheep emaciate and the carcass should be condemned, which causes heavy losses to farmers. The positive relationship between high productivity of sheep (meat and milk production) and occurrence of this disease in this trial needs further investigation.

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## Challenges, problems and solutions encountered when initiating the Yorkshire and Humberside health pig scheme

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**Introduction** The Yorkshire and Humberside Pig Health Scheme (YHH) is an industry-led initiative aimed at finding and mapping all pigs within the region and identifying their endemic disease status as a means to improve both pig health and productivity. This initiative has chosen four diseases to be targeted; enzootic pneumonia (EP), porcine reproductive and respiratory syndrome (PRRS), swine dysentery and mange which have been prioritised over other diseases due to their economic impact, the feasibility of control and the diagnostic tests currently available. Due to the potential size of the initiative it has been split into two stages, planning in stage one which is expected to take around a year and action/implementation in stage two which will continue for several years thereafter. The scheme aims to create a 'map' of all pig farms currently in production and provide a health status based upon the presence or absence of these four diseases. Farms will be placed into geographic 'clusters' and will manage their own cluster, in order to make it 'disease free'. This will be achieved in various ways including increased biosecurity, tracking the movements of all livestock and anything affiliated with the farms that could potentially contaminate their farms and cause a disease outbreak.

**Materials and methods** Within stage one 'cold calling' of producers taken from known database lists, publications in the local press, on the internet and word of mouth have all been employed to increase publicity and encourage involvement in the YHH. A Steering Committee has been created in order to achieve the goals of the YHH whilst also providing the tools to producers so they can reduce production costs by £8 a pig (<http://www.pigworld.co.uk/Pages/Y&HHIS.html>). The Committee represents all involved in the industry as it involves producers, specialist pig-vets, relevant allied trades and support from BPEX; it meets on a regular basis to discuss where the scheme is heading, any problems encountered and what still needs to be achieved. Parallel to this a vet pilot scheme is also running in order to establish the reliability of the diagnostics currently in use, to determine disease protocols for the scheme and as an additional way of gaining support from producers. Stage two protocols will be determined depending upon the success of the stage one vet pilot scheme protocols.

**Results** Foremost the scheme has made a promising start with 50 producers already involved accounting for approximately 60% of all pigs in the region (personal communication, Steering Committee Meeting). However, it is proving difficult to locate all the remaining pigs due to inaccuracies in current databases and the fact that small hobby farms are generally not interacting regularly with any authorities. If these farms remain hidden it means the initiative may be compromised, although the level of risk posed by small units has yet to be accurately estimated. Secondly, some producers have proved remarkably obstinate and have avoided involvement in the scheme. This could potentially jeopardise the initiative, for example having an infected farm within a 'cluster' not involved in the scheme increases the risk of a breakdown on the other units. Even when given the correct contact information of the producers, those that are contacted are often unavailable or unwilling to involve themselves; primarily due to insecurities over the disclosure of their farms health status or a lack of time. The vet pilot scheme has had further problems, primarily in validating the diagnostics used. For example, some farms have been reluctant to be involved with this stage of the pilot due to the perceived level of high inaccuracies in the PCR tests undertaken to determine the presence of swine dysentery on a farm and because of the implications that would follow a false positive result. Another, considerably large problem has been finding an appropriate way to obtain blood samples. When taken from a live pig the sample collection needs to be carried out by a vet, this is expensive and not always possible. The alternative option is taking blood from a dead pig in the slaughter house, however for reasons of health and safety it has proven very difficult to gain access here; this is yet to be resolved. Whilst working on the scheme however one of the most obvious problems is concisely defining any issues with implementing the scheme and efficiently finding solutions for them. Although it is imperative that all involved in pig production are represented when designing the initiative, this can sometimes lead to a larger amount of discussions and not enough actions. Because of these problems in this area the scheme will struggle to make its original targets in the time span first suggested. However, considerable progress has been made in generating producer and veterinary interest in improving health status, rationalising databases and in the application of GIS to generate the first ever map of where pig units are in the region.

**Conclusion** This pilot scheme for a regional multi-disease eradication scheme will as it progresses hopefully demonstrate its value in aiding the British Pig Industry to remain internationally competitive in today's market. However it also demonstrates the difficulties when creating a scheme of this magnitude; in future schemes it is hoped that some of these difficulties would not be faced again and a more efficient initiative would follow. It also demonstrates what still needs to be done for this scheme to be deemed successful - a more decisive approach to problems, assessing the diagnostics used in protocols and continuing to try and get producers involved.

**Acknowledgements** The author wishes to thank the Bishopton Veterinary Group, Veterinary Laboratories Agency in Thirsk and all those on and involved in the initiative, especially the Steering Committee members. Jen Waters is sponsored by BPEX.

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## Interactions between neonatal lamb vigour and faecal soiling at weaning in three breeds of sheep

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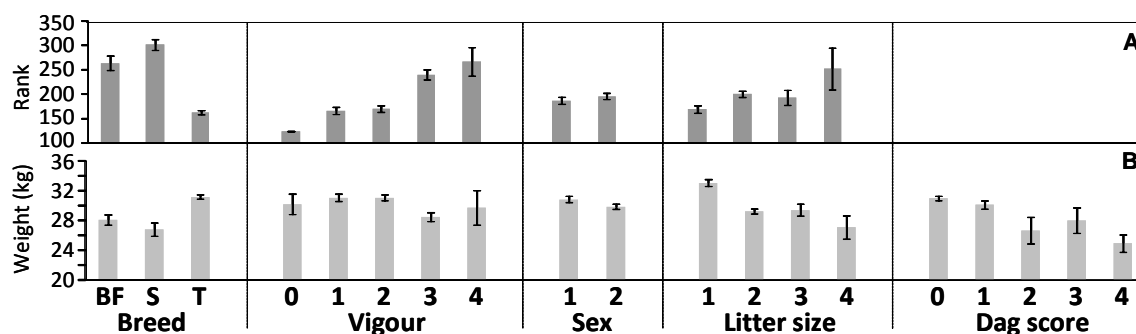
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**Introduction** Faecal soiling, i.e. the accumulation of faeces in the wool of the breech area (tail, perineum and anus), is a factor predisposing sheep to cutaneous myiasis (flystrike). Soiled wool clusters together into 'dags' which is associated with longer wool fleeces and has contamination implications for meat processing and the wool industry. However, the basis of faecal soiling is not clearly understood and may include infectious agents, genetic and environmental factors. Thus breed and early nutritional environment, and the lamb's response to this, may affect faecal soiling. For example, ewe nutrition, and larger litter size, has an effect on foetal growth and subsequent lamb vigour after birth, as well as on the level of parasitism. The aim of this study was to investigate whether breed, litter size and poor vigour at birth result in higher levels of faecal soiling at weaning.

**Materials and methods** Data from lambs from 284 ewes: 32 Scottish Blackface (BF), 29 Suffolk (S) and 203 Texel (T), born in the 2008 breeding season, were collected from birth to weaning. Ewes were group-housed on straw-bedded large pens prior to lambing, and were fed a concentrate diet and *ad libitum* hay to satisfy nutrient requirements. All ewes were allowed to give birth unaided, as far as possible, according to a standard lambing protocol. Ewes gave birth to 382 lambs (BF 43, S 49, T 291). All births were recorded live by observers and lamb sex, litter size, amount of birth assistance, vigour score (at 5 minutes of age), sucking assistance and birth weight were recorded for each lamb. Ewes and lambs remained indoors for the first 3 days after birth and then were moved outdoors until weaning (approximately 16 weeks of age). At weaning lambs were weighed and dag scored, using a pictorial scoring system (ranging from 0–4). Neonatal scores (birth assistance, lamb vigour, sucking assistance) were analysed using Kruskal-Wallis non-parametric one-way-ANOVAs while birth weight was analysed using a one-way-ANOVA (Genstat 11). Dag scores and weaning weights were ranked and then analysed via Restricted Maximum Likelihood (REML) in Genstat, fitting lamb vigour (and dag score for weaning weight,) as a covariate and breed, sex and litter size as fixed effects; ewe identity was fitted as a random effect.

**Results** There were significant effects of lamb vigour score (Wald=61.48, d.f.=1,  $P<0.001$ ), breed (Wald=94.14, d.f.=2,  $P<0.001$ ) and litter size (Wald=11.80, d.f.=3,  $P=0.01$ ) on dag rank (figure 1A) but there was no effect of lamb sex (Wald=2.63, d.f.=1,  $P=0.105$ ). There was an interaction of breed\*litter size (Wald=9.06, d.f.=3,  $P=0.03$ ) but no interaction of lamb vigour\*breed (Wald=3.66, d.f.=2,  $P=0.162$ ). For weaning weight, lower vigour lambs were lighter than higher vigour lambs (figure 1B; Wald=6.90, d.f.=4,  $P=0.009$ ); dag score, with dirtier scoring lambs being lighter than cleaner lambs (Wald=7.93, d.f.=4,  $P=0.005$ ); breed, with T being heavier than S or BF (Wald=15.52, d.f.=2,  $P<0.001$ ); litter size, with singles being heavier than multiples (Wald=39.34, d.f.=4,  $P<0.001$ ); and sex, with males being heavier than females (Wald=6.96, d.f.=1,  $P=0.008$ ). There was also an interaction of dag score\*sex\*litter size (Wald=5.96, d.f.=2,  $P=0.051$ ).



**Figure 1** (A) Effects of breed, lamb vigour, lamb sex and litter size on dag rank at weaning. (B) Effects of breed, lamb vigour, lamb sex, litter size and dag score on weaning weight. Values are mean±SEM.

**Conclusions** Factors affecting neonatal lamb vigour appear to have continuing effects throughout later life as suggested by the relationship between lamb vigour and dag score, and dag score and weaning weight. The positive relationships found between vigour and dag scores may arise because high vigour lambs are quicker to stand and suck, resulting in greater intake of colostrum and better immunity in later life. As dag score may be associated with gastro-intestinal parasitism, the high vigour lambs may be better able to deal with worm infection in later life. Additionally, higher vigour lambs, by sucking quickly, may also be better bonded to their dams resulting in increased opportunities to learn to avoid faecal-contaminated pasture, and thus may have a reduced risk for ingesting worm larvae. Studies suggest that dag score is correlated with the level of parasite exposure and may explain the negative correlation between dag score and weaning weight, as parasite exposure and weight gain are negatively correlated.

**Acknowledgements** This work was supported by the Scottish Government (RERAD). SMM is supported by a BBSRC CASE studentship in association with the Suffolk Sheep Society. Farm and technical staff at SAC sheep unit Woodhouselee Farm assisted in collecting data.

## Use of an ice vest to elicit a cold response in neonatal lambs

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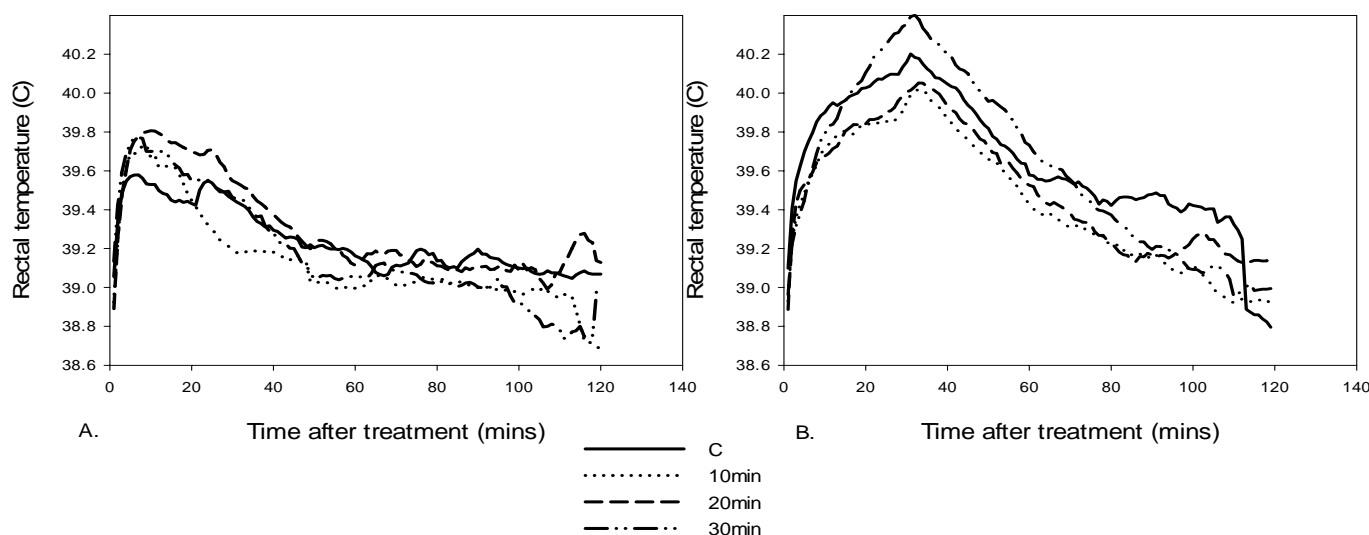
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**Introduction** The ability of the neonatal lamb to respond to cold stress is considered vital to the likelihood of its survival in many sheep production systems. Various methods have been used to assess the neonatal lamb cold responses including progressively cooled water baths (Slee *et al.* 1990), temperature controlled chambers (Alexander 1961) and noradrenaline and other drug treatments which stimulate brown fat metabolism thereby mimicking a real cold exposure (Slee *et al.* 1987). This study compared body temperature responses induced by an ice vest to those induced by a noradrenaline challenge as a means of assessing individual lamb variation in cold responses.

**Materials and methods** The ice vest (IV) was similar to a small dog coat with an ice pack inserted to cover the lamb dorsally. Four treatments were used: C (control; coat with no ice insert worn for 20 minutes; n=10); 10min (ice vest worn for 10 minutes; n=10); 20min (ice vest worn for 20 minutes; n=10); 30min (ice vest worn for 30 minutes; n=10). All IV treatments occurred between 3 and 6 hours after birth. At 12 hours after birth, each lamb was given a 150µg/kg birth weight injection of noradrenaline (NA). Rectal temperature (RT) was logged every minute to measure the lamb's response. From the temperature response curves (Figure 1A and B), peak RT, time to peak RT, length of response, area under the curve, difference between peak and basal RT and time to reach basal +2sd RT were calculated. Data were analysed using PROC GLM in SAS 9.1. A Spearman rank correlation coefficient was calculated for these parameters to compare rankings between the IV and NA challenges.

**Results and Discussion** RT response curves are shown in Figure 1. For the IV challenge peak RT and time to reach peak RT did not differ across treatments. However the difference between peak and basal RT was significantly lower ( $P<0.05$ ) for C lambs compared to 10min, 20min and 30min ( $C=0.46^{\circ}\text{C}$ , 10min =  $0.73^{\circ}\text{C}$ , 20min =  $0.72^{\circ}\text{C}$ , 30min =  $0.77^{\circ}\text{C}$ ;  $se=0.06$ ). Length of response and AUC was significantly higher for 20min lambs compared to C and 10min lambs however it was not significantly different to 30min lambs. This was possibly due to the latter group beginning to approach their basal metabolic rate while the ice vest was worn as the time to reach basal +2sd following ice vest removal was significantly shorter for 30min lambs compared to all other lambs (30min=2.9 mins, C= 9.8 mins, 10min= 16.4 mins, 20min= 19.6 mins;  $se= 3.24$  mins). The NA challenge elicited a significantly higher response compared to the cold challenge for peak RT ( $40.20^{\circ}\text{C}$  vs  $39.79^{\circ}\text{C}$ ), time to peak RT (23.98min vs 8.51 min), length of response (59.78min vs 32.18 min), AUC (2344.50 vs 1234.63) and difference between peak and basal RT ( $0.94^{\circ}\text{C}$  vs  $0.63^{\circ}\text{C}$ ). This suggests that an individual lamb's response to the NA challenge may not accurately reflect their ability to respond to a cold challenge. The non-significant Spearman rank correlation between the two challenges further supports this.



**Figure 1** Rectal temperature response curves for each cold challenge (A) and noradrenaline challenge (B).

**Conclusion** From this study it appears that the ice vest could be a useful technique in determining the relative cold response capacity of a neonatal lamb but longer exposure times may be necessary. It appears that a single injection of noradrenaline at a dose of 150µg/kg may not accurately reflect the capacity of a lamb to respond to cold exposure.

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## Beef primal cuts expressed as proportions of overall matured carcass weights (carcass balance) in Aberdeen Angus cross and Limousin cross steers and heifers

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**Introduction** “Carcass balance” or the proportion of overall beef carcass weight that is present in various primal cuts in both the hindquarter (HQ) and forequarter (FQ) segments has a considerable effect on the commercial value of each carcass. As part of a wide ranging study to examine animal performance and quality attributes of beef cattle, the objective of the current experiment was to quantify the proportions of various primal cuts within beef carcasses from both Aberdeen Angus cross (AAx) and Limousin cross (LIMx) steers and heifers slaughtered through a commercial abattoir.

**Materials and methods** Nine AAx steers and seven each of AAx heifers, LIMx steers and LIMx heifers from a range of dam types were used in this study where one side of the slaughtered carcasses were cut into a total of eleven commercial primals, vacuum packed and frozen at -20 °C. Although the 11 primals were further sub-divided for other experimental procedures, the weight (kg) of each commercial primal was then expressed as a proportion of the total matured carcass side weight (g/kg carcass weight) for the purpose of this “carcass balance” study. Nineteen of the carcasses were sourced from the Beef Research Centre at SAC, Edinburgh and the remaining eleven carcasses were sourced at the commercial abattoir as they arrived for slaughter. Animals were slaughtered in eight batches during the autumn and winter period of 2007-2008 and the cattle represented the offspring of nine AA and nine LIM sires. Data for each of the 11 primals expressed on a proportional basis were statistically analysed using the REML procedure in Genstat 11 to determine breed (B) and sex (S) effects as well as their interaction (BxS).

**Results** Average age at slaughter was 587 (s.e. 11.1) days (range 518 – 747) whilst average matured carcass weight was 332.5 (s.e. 5.08) kg (range 276.4 – 402.2) across all animals in the study. HQ shin, topside, rump, flank and sirloin along with the FQ flank, ribs, brisket, shin, neck and clod proportions are given in Table 1. The total hindquarter and total forequarter proportions are also shown. The main results for individual primals are as follows:- AAx animals had a lower proportion ( $P<0.05$ ) of HQ topside compared with LIMx animals (204 vs 214 g/kg carcass weight) whilst steers had lower proportions ( $P<0.05$ ) of HQ topside, rump and sirloin and higher proportions of FQ shin ( $P<0.001$ ) and clod ( $P<0.05$ ) compared with heifers.

**Table 1** Primal cuts expressed as a proportion of total carcass weight (g/kg) in AAx and LIMx steers and heifers

	AAx		LIMx		Breed (B)		Sex (S)		s.e.d		Sig. of effects		
	Steer	Heifer	Steer	Heifer	AAx	LIMx	Steer	Heifer	B&S	BxS	B	S	BxS
HQ shin	50	48	50	49	49	49	50	48	1.14	1.62			
HQ topside	200 <sup>a</sup>	208 <sup>ab</sup>	210 <sup>ab</sup>	218 <sup>b</sup>	204	214	205	213	3.83	5.41	*	*	*
HQ rump	78 <sup>a</sup>	86 <sup>b</sup>	81 <sup>ab</sup>	82 <sup>ab</sup>	82	81	79	84	2.08	2.94		*	*
HQ flank	83	85	82	79	84	80	82	82	3.47	4.90			
HQ sirloin	74 <sup>a</sup>	80 <sup>b</sup>	77 <sup>ab</sup>	81 <sup>b</sup>	77	79	76	80	1.92	2.72		*	*
FQ flank	66	65	63	64	65	63	65	64	1.77	2.50			
FQ ribs	205	205	207	208	205	207	206	207	2.22	3.15			
FQ brisket	81	80	83	80	81	82	82	80	1.92	2.71			
FQ shin	33 <sup>a</sup>	30 <sup>b</sup>	33 <sup>a</sup>	30 <sup>b</sup>	32	31	33	30	0.73	1.03		***	*
FQ neck	66	60	59	58	63	59	63	59	2.21	3.13			
FQ clod	62 <sup>a</sup>	54 <sup>b</sup>	56 <sup>ab</sup>	52 <sup>b</sup>	58	54	59	53	2.36	3.34		*	*
Total hindquarter	486 <sup>a</sup>	507 <sup>c</sup>	498 <sup>b</sup>	509 <sup>c</sup>	496	504	492	508	2.67	3.78	*	***	*
Total forequarter	514 <sup>a</sup>	493 <sup>c</sup>	502 <sup>b</sup>	491 <sup>c</sup>	504	496	508	492	2.67	3.78	*	***	*
Total carcass weight (kg)	356.0 <sup>a</sup>	299.6 <sup>b</sup>	360.3 <sup>a</sup>	313.8 <sup>b</sup>	327.8	337.1	358.2	306.7	10.16	14.37		***	**

Within the BxS interaction, values not sharing common superscripts differ significantly ( $P<0.05$ ).

**Conclusion** The results show that steers have a greater proportion of forequarter (shin and clod) and lower proportion of hindquarter (topside, rump and sirloin) in their carcasses compared with heifers. This may be a function of circulating hormone levels. LIMx animals have a higher proportion of hindquarter compared with AAx animals with the differences being larger between LIMx and AAx steers compared with the difference between LIMx and AAx heifers. These proportional differences amongst “carcass balance” can have a considerable effect on the overall value of animal breed types and sexes due to differential pricing of the various primals.

**Acknowledgements** SAC is grateful to the Scottish Government for funding this research and to Scotbeef, QMS, BCF and Signet for their substantial support.

## Concentrations of specific unsaturated fatty acids in *semitendinosus* muscle of early and late maturing heifer calves offered concentrates containing either safflower oil or ruminally-protected fish oil while at pasture

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**Introduction** There is increasing evidence that diet has a major role in the development of chronic diseases in humans. Ruminant derived foods are a major source of fat in the human diet and therefore there is increasing interest in enhancing the nutritional value of these foods to improve long-term human health. Beef from cattle reared on grass is known to contain appreciable concentrations of fatty acids that exhibit positive effects including oleic acid (OA, *cis*-9 18:1), the long chain n-3 fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and *cis*-9, *trans*-11 conjugated linoleic acid (CLA) (Nuernberg *et al.*, 2002). Supplementation with plant oils or marine lipids can be used to enhance tissue CLA concentrations while significant enrichment of EPA and DHA in beef requires the use of rumen protected fish oil due to the extensive metabolism of long chain n-3 fatty acids in the rumen. This experiment was designed to examine the potential of concentrate supplements containing safflower oil (SAFF) as a rich source of 18:2n-6 (Boles *et al.*, 2005; Dinius *et al.*, 1974) or rumen protected fish oil (RPFO) to increase muscle concentrations of *cis*-9, *trans*-11 CLA and n-3 LC-PUFA, respectively, in grazing heifer calves.

**Materials and methods** Ninety-six 4-month old heifer calves, of which 48 were early-maturing Aberdeen Angus × Friesian (AAF) and 48 were late-maturing Belgian Blue × Friesian (BBF) were recruited to the experiment and randomly assigned to a control (grazing only) treatment (CONT), or supplementation with concentrates containing either SAFF or a proprietary RPFO supplement (920g dry matter (DM)/kg, 302g lipid/kg DM, 3.47g EPA/100g and 5.74g DHA/100g). Biopsy samples of *semitendinosus* muscle were recovered after 4 months on diet using a spring-loaded biopsy gun (Biotech PPB-U, Nitra, Slovakia) following local administration of adrenacaine (Norbrook Laboratories, Ltd., Newry, BT35 6JP, Northern Ireland) and stored under N<sub>2</sub> at -20°C until analysed. Extraction of tissue lipids and conversion to fatty acid methyl esters (FAME) was performed according to standard procedures (Noci *et al.*, 2005). The composition of FAME and determination of tissue fatty acid content was determined by GC-FID using a Varian CP3800 chromatograph with a CP8400 autosampler and employing a CP-Sil 88 cyanopropyl fused capillary column (100m × 0.25mm i.d. × 0.2µm film thickness, Chrompack, Middelburg, The Netherlands) with H<sub>2</sub> as carrier gas (Shingfield *et al.*, 2003). Fatty acid concentrations (mg/100g muscle) were calculated according to the formula of Golay *et al.* (2006). Data were subjected to Analysis of Variance for a randomised block design with a 2 (breeds, B) × 3 (diets, D) factorial arrangement of treatments.

**Results** Concentrations (mg/100g muscle) of EPA, DHA and *cis*-9, *trans*-11 CLA in *semitendinosus* muscle are presented in Table 1. There was no effect (P>0.05) of breed on EPA (15.42 v. 17.28 for AAF and BBF, respectively; SED=1.641) or DHA (6.07 v 6.61 for AAF and BBF, respectively; SED=0.505) concentrations. For EPA, there was no difference between CONT and SAFF, while RPFO resulted in a significant (P<0.001, SED=2.010) 3-fold enrichment. Supplements of RPFO also resulted in significantly (P<0.001, SED=1.229) higher concentrations of DHA compared with CONT or SAFF. There was no effect of breed (P=0.159, SED=0.671) or diet (P=0.386, SED=0.822) on muscle *cis*-9, *trans*-11 CLA content.

**Table 1** Concentrations (mg/100g muscle) of EPA, DHA and *cis*-9, *trans*-11 CLA in *semitendinosus* muscle from heifer calves

Fatty acid	AAF			BBF			P values		
	CONT	SAFF	RPFO	CONT	SAFF	RPFO	B	D	B×D
EPA, C20:5n-3	10.49	9.39	26.36	8.92	9.45	33.47	0.259	<0.001	0.078
DHA, C22:6n-3	6.75	2.85	8.61	5.88	3.35	10.59	0.292	<0.001	0.078
<i>cis</i> -9, <i>trans</i> -11CLA	4.74	3.55	3.48	3.27	3.38	2.25	0.159	0.386	0.706

**Conclusion** Provision of a rumen-protected fish oil supplement to heifer calves for 4 months while at pasture was an effective strategy for enhancing muscle EPA and DHA content, whereas provision of a concentrate containing safflower oil had no effect on *cis*-9, *trans*-11 CLA abundance in muscle in heifer calves of this age.

**Acknowledgements** This research was supported by ProSafe Beef, an EU 6<sup>th</sup> Framework Programme project (2007-2012). The donation of the rumen-protected fish oil supplement by the Farmright Group, Ltd., UK is gratefully acknowledged and appreciated. Statistical analysis was performed by Paula Reid, Teagasc, Ashtown, Dublin.

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## Effect of sex type, feeding regime and fat cover on eating quality of lamb

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**Introduction** Research conducted in the last 20 years on lamb eating quality has shown that pre-slaughter factors such as lamb sex (Arsenos *et al*, 2002), diet (Priolo, 2001) and fat content and composition (Sanudo *et al*, 2000) can influence lamb acceptability to consumers. These factors and the interactions between them have highlighted the importance of the production system used to finish lambs on the eating quality of the meat, as perceived by consumers. This experiment was conducted with the aim of increasing the understanding of the role of sex, feeding regime and fat content and composition on the eating quality and nutritional attributes of lamb meat.

**Material and methods** Seven experimental groups (of 10 carcasses each) were selected according to their fat classification (EU classification), sex and feeding regime. All the lamb came from one farm in Wales where the sex types were finished separately. All the female lambs were finished with “new field (ryegrass) and rapeseed” (Nfr), the castrate lambs were finished either with “aftermath fields of cereal and concentrates” (Amc) or Nfr and the entire males were finished with Amc (Table 1). The carcasses were electrically stimulated with high voltage, fast chilled at 1°C for 4 hours and then stored at 2°C for 24 hours. The carcasses were boned and the *m. longissimus dorsi* and the leg were vacuum packed and aged for 7 days before being blast frozen. The *m. l. dorsi*, the *m. adductor* and *vastus lateralis* from the leg, were used for acceptability panels, while the *m. l. dorsi* and *v. lateralis* were used for fatty acid profiles and sensory profiling. In the hedonic panels 56 assessors scored the lamb on a scale of 1 (extremely acceptable) to 8 (extremely unacceptable). The sensory data (profiling and acceptability) and fatty acid profile were analysed by Random Effect Model.

**Results** Despite the differences in fat class, there were no differences in total intramuscular fat content ( $P>0.05$ ) between the treatment groups. The fatty acid profiling gave statistically significant differences ( $P<0.05$ ) in the content of some polyunsaturated fatty acids in both muscles. Entire males fed with concentrates had significantly higher contents of linoleic (C18:2), significant lower contents of linolenic acid (C18:3) and a significantly higher n-6/n-3 ratio compared with the rest of the groups. There were very few differences in sensory profiling results. In the acceptability panel of the *m. Longissimus dorsi*, statistically significant differences were found in the acceptability of flavour, texture in mouth, aftertaste and overall acceptability (Table 1). For all of these attributes, the meat from entire males was found to be less acceptable than the other experimental groups. There were no statistically significant differences ( $P<0.05$ ) between the experimental groups in the consumer panels of the muscles *adductor* or *v. lateralis*, where the meat of all the groups were considered as “acceptable” for the panellist (score between 2.5 to 3.5)

**Table 1** Mean consumer acceptability (1 extremely acceptable to 8 extremely unacceptable) of grilled lamb steaks from the *m. longissimus dorsi* for the experimental groups studied

In. longissimus dorsi for the experimental groups studied									
Lamb sex	Experimental treatments							S.E.M.	Sig
	Male entire	Male castrate				Female			
Fat Classification	2-3L	2-3L	3H-4L	2-3L	3H-4L	2-3L	3H-4L		
Finishing system	Amc	Amc	Amc	Nfr	Nfr	Nfr	Nfr		
Acceptability of									
Appearance	2.5	2.6	2.7	2.7	2.6	2.6	2.6	0.132	ns
Aroma	2.8	2.5	2.6	2.6	2.5	2.5	2.5	0.115	ns
Flavour	3.2 <sup>c</sup>	2.8 <sup>b</sup>	2.5 <sup>ab</sup>	2.7 <sup>ab</sup>	2.7 <sup>ab</sup>	2.8 <sup>ab</sup>	2.4 <sup>a</sup>	0.138	*
Texture in the Mouth	3.4 <sup>c</sup>	2.7 <sup>ab</sup>	2.6 <sup>ab</sup>	2.9 <sup>b</sup>	2.6 <sup>ab</sup>	2.9 <sup>b</sup>	2.4 <sup>a</sup>	0.150	**
After taste	3.4 <sup>c</sup>	2.9 <sup>ab</sup>	2.9 <sup>ab</sup>	3.1 <sup>bc</sup>	3.1b <sup>c</sup>	3.0 <sup>b</sup>	2.6 <sup>a</sup>	0.132	*
Overall acceptability	3.3 <sup>c</sup>	2.7 <sup>ab</sup>	2.6 <sup>ab</sup>	2.9 <sup>ab</sup>	2.8 <sup>ab</sup>	2.9 <sup>b</sup>	2.5 <sup>a</sup>	0.146	*

\* $P<0.05$ ; \*\* $P<0.001$ ; S.E.M.=standard error of means; <sup>a, b, c</sup> Values within a row that do not carry a common superscript are significantly different ( $P<0.05$ ); ns= $P>0.05$

**Conclusions** All muscles tested received good scores for acceptability, with no differences in fat content and few in fatty acids or sensory profiling attributes due to fat cover, sex or diet. Only the entire males gave slightly less acceptable loin and lower levels of n-3 fatty acids. Whether these were a result of gender or dietary differences would require further study.

### Acknowledgments

The authors gratefully acknowledge funding from Dunbia

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## Comparison of systems for assuring the eating quality of beef

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**Introduction** A number of quality assurance schemes have been developed to grade the eating quality of beef. The UK MLC Blueprint (UK BP) and New Zealand QMark (NZ QM) systems aim to improve eating quality through process control of factors such as carcass suspension, electrical stimulation and ageing. These systems select those carcasses expected to provide consumers with good eating quality. The USDA system classifies beef carcasses into quality grades based on the degree of maturity and intramuscular marbling. The Australian MSA system uses process control and carcass characteristics but also classifies individual beef muscles into eating quality grades derived from consumer perceptions, depending on cooking method.

**Materials and methods** A series of experiments were conducted to assess the role of factors including gender, breed, hanging method, ageing, electrical stimulation/chilling, muscle and position within muscle on eating quality. In total, 192 animals were used, giving 36000 beef samples which were assessed by 6000 consumers. Breed, sex, hot standard carcass weight and EUROP grade were recorded as were hump height, ossification, rib fat, marbling, meat colour, fat colour, ultimate pH and temperature. Grilled and roasted beef muscles were assessed by consumers who gave it a quality rating: 'unsatisfactory', 'satisfactory everyday quality', 'better than everyday quality' and 'premium' (Farmer *et al.*, 2009). The information recorded enabled the meat to be accorded a classification under several trial quality systems (Table 1). These were very similar to or were modifications of those available internationally. The distribution of the consumer scores for each muscle for each grade was evaluated statistically using a combination of Wald analyses (Genstat) with visual inspection of the distributions to establish the direction of any differences.

**Table 1** Grading systems for delivery of good eating quality for NI beef

	MSA	MSA-B	USDA	UK BP	UK BP-C	NZ QM
Classifications for statistical evaluation.	Ungraded, Unsatis., 3*, (4* + 5*)	Ungraded, Unsatis., 3*, (4* + 5*)	Ungraded, Utility, Standard, Select, (Choice + Prime)	Ungraded, BP, BP+21d	Ungraded, BP, BP+21d	Ungraded, QMark
Basis for classification	Processing & muscle & cooking method	animal data,	Maturity and marbling	Processing & animal data	EUROP grade	Processing data
Amendments	-	Bulls included	-	-	'O' included	-

**Results and Discussion** Table 2 identifies where consumer quality ratings were significantly different between grades and where the more highly graded meat gave better eating quality (o) or not (~). No system was perfect. Beef passing the NZ and US systems delivered improved eating quality inconsistently and performed poorly for roasts. These systems do not consider hanging method and the US system focuses on marbling, which is generally much lower in European carcasses. The UK Blueprint performed well only when low conformation grades were included (UKBP-C). Versions of the MSA system performed well for the greatest proportion of muscles and for both cooking methods. The inclusion of bulls (MSA-B) improved performance for some muscles.

**Table 2** Ability of beef eating quality systems to show differentiation between different levels of consumer satisfaction

Cooking Muscle	MSA	MSA-B	USDA	UKBP	UKBP-C	NZQM
Grilled						
Striploin – anterior ( <i>l. dorsi</i> )	*** o	bx	ns ~	*	o	ns ~
Striploin – mid ( <i>l. dorsi</i> )	*** ~	ns	**	o	ns	*** o
Striploin – posterior ( <i>l. dorsi</i> )	*** o	**	o	ns	o	*** o
Rump flat (biceps femoris)	* o	*	o	ns	~	ns o
Rump (gluteus medius)	*** o	***	o	ns	o	*** o
Rump (gluteus medius eye)	ns o	*	o	*	o	ns ~
Roast						
Silverside eye	ns ~	ns	~	ns	~	ns ~
Silverside	ns o	**	o	ns	~	* o
Rump (gluteus medius eye)	*** o	***	o	ns	o	*** o
Topside (semimembranosus)	*** o	bx	ns	~	*	o

Statistical significance of distribution: ns, \* =  $P < 0.05$ , \*\* =  $P < 0.01$ , \*\*\* =  $P < 0.001$ ; direction of eating quality indicated by: o where higher grades received better consumer scores or ~ no consistent relationship; bx = no bulls tested

**Conclusions** Of the quality assurance systems tested, the standard MSA system performed best for NI beef before amendment. Both MSA and MLC Blueprint performed well with amendments. MSA allows grading of individual muscles which improves versatility.

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## Preliminary comparison between two colour measuring instruments of different optical geometries when used to measure bovine adipose and muscle tissue colour

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**Introduction** As with most food products, colour of beef is an important quality characteristic. Subjective assessment of colour is open to bias but environmental conditions, particularly lighting, can also strongly influence colour perception. Hence, colour measuring devices such as tristimulus colorimeters (e.g. the Minolta CR series) and spectrophotometers (e.g. the HunterLab UltraScan, ColorQuest and LabScan series), are frequently used to measure beef tissue colour. Numerical values can be applied conveniently to any perceived colour and hence, colours can be described objectively. However, despite the advantages of this approach, there are still some relative weaknesses. When different colour measuring devices/instruments have been used to measure colour (a cursory review of relevant literature reveals that, at least 10 devices have been commonly utilised), different data have been generated, even when measuring the same tissue, and even under the same environmental and instrument (colour space, standard illuminant) conditions. Different optical geometries of different instruments are an important source of variation in this regard (MacDougall, 1994). That the choice of instrument used to measure food colour affects the colour coordinates generated has been recognised (Baardseth *et al.*, 1988) but not widely documented. Therefore, the issue of concern was how data from different instruments related to each other such that values from one instrument could be used to effectively 'predict' values from another, if such a necessity became unavoidable due to practical and/or logistical exigencies.

**Materials and methods** Samples of subcutaneous fat (SCF) were recovered at either 24 or 48 hours *post-mortem* from over the *Musculus longissimus dorsi* (LD) between the 9<sup>th</sup> and 13<sup>th</sup> ribs or were measured directly on the carcass at these times. The colour of LD muscle was measured by cutting a steak, 2.5cm thick, between the 10<sup>th</sup> and 13<sup>th</sup> ribs, removing adhering adipose and connective tissue and overwrapping with oxygen-permeable PVC film and permitting to bloom at 4°C, in darkness, for 3 hours. Readings of 'L' (lightness), 'a' (redness) and 'b' (yellowness) were made on SCF within 48 hours *post-mortem* and on bloomed LD at 48 hours and 14 days *post-mortem*, the latter following ageing in darkness under vacuum-packaging at 4°C. Measurements were made using two instruments; the portable Minolta chromameter, model CR300 (CR300) and the benchtop HunterLab UltraScan XE spectrophotometer, equipped with Universal software Version 2.2.2 (Hunter Associates Laboratory Inc., Reston, Virginia 22090, USA) (HLabXE) coupled to a personal computer. Instruments were calibrated using their standard white calibration tiles according to manufacturer's guidelines. The measuring aperture areas of the CR300 was 8mm and that of the HLabXE was 25.4mm. All measurements were made in the Hunter *L a b* colour space and the D<sub>65</sub> standard illuminant was used throughout. Where appropriate, tissue 'H' (hue) and 'C' (chroma/saturation) were calculated as  $\tan^{-1}(b/a)$  and  $\sqrt{a^2 + b^2}$ , respectively (McLaren, 1987). Final conversion of hue from radians to degrees was achieved by multiplying  $\tan^{-1}(b/a)$  by  $180/\pi$  (Liu *et al.*, 1996). The CR300 and the HLabXE were compared when measuring SCF colour ('b' and 'C' values) and all colour coordinates of LD muscle at 2day (48 hours) and 14day *post-mortem*. For each comparison, variables were compared using simple linear regression, including terms for the regression model and error variances. Data are presented in Table 1.

### Results

**Table 1** Relationship between the HunterLab UltraScan XE colorimeter and the Minolta CR300 chromameter when used to measure beef carcass tissue colour.

SCF	Equations <sup>1</sup>				r	s.e.	P-value
	y	Slope,m	x	c			
'b' value	CR300 b	0.339	(HlabXE b)	4.936	0.54	1.908	<0.001
'C' value	CR300 C	0.294	(HlabXE C)	6.422	0.58	1.822	<0.001
<b>Muscle</b> (2d and 14d pooled data)							
'L' value	CR300 L	0.062	(HlabXE L)	34.62	0.16	2.061	0.054
'a' value	CR300 a	0.289	(HlabXE a)	8.082	0.65	1.935	<0.001
'b' value	CR300 b	0.192	(HlabXE b)	3.53	0.30	1.517	0.0002
'H' value	CR300 H	0.303	(HlabXE H)	12.19	0.20	4.454	0.016
'C' value	CR300 C	0.277	(HlabXE C)	9.37	0.63	2.120	<0.001

**Conclusion** The present study while by no means exhaustive, indicates that despite a relatively small data set, there is potential to use colour coordinates generated by one instrument to predict those that would be generated by a different instrument on the same samples under the same conditions. The data also emphasise that reporting the instrument used to generate colour data is important for interpretative purposes.

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## The effects of sire breed type (Charolais and Aberdeen Angus) on production and carcass quality from an organic continental cross spring calving herd

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**Introduction** The Irish 'Department of Agriculture Fisheries and Food' Organic Farming Action Plan 2008-2012 stated that the UK imports 4,000 tonnes of organic beef per year. For Ireland to supply the home and UK market it would need three times the amount of organic beef currently produced. The aim of the study located at the Teagasc Johnstown Castle Environmental Research Centre, was to determine the effects of sire breed type (Charolais and Aberdeen Angus) on production and carcass quality in organic beef production.

**Material and methods** A 44-cow continental-cross spring-calving herd made up of Limousin x and Simmental x cows was established to produce cross-bred calves. This herd was maintained by bringing in mature cow replacements of the same breed type. Using a representative group of sires from each breed 50% of the cows were each bred to Aberdeen Angus or Charolais sires, which were ranked similarly within breed for genetic merit. AI was used to the greatest extent possible with two natural service bulls being used to cover repeat matings. This study was carried out on using the progeny born in each of two years (2006 and 2007) and all animals were taken to slaughter. The cow-calf herd followed a rotational grazing system in a designated area of the 60 ha farm. The yearling heifers and steers also had a rotational grazing programme on a different land area of the same land unit. During the winter months animals were accommodated on straw bedded sheds according to organic standards. Animals were slaughtered at 20 or 22 months (heifers) and 22 or 24 months (steers). Data was analysed using the general linear models procedure of the Statistical Analysis Institute (SAS, 2001). Data on live weight, slaughter and carcass traits were analysed as a 2 x 2 x 2 factorial with terms in the model for breed, sex, slaughter date, their interactions and year.

**Results** The Charolais calves were approximately 10 kg heavier at birth than the Aberdeen Angus calves. The male calves in each breed were approximately 4 kg heavier at birth than the female calves (Table 1). The growth advantage from birth to weaning of the steers over the heifers and that of Charolais over Aberdeen Angus (Table 1) was comparable to that achieved in conventional production systems for matings with mature continental cows. The final live weights of the Aberdeen Angus and Charolais heifers and steers was 523, 545, 640 and 641 kg respectively (Table 2) The slaughter data generated showed the difference between Aberdeen Angus and Charolais heifer carcasses was 24 kg in favour of the Charolais sires (Table 2). The corresponding difference for the steers was 11 kg (Table 2).

**Table 1** Effect of sire breed on calf birth weight, weaning weight and live weight gain birth to weaning

	AA		CH		s.e.d
	Female	Male	Female	Male	
Birth wt (kg)	40.6 <sup>a</sup>	44.9 <sup>b</sup>	50.2 <sup>c</sup>	54.6 <sup>d</sup>	1.35
Weaning wt (kg)	255.6 <sup>a</sup>	288.7 <sup>b</sup>	285.9 <sup>b</sup>	302.5 <sup>b</sup>	6.51
Daily live weight gain kg/day	1.04 <sup>a</sup>	1.17 <sup>b</sup>	1.13 <sup>b</sup>	1.19 <sup>b</sup>	0.028

<sup>abcd</sup>Means with different superscripts within rows differ significantly (P<0.05)

**Table 2** Effect of sire breed and sex on carcass characteristics of calves born in spring 2006 and 2007

	AA		CH		s.e.d
	Female	Male	Female	Male	
Birth wt	40.6 <sup>a</sup>	44.9 <sup>b</sup>	50.2 <sup>c</sup>	54.6 <sup>d</sup>	1.35
Final wt	523.3 <sup>a</sup>	639.9 <sup>b</sup>	545.3 <sup>a</sup>	640.5 <sup>b</sup>	10.84
Carcass wt	278.2 <sup>a</sup>	348.8 <sup>b</sup>	301.7 <sup>a</sup>	359.7 <sup>b</sup>	6.51
Carcass birth to slaughter	0.41 <sup>a</sup>	0.47 <sup>c</sup>	0.44 <sup>b</sup>	0.48 <sup>c</sup>	0.009
KO%	53.2 <sup>a</sup>	56.1 <sup>b</sup>	55.2 <sup>ab</sup>	57.4 <sup>b</sup>	0.48
Conformation	2.99 <sup>ab</sup>	2.75 <sup>a</sup>	3.18 <sup>b</sup>	3.05 <sup>b</sup>	0.095
Fat score	3.50 <sup>a</sup>	3.56 <sup>a</sup>	2.86 <sup>b</sup>	2.63 <sup>b</sup>	0.128

<sup>abcd</sup>Means with different superscripts within rows differ significantly (P<0.05)

<sup>1</sup>Conformation score E = 5, U = 4, R = 3, O = 2, P = 1

<sup>2</sup>Fat score 5 = Fattest, 1 = Leanest

There were no interactions

**Conclusion** The results to date, from this contrasting sire breed and sex comparison study, indicates that is possible to achieve animal performance comparable with well managed conventional suckler calf to beef systems (Drennan and McGee, 2009).

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## Qualitative characteristics of the carcass of goats finished in native pasture: effects of the genotype

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**Introduction** The qualitative characteristics of the carcass are evaluated by conformation, marbling, color and texture scores of the meat. An exhaustive description of these characters is necessary, since the consumer makes a selection based on these attributes. Moreover, changes to these characteristics can add value to the end item. The objective of this work was to evaluate the effect of the genotype on the qualitative characteristics of the carcass of goats finished on native pasture.

**Material and methods** This study was carried out in EMEPA (Empresa de Pesquisa Agropecuária da Paraíba SA) "Pendência Experimental Station", in Soledade, PB. Twenty one goats of different genotypes, with an average age of 280 days and 32 kg of live body weight, were used. After a fasting period for 18 hours, the animals were slaughtered and the carcasses were weighed and subsequently placed in plastic bags. They were transported to a cold storage at 4 °C, where they remained hanging by the tendons of the leg for 24 hours. After the cooling period, the carcasses were scored for conformation using an X point scale where 1= poor and X=excellent And for fat cover using an X point scale where 1=very thin and X=very fat.

The carcasses were split in half longitudinally and the left half was evaluated for loin eye area (LEA), marbling, colour and texture of this muscle on the cut surface between the 12th and 13th ribs, using X point scales from 1 = none to X = excessive, 1= Light pink X= Dark red and 1= fine X = very rough (respectively) using the methodology described by Osorio and Osorio (2003).

**Results** Treatments were assigned to the animals according to a completely random design with three treatments (non defined breeds - NDB, ½ Boer, and ¾ Boer genotypes) and seven replications. The ¾ and ½ Boer animals presented a better carcass conformation than the SRD animals ( $P<0.05$ ). Meat marbling was similar to all genotypes ( $P>0.05$ ), so that the expected superiority of the ½ and ¾ Boer genotypes was not confirmed in this study ( $P>0.05$ ) and the values observed for the intramuscular fatness were 1.87, 1.71, and 1.83 to the NDB, ½ Boer and ¾ Boer genotypes, respectively. The meat from NDB animals presented a darker coloration (2.87) than the meat from ½ and ¾ Boer genotype animals (2.14 and 2.00, respectively), suggesting a better quality of the meat from these last two genotypes.

**Table 1** Means values of the qualitative characteristics of the carcass of goat in function of the genotypes, kept in native pasture

Parameters	Genotype			Sig.	VC (%)
	NDB	½ Bôer	¾ Bôer		
Conformation	1.50 <sup>b</sup>	1.74 <sup>a</sup>	2.66 <sup>a</sup>	*	42.73
State of fat					
Finishing	1.00	1.00	1.16	Ns	20.54
Perirrenal and Pélvic Fat	1.00	1.00	1.20	Ns	20.24
Section of muscle <i>L. dorsi</i>					
Marbling of the meat (quant.)	1.87	1.71	1.83	Ns	39.74
Texture of the meat	3.75	4.00	3.67	Ns	23.83
Coloration of the meat	2.87 <sup>a</sup>	2.14 <sup>b</sup>	2.00 <sup>b</sup>	*	13.02

\*Significant ( $P<0.05$ ), NS: Not significant

**Conclusions** Based on the carcass qualitative evaluation, it was verified that the ½ and ¾ Boer genotypes present a carcass with a higher content of lean meat denoted by a better carcass conformation, which is the main indicator of muscular performance; and a better lean meat, demonstrated by the lighter muscle coloration, one of the main meat tenderness indicators.

**Acknowledgements** The authors would like to thanks EMEPA (Empresa de Pesquisa Agropecuária da Paraíba SA) for help in this study..

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## Gene expression profiles of pork samples divergent in intramuscular fat content

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**Introduction** Intramuscular fat (IMF) (also referred as marbling) is related to meat eating quality (e.g. flavour, juiciness and tenderness of meat) and IMF levels of pig meat are very important for consumers in relation to healthier options in food choices (Hocquette, *et al*, 2009; Harper and Pethick, 2004). It has been reported that restriction of lysine in the diet of grower-finisher pigs increases IMF deposition in porcine muscles such as M. semimembranosus (SM) (Zhang *et al.*, 2008). The aim of the present study was to identify the diet-associated gene expression profiles in porcine muscle.

**Materials and methods** This study involved an experimental diet and a control diet. 11 Duroc origin pigs (female n=6, male n=5) were placed on the experimental diet which was restricted in lysine (0.7 g lysine /kg feed) and 11 Duroc origin pigs (female n=6, male n=5) were placed on the control diet which was an isoenergetic but not lysine-restricted diet (1.3 g lysine /kg feed). Animals were fed from a mean weight of 46 kg to slaughter at commercial weight. SM muscle tissues were removed at day 2 post-mortem and IMF % levels assessed according to the protocol (Bostian, *et al*, 1985). In parallel, SM tissue was preserved in RNAlater® within 10 minutes of slaughter for microarray analysis. High quality RNA was extracted and hybridised to the Affymetrix GeneChip® porcine array. Rosetta Resolver® was used to estimate fold changes and p-values, and ontologies of differentially expressed genes classified using Panther Protein Classification System (Mi, *et al*, 2007; Thomas *et al*, 2003).

**Results** M. semimembranosus from lysine restricted pigs had significantly higher levels of IMF in comparison to control diet pigs ( $3.24 \pm 0.25$  % versus  $1.73 \pm 0.26$ %) (Table 1) but there was no difference in IMF level between male and female-derived muscle. When analysed separately by gender, IMF levels in SM muscle of Duroc females were significantly affected by restricted diet. Although males on the restricted diet had higher levels of IMF, this was not significant (Table 1). The analysis of the microarray data revealed 477 transcripts were differentially expressed between the restricted diet and the control diet groups. Of the 477 transcripts, 240 and 246 transcripts displayed greater than 1.5-fold increase or decrease in Duroc female and male, respectively at the 0.05 level. 52 differentially expressed genes were identified by using Panther System. Among these 52 genes modulated by diet across genders, several biological processes and molecular functions were significantly over-represented compared to the entire NCBI reference list of human genome (P-value  $\leq 0.05$ ). These biological processes included amino acid metabolism, signal transduction and lipid, fatty acid and steroid metabolism process whilst over-represented molecular functions included peptide hormone, oxidoreductase, signalling molecule and oxygenase. Gene expression levels and their association with pork quality will be validated using real-time PCR.

**Table 1** IMF levels in SM muscle in Duroc population

	Restricted_diet_(0.7 g lysine /kg feed)	Control_diet_(1.3 g lysine /kg feed)	Diet
	Mean $\pm$ S.E. (%)	Mean $\pm$ S.E. (%)	P-value
Duroc (female + male)	$3.24 \pm 0.3$	$1.73 \pm 0.3$	< 0.001
Duroc female	$3.60 \pm 0.4$	$1.92 \pm 0.4$	0.02
Duroc male	$2.87 \pm 0.4$	$1.52 \pm 0.4$	0.17
Gender P-value	0.85	0.99	

**Conclusions** These results show that lysine restricted diet resulted in higher levels of IMF content in a commercially important porcine muscle in comparison to control groups. However female derived muscle SM tissue appeared to have greater response to the diet in comparison to male derived muscle in the Duroc breed. Biological processes relevant to fat deposition and amino acid metabolism were significantly over-represented among differentially expressed genes.

**Acknowledgements** This research was funded by funded by the Irish Department of Agriculture, Fisheries and Food, through the Food Institutional Research Measure.

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## The effects of dried pistachio epicarp on lambs' performance

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**Introduction** Pistachio by-product or the residue of pistachio after peeling has potentially high nutritive value but its biological effects in ruminants have not been studied extensively. There is about 430000 hectares of pistachio garden in Iran and annual wet and dry pistachio production is 755400 and 253000 tones respectively. This by-product contains: %64.5 epicarp, %25 cluster, %10 leaf and %0.5 nut and shell. Problems with this by-product are including: 1-deterioration in less than 24 hours so it could not stored for long term. 2-Pistachio epicarp contains high level of tannins and other phenolic compounds. 3- This by-product is potentially an environmental pollutant and cost effective for disposal. Introducing of pistachio epicarp as feedstuff was the aim of this project.

**Materials and methods** This experiment was carried out using 70 male lambs with initial weight of 35.9±1.21 Kg in a completely randomized design Seven groups of ten male lambs were fed for 90 days, iso-caloric, iso-nitrogenous diets, containing either no added pistachio epicarp (control) and added levels 10, 15, 20, 25, 30 and 35 percentage pistachio epicarp in rations. After 90 days feeding, the lambs were slaughtered. Kidneys and livers were fixed in formalin and used for histology and pathology tests. The right half of each carcass was separated into six primal cuts: neck, shoulder, breast, leg, loin and rump. Each cut was dissected into components of lean meat plus bone, fat and residue, which were weighted and calculated as percentage of the whole cut. Data were analyzed with SAS software in proc ANOVA.

**Results** Inclusion of 10 percent of pistachio epicarp in ration showed a better performance compared to other experimental groups. Inclusion of pistachio epicarp up to 25 percent of ration did not affect final body condition score, total dry matter intake, total gain, feed conversion ratio, final weight and average daily gain. Inclusion of 30 and 35 percentage pistachio epicarp in the ration (treatment 6,7) negatively affected performance of animals. No significant differences were found in weight of stomach and intestine to body weight which indicates that all groups of animals had the same capacity of digestive tract. Measurement of feed intake, showed a high tendency to feed intake in treatment two (10%). Microscopic and macroscopic pathology of kidneys and livers did not show any damage in all treatments. Except for group two, no significant changes in neck, rump and legs percentage (to cold carcass), were found between experimental groups. Use of pistachio epicarp did not affect the percentage of shoulder. An exception was observed in group seven (35% pistachio epicarp) which breast and loin were different with other six treatments. The percentages of lean meat plus bone were not different among all groups except for treatments two and seven. The aforementioned result was observed fat. The lowest percentage of fat was observed in group 7 and the highest was belonged to group two.

**Table 1** Mean and standard deviation of feed intake, feed conversion, gain, initial and final weight and body condition score

	Rations						
	1(0%)	2(10%)	3(15%)	4(20%)	5(25%)	6(30%)	7(35%)
Total Intake(kg)	183.63±3.75 <sup>b</sup>	197.71±5.81 <sup>a</sup>	182.30±4.36 <sup>b</sup>	180.28±3.95 <sup>bc</sup>	180.18±5.19 <sup>bc</sup>	177.51±2.48 <sup>c</sup>	168.24±5.36 <sup>d</sup>
FCR	8.56±0.063 <sup>b</sup>	8.23±0.043 <sup>a</sup>	8.60±0.064 <sup>b</sup>	8.54±0.049 <sup>b</sup>	8.60±0.072 <sup>b</sup>	9.18±0.001 <sup>c</sup>	9.32±0.059 <sup>c</sup>
Total Gain(Kg)	21.45±0.50 <sup>b</sup>	24.02±0.73 <sup>a</sup>	21.19±0.52 <sup>b</sup>	21.10±0.43 <sup>b</sup>	20.97±0.64 <sup>b</sup>	19.34±0.17 <sup>c</sup>	18.23±0.60 <sup>d</sup>
ADG (gr)	238.32±5.53 <sup>b</sup>	266.87±8.08 <sup>a</sup>	235.42±5.78 <sup>b</sup>	234.45±4.73 <sup>b</sup>	232.94±7.14 <sup>b</sup>	214.90±1.90 <sup>c</sup>	202.58±6.65 <sup>d</sup>
IW (Kg)	35.30±1.06 <sup>a</sup>	35.85±0.71 <sup>a</sup>	35.00±1.45 <sup>a</sup>	35.75±1.32 <sup>a</sup>	35.00±1.15 <sup>a</sup>	35.05±1.50 <sup>a</sup>	35.05±1.17 <sup>a</sup>
FW (Kg)	56.75±1.22 <sup>b</sup>	59.87±1.21 <sup>a</sup>	56.19±1.84 <sup>b</sup>	56.85±1.18 <sup>b</sup>	55.97±1.27 <sup>b</sup>	54.39±1.42 <sup>c</sup>	53.28±1.39 <sup>c</sup>
Final BCS	3.45±0.23 <sup>a</sup>	3.55±0.33 <sup>a</sup>	3.45±0.23 <sup>a</sup>	3.53±0.34 <sup>a</sup>	3.47±0.42 <sup>a</sup>	3.45±0.37 <sup>a</sup>	3.48±0.36 <sup>a</sup>

ADG= Average Daily Gain(gr)- BCS= Body Condition Score- IW and FW= initial and final weight- FCR= feed conversion ratio

Numbers in a row with different letters (a, b and etc) differ significantly ( $P < 0.05$ ).

**Conclusions** High tendency to feed intake and better performance in treatment two (10%) may have three reasons: 1- Increasing level of pistachio in the diet increased level of phenolic compounds in the ration. Negative effect of tanning on feed intake has well been documented. In treatment 2 (10%) the level of phenolics was not high enough to affect feed intake but at the mean time animals may had been encouraged to more intake due to better taste. 2- All rations were tested in an Gas-Test experiment and the gas production curve showed a positive synergistic effect on dry matter and organic matter digestibility (plus we did another degradation *in vitro* test by using Tilley and Terry method and that's results showed high digestibility in diet two. 3- As tannins could binding to feedstuffs protein and by-pass them to intestine, a low level of tannin like treatment two can supply an approximate by-pass protein for animals but the higher levels of tannin results to high amount of UDP and low amount of RDP in animals. It could be suggested that the feeding pistachio epicarp up to 25% of total dry matter intake had no negative effects on performance, carcass characteristics, meat quality and health of lambs. This by-product could be used up to 25%, without negative effect on performance and animal health.

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## Performance of growing lambs fed with increasing levels of sorghum grains containing tannins

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**Introduction** Sorghum (*Sorghum bicolor* (L.) Moench) has a great importance in animal nutrition as substitute to corn, due to the similarity of the chemical composition and availability (Ruskin *et al.*, 1996). Comparisons have shown that sorghum represents 95% of the nutritional value of corn (Oliveira *et al.*, 2003). However, some sorghum grains may have tannins which can affect intake and digestibility (Reed, 1995; Cannas, 1999 and McDonald *et al.*, 1995). The purpose of this work was to evaluate the replacement of corn by sorghum grains containing tannins on growing lamb performance.

**Material and methods** Twenty male Santa Ines lambs with a mean body weight  $17.80 \pm 1.02$  kg were adapted for two weeks with a basal diet (Tifton hay – 40% and corn plus soybean meal – 60%) prior to the experimental period. Animals were randomly divided into five (n = 4) treatments (0, 25, 50, 75 and 100% replacement of corn by sorghum (cultivar BR 701 containing 29 g/Kg DM of condensed tannins), for 75 days. The animals were allocated in individual pens with an individual feeding system (3% body weight), the water was offered *ad libitum*. Feed offered and refusals were measured everyday and animals were weighed weekly to calculate the average feed intake (kg/d), average daily weight gain (kg/d) and feed conversion (kg/kg gain). All the results were analysed using the GLM procedure, than the sum of squares was partitioned into orthogonal polynomials (linear, quadratic, cubic and quadratic components) using SAS system (2001).

**Results** The performance of animals was not affected ( $P > 0.05$ ) by the level of replacement of corn by sorghum containing tannins (Table 1).

**Table 1** Effect of replacement of corn by sorghum with tannins in growing lambs performance

% Sorghum	Initial body weight (kg)	Final body weight (kg)	Average Feed intake (kg/d)	Average daily weight gain (kg/d)	Feed conversion (kg/kg gain)
0	17.20	21.90	0.55	0.06	6.62
25	18.75	24.25	0.59	0.07	8.35
50	18.00	24.00	0.63	0.08	7.95
75	17.50	22.87	0.59	0.07	10.17
100	17.66	22.16	0.59	0.05	10.16
S.E.D	-	4.66	0.13	0.03	3.80
P <sub>≤</sub>	-	0.48	0.69	0.53	0.60

**Conclusions** The results showed that the sorghum containing tannins may be used in growing lamb diets, but more studies are needed over long periods to ensure safety use in sheep and maintenance of production levels.

**Acknowledgements** This experiment was supported by FAPESP, project nº 08/53782-8 and Mr. Joaquim Everaldo dos Santos for help during the experiment.

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## Influence of season and finishing diet on the fatty acid composition of lamb longissimus dorsi muscle

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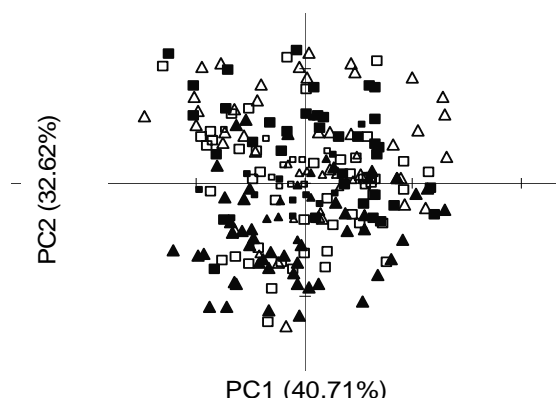
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**Introduction** The fatty acid (FA) composition of ruminant meat is influenced by many aspects of the production system including the animal finishing diet. Lamb produced from grass-fed animals contains greater concentrations of long-chain (LC) n-3 polyunsaturated fatty acids (PUFA) than lamb produced from concentrate-fed animals (French *et al*, 2000). Since the animal diet is likely to vary over the farming year (McAfee *et al*, 2009), it is possible that the season of animal slaughter will also influence quantities of LCn-3 PUFA found in meat and subsequently available for human consumption. The aim of this study was to determine the FA composition of lamb produced in Northern Ireland, examining the effects of both season of slaughter and reported finishing diet.

**Methods** Samples of lamb longissimus dorsi (LD) muscle (n= 217) were collected fortnightly from a commercial abattoir over a 12 month period. Producers were identified and contacted to obtain information on whether the finishing diet provided in the month prior to slaughter was grass, concentrates, grass supplemented with concentrates or silage supplemented with concentrates. Total lipid was extracted from lean tissue according to the Folch method (Folch *et al*, 1957) and FA methyl esters were analysed using gas chromatography. Principle components analysis (PCA) was performed on the proportional FA data (% w/w) to investigate the influence of season and reported finishing system on FA profiles. To analyse for the effect of season and diet, a factorial ANOVA (SPSS v. 11.5) was used including season and diet as well as their interaction as fixed effects. Bonferroni post hoc test was used to adjust for multiple comparisons. Seasons defined as Spring: March – May; Summer: June-August; Autumn: September – November; Winter: December – February.

**Results** The intramuscular fat content of the LD muscle was not significantly affected by season. Lamb finished in summer had significantly higher concentrations of LCn-3 PUFA and total conjugated linoleic acids (CLA) than any other season ( $P < 0.05$  season and diet for docosapentaenoic acid (C22:5n-3) ( $P < 0.01$ ) in lamb sampled in summer. This interaction showed that in summer animals that were reared on grass had higher concentrations of this LCn-3 PUFA compared to those finished on concentrates alone or concentrates supplemented with silage. PCA analysis explained 74% of total FA variation (PC1 41%, PC2 33%). The PCA scores plot (Fig 1) showed that samples of LD muscle from lamb produced in summer (▲) were grouped mainly in the lower half section. The loadings plot showed that summer produced lamb was associated with lauric acid, myristic acid, palmitic acid, palmitoleic acid, CLA c9, t11 isomer, linoleic acid (C18:2n-6), alpha-linolenic acid (C18:3n-3), arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3). There is no clear separation between the other seasons on the PCA scores plot (Fig 1).



**Figure 1** PCA scores plot for FA analysis.  
△ Spring; ▲ Summer; □ Autumn; ■ Winter.

**Conclusions** Results of this study provide evidence that there is seasonal variation in the concentration of a number of FA in lamb produced under a range of commercial production systems. The higher concentrations of LCn-3 PUFA and total CLA in summer-produced lamb may have potential benefits for consumer health. Further research is needed however, to determine the time course of these changes in order to optimise conversion of the C18:3n-3 from grass to LCn-3 PUFA in the lamb muscle.

**Acknowledgements** The authors gratefully acknowledge funding from LMC, AgriSearch and DEL Co-operative Award in Science and Technology (CAST).

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## Effects of various levels of wheat bran on weight gain of Baluchi sheep breed of Iran

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**Introduction** Using agricultural by products could result in decreasing feeding costs of animal husbandry enterprise. Wheat bran, as an important by product from milling industry, could be utilized as feed ingredient in animal nutrition due to high content of protein as well as minerals particularly P and B complex vitamins (Bartink and Jakubczyk, 1989). A huge amount of wheat bran is annually produced in developing countries. Application of the wheat bran is restricted in poultry diet due to its high fibre percentage (Abll *et al.*, 1992). Major use of wheat bran has been in ruminant nutrition because of high fibre digestibility (Gravel *et al.*, 1978). The main objective of the present research was to determine the effects of various levels of wheat bran on fattening performance of Baluchi sheep breed of Iran.

**Materials and methods** The experiment was undertaken using fifteen male Baluchi lambs in a completely randomized statistical design to evaluate the use of different levels of wheat bran on fattening performance. Average initial body weight of the lambs was 21.96 Kg (SD=1.92 Kg). Three treatments were applied in the experiment with five replicates for each treatment. The Experimental treatments were: 1.10% wheat bran (control), 20% wheat bran and 30% wheat bran (DM% in diet). Wheat bran was used instead of corn and barley ingredients in treatments two and three. The metabolisable energy and protein content of the diets were approximately 2.5 MJ/kg and 14%, respectively. The experiment was carried out for a period of 90 days. During this time, feeding trial fresh feed was offered to the animals in excess of their consumption twice a day as TMR. Weighing was carried out after a 16-hour fasting once fortnightly. Feed intake was daily measured during the experimental period. The data were analysed using SAS programme.

**Results** The results obtained from the present research indicated that no significant differences were found among different treatment for average final weight, daily weight gain, dry matter intake and feed conversion ratio (Table 1).

**Table 1** Statistical comparison of the fattening performance among different treatments

Performance characteristics	Levels of wheat bran (%)			SEM
	10	20	30	
Final weight (kg)	39.71	37.44	36.55	1.38
Daily weight gain(g)	197.24	171.96	162.09	15.46
Dry matter intake(g/day)	1142.9	1099.92	1077.26	55.11
Feed conversion ratio	5.86	6.60	6.78	0.33

**Conclusions** From nutritional point of view, no significant differences among the treatments may be attributed to high digestibility of wheat bran leading the same provided energy as compared to control diet. As a consequence, we can not firmly say whether wheat bran could be utilized up to 30% DM of a diet, which in turn resulting in a decrease cost of animals feedlot. Further studies with more experimental animals are needed.

**Acknowledgements** The authors would like to express their especial thanks to the University of Birjand for the financial support provided for this research.

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## The effect of rumen isolated bacteria on degradation of sugarcane pith processed with steam and or exogenous enzyme in *in vitro* culture condition

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**Introduction** Rumen cellulolytic bacteria, such as *Ruminococcus albus* have a key role in ruminal digestion of plant cell walls, due to their numerical predominance and metabolic diversity (Cheng *et al.*, 1991). Many methods have proved successful in disrupting cell wall material e.g. using enzyme (Eun and Beauchemin, 2007), and or steam (Castro and Machado, 1990). Steam explosion resulted in to disrupt lignocellulosics and partial or complete hydrolysis of hemicellulose fraction in a way which allows improved utilization of cell wall polysaccharides by rumen microbes and improving enzymatic accessibility and digestibility (Castro and Machado, 1990). Researchers reported increasing of *in vitro* DM digestibility for sugarcane pith treated with steam by about 14% (Chaji and Naserian, 2006). The objective of this experiment was to estimate the *in vitro* disappearance of dry matter (DM) and neutral detergent fiber (NDF) of untreated sugarcane pith and treated with steam and or enzyme (4 g/kg DM) by rumen isolated bacteria.

**Material and methods** Four fistulated sheep was used to collect rumen fluid which fed 250 g concentrate, 550 g lucerne hay and 200 g wheat straw, then centrifuged (1000 rpm, 10 min). Supernatant was used to grow bacteria in medium containing fungicides (benomyle: 500 ppm/ml medium and metalaxyle: 10 mg/ml medium) under anaerobic conditions at 39 °C for 24 h. These isolates were then used as a source of inoculum for culturing bacteria in a serum bottle containing 45 ml of culture medium of rumen bacteria (Galdwell and Bryant, 1966) and 1g of sugarcane pith as untreated (USP), treated with steam (SSP, at 19 bar for 3 min 70% moisture) and or with exogenous enzyme (ESP, 4 g/kg DM, the enzyme mixture composition was Cellulase, Xylanase, Betaglucanase, Alpha amylase, Pectinase, Phytase, Protease and Lipase as 0.03, 6.6, 10, 0.7, 0.7, 0.07, 0.5 and 3 MU/kg, respectively; Bioproton Pty. Ltd. Co.) under anaerobic conditions (using three times subculture), at 39 °C for 12, 24, 48, 72 and 96 h (3 replicates per time). The residual substrates of each bottle were then filtered and used to determine the DM and NDF concentrations. Data of DM and NDF disappearance in different times were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare the means at  $P < 0.05$ .

**Results** Disappearance of DM and NDF of samples using rumen isolated bacteria culture are given in Table 1. Sugarcane pith treated with steam had the highest disappearance rate of DM and NDF of in each incubation time ( $P < 0.05$ ) in compared with the other samples, using *in vitro* bacterial culture.

**Table 1** Disappearance of DM and neutral detergent fibre of sugarcane pith treated with steam and or enzyme by rumen bacteria

Incubation time (h)	DM disappearance (g /100g)					NDF disappearance (mg/ g)				
	USP	SSP	ESP	s.e.d	P	USP	SSP	ESP	s.e.d	P
12	34.2 <sup>c</sup>	45.0 <sup>a</sup>	39.3 <sup>b</sup>	0.65	<.0001	87.2 <sup>c</sup>	148.0 <sup>a</sup>	105.3 <sup>b</sup>	0.62	<.0001
24	36.4 <sup>c</sup>	51.1 <sup>a</sup>	45.6 <sup>b</sup>	0.51	<.0001	164.4 <sup>c</sup>	217.1 <sup>a</sup>	199.6 <sup>b</sup>	0.71	<.0001
48	47.2 <sup>c</sup>	63.2 <sup>a</sup>	55.1 <sup>b</sup>	0.72	<.0001	236.2 <sup>c</sup>	302.2 <sup>a</sup>	271.1 <sup>b</sup>	0.80	<.0001
72	56.1 <sup>c</sup>	71.1 <sup>a</sup>	60.2 <sup>b</sup>	0.70	<.0001	244.1 <sup>c</sup>	314.1 <sup>a</sup>	285.2 <sup>b</sup>	0.75	<.0001
96	58.3 <sup>c</sup>	73.3 <sup>a</sup>	63.3 <sup>b</sup>	0.42	<.0001	247.3 <sup>c</sup>	317.3 <sup>a</sup>	287.3 <sup>b</sup>	0.62	<.0001

Means with different letters within each row, differed significantly ( $P < 0.05$ )

**Conclusions** The present experiment showed *in vitro* bacterial digestion of DM and NDF of sugarcane pith was increased by steam or enzyme, and the effect of steam was more than enzyme. Results of the present study confirmed results Nsereko *et al.* (2000) that reported enzyme products containing xylanases and esterases had stimulatory effects on fibre degradation of alfalfa hay. Also Toussaint *et al.* (1991) reported the increase in enzymic hydrolysis after steam treatment. Results of the present study indicated DM and NDF digestion of sugarcane pith by bacteria could be improved by steam and or exogenous enzyme.

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## The effect of dietary level of steam treated sugarcane pith on digestibility and ruminal passage rate in Iranian Baluchi sheep

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**Introduction** Sugarcane (*Saccharum officinarum*) is an important crop in the southwest of Iran as well as tropical and subtropical regions of the world. One potential use of sugarcane pith (the fibrous residue following juice extraction and separation of the rind from bagasse) is as a feedstuff for ruminants. The low digestibility, high lignin and very low nitrogen content are considered as the main reasons for unsatisfactory performance of animals fed these roughages. Steam treatments improve voluntary intake and nutritive value of low-quality roughages. In order to better understand the processes of digestion, knowledge of the mean retention time (MRT) and rate of removal of particular components of the diet from gastro-intestinal tract (GIT) is required. In experiments on digestive function in ruminants, markers can be used to estimate the rate of movement of digesta within the GIT. The objective of this study was to evaluate the effect of level of steam treated sugarcane pith (STP) diet inclusion on apparent digestibility of total GIT, and rumen passage parameters.

**Material and methods** Four Iranian Baluchi wethers (30±2 kg wt.) fitted with rumen (the dorsal sac) and abomasal cannulae were used. They were housed indoors and confined in metabolism cages. The ration was given in equal portions at intervals of 3 h by an automatic interval feeder. Experimental treatments consisted of 1) the control diet (without STP, STP0), 2) 4% STP (STP4) 3) 8% STP (STP8) 4) 12% STP (STP12) per kg DM of diet. Incremental inclusion of STP replaced wheat bran in the control diet. The experiment was carried out in a change over design; 4 Baluchi wethers were allotted to 4 diets in a 4×4 Latin square design. The duration of each period consisted of 14 d of adaptation, 7 d of total faecal collection, 6 d of continuous ruminal injection of marker (Cr-EDTA) and 2d of rumen fluid sampling. The Cr-EDTA was prepared as described by Binnerts *et al.* (1968). The abomasal samples were obtained on 2 successive days (5<sup>th</sup> and 6<sup>th</sup> d) during marker injection at 3 h interval. When injection ceased, rumen digesta was collected at 0, 3, 6, 9, 12, 15, 18, 24, 48 h. Using the chemical components of diets and faeces, intake and digestibility of nutrients were calculated. The recommended equations of Weston and Hogan (1967) under steady state conditions were used to calculate passage rate parameters. Digestibility and ruminal digestion kinetics data were statistically analyzed in accordance with a standard latin square design using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

**Results** Intake and apparent digestibility of dry matter (DM) were not affected by treatment but the high pith treatment had the highest digestibility of NDF (61%) and ADF (51%) (P<0.05). Ruminal fluid dilution rate (RFDR), rumen turnover time (RTT), ruminal out flow rate (ROFR) and MRT were all affected by treatment in a linear direction (P<0.05). STP0 in comparison with STP12 had the highest RFDR (8.35 vs. 7.09) and ROFR (0.47 vs. 0.36 L/h) from rumen.

**Table 1** Effect of dietary inclusion level of steam treated sugarcane pith on feed intake and diet digestibility

	STP (g/kg DM of diet)				s.e.m	P-Value
	0	40	80	120		
DM intake (g/d)	831.37	823.37	860.57	839.83	11.83	NS
OM intake (g/d)	775.50	769.52	801.88	780.20	15.61	NS
DM digestibility (g/kg)	600.1	604.4	605.9	629.7	6.90	NS
OM digestibility (g/kg)	696.5	700.3	714.1	740.3	4.90	NS
NDF digestibility (g/kg)	507 <sup>a</sup>	540.1 <sup>b</sup>	563.7 <sup>b</sup>	608.8 <sup>c</sup>	6.90	L*
ADF digestibility (g/kg)	429.9 <sup>a</sup>	467 <sup>ab</sup>	497.8 <sup>b</sup>	568.9 <sup>c</sup>	11	L*

OM: Organic matter; L: Linear effect; NS: Non significant; \*P<0.05

**Table 2** Effect of dietary inclusion level of steam treated sugarcane pith on ruminal digestion kinetics

	STP (g/kg DM of diet)				s.e.m	Effect
	0	40	80	120		
Ruminal fluid dilution rate (%/h)	8.53 <sup>a</sup>	7.66 <sup>b</sup>	7.42 <sup>b</sup>	7.09 <sup>b</sup>	0.22	L*
Rumen turnover time (h)	11.97 <sup>b</sup>	13.14 <sup>a</sup>	13.53 <sup>a</sup>	14.17 <sup>a</sup>	0.34	L*
Ruminal Outflow rate (L/h)	0.47 <sup>a</sup>	0.397 <sup>ab</sup>	0.374 <sup>b</sup>	0.36 <sup>b</sup>	0.03	L*
MRT (h)	12.39 <sup>b</sup>	13.19 <sup>ab</sup>	13.50 <sup>a</sup>	14.03 <sup>a</sup>	0.40	L*

L: Linear effect; NS: non significant; MRT: Mean retention time; \*P<0.05

**Conclusion** The results of this experiment suggest that STP can be included up to 12% of dietary DM and leads to lower ROFR, RFDR and RTT and higher MRT when compared with a wheat bran based diet (STP0). The greater digestibility of NDF and ADF fractions suggest that steam treatment can improve the nutritive value of sugarcane pith.

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## Quantification of cellulolytic bacteria using *in vitro* culture containing treated or untreated cottonseed hulls determined by real-time polymerase chain reaction

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**Introduction** Treatment of lignocellulosic substances with an alkali solution removes lignin and decreases the crystallinity of cellulose, thereby increasing the biodegradation of cell walls by fibrolytic micro organisms located in the rumen (Gould, 1984; Krause, 2003). Major fibrolytic bacteria are the gram-negative *Fibrobacter succinogenes*, and two species of gram-positive bacteria, *Ruminococcus albus* and *Ruminococcus flavefaciens* (Krause, 2003). The objective of the present experiment was to quantify the cellulolytic bacteria population using *in vitro* culture containing sodium hydroxide treated or untreated cottonseed hulls (CH) determined by real-time polymerase chain reaction (RT-PCR).

**Material and methods** Cottonseed hulls were used as untreated or treated with NaOH as 20 g/kg DM [a 20% solution of NaOH was sprayed on CH and kept for 48 h (CH2S48) at room temperature]. Samples were incubated in medium prepared as described by Arroquy *et al.* (2005). Forty-five ml of medium was supplied into a 100 ml bottle containing 0.45 g of the feed sample (3 replicates). Then, each bottle was inoculated under carbon dioxide with 5 ml of isolated rumen bacteria. Rumen fluid was obtained from three sheep (49.5±2.5 kg body weight) fitted by rumen fistulae, before the morning feeding. The animals fed 1 kg/d DM of lucerne hay and 0.3 kg/d DM of concentrate (165 g CP/kg DM). Rumen fluid was immediately strained through four layers of cheesecloth. Then, the rumen fluid was centrifuged (10 min, 3000 rpm) and a solution of cycloheximide was added to protozoa free supernatant. The bottles were incubated for 96 h at 38.6°C. After the incubation, 1 ml of each bottle was sampled for DNA extraction. The extraction was done using Bioneer Accuprep Genomic DNA Extraction Kit. The 16S rRNA gene-targeted primers sets used in the present study were forward: 5'-GTGSTGCA YGGYTGTCTCA-3', 5'-GTTTCGGAATTACTGGGCGTAAA-3', 5'-CCCTAAAAGCAGTCTTAAGTTTCG-3' and 5'-CGAACGGAGATAATTTGAGTTTACTTAGG-3' for total bacteria, *Fibrobacter succinigenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*, respectively, and reverse: 5'-ACGTCRTCCMCACCTTCCTC-3', 5'-CGCCTGCCCTGAACATC-3', 5'-CCTCCTTGCGGTTAGAACA-3', 5'-CGGTCTCTGTATGTTATGAGGTATTACC-3' for total bacteria, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens*, respectively. Then, quantification of cellulolytic bacteria was carried out using RT-PCR (2 replicates for each primer). Bacterial rDNA concentrations were measured relative to total bacteria amplification ( $\Delta\Delta Ct$ ). Data were analyzed using the GLM procedure of SAS 9.1 and the means were compared by the Tukey test ( $P < 0.05$ ).

**Results** Quantity of the major species of cellulolytic bacteria existing in the *in vitro* culture relative to total bacteria population is shown in Table 1. Chemical treatment had no significant effect on the quantity of cellulolytic bacteria under present experimental condition.

**Table 1** Quantity of the major species of cellulolytic bacteria existing in the *in vitro* culture relative to total bacteria population

Items	Bacteria		
	<i>Fibrobacter succinogenes</i> × (10 <sup>-4</sup> )	<i>Ruminococcus flavefaciens</i> × (10 <sup>-7</sup> )	<i>Ruminococcus albus</i> × (10 <sup>-4</sup> )
Untreated CH	12	950	17
NaOH-treated CH	13	560	14
s.e.m	0.002	0.003	0.004
P	> 0.05	> 0.05	> 0.05

**Conclusions** Results of the present study indicate that the *in vitro* relative quantity of the major species of cellulolytic bacteria was not influenced by sodium hydroxide treatment of CH. Therefore, it was concluded that the treatment of CH with NaOH solution, as done in the present study might not alter the fibrolytic bacteria population. It was previously indicated that the digestibility of fibrous materials is generally related to rumen bacterial populations which are capable of producing wide range of fibrolytic enzymes (Krause, 2003). Therefore, it is not reasonable to get significant difference in digestibility of fibrous materials when treated with NaOH as obtained by Petersen *et al.* (1981), who reported no significant differences in OM digestibility of the roughages treated with NaOH at 4% of DM.

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## ***In vitro* gas production parameters of sesame (*Sesamum indicum*) straw treated with sodium hydroxide, urea or sulphuric acid**

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**Introduction** Sesame (*Sesamum indicum*) stover (SS) is the most abundant residue of sesame cultivation in semi-arid regions of Iran, and traditionally used as a basal feed in sheep and goat rations. However, voluntary feed intake and total tract digestibility of this straw are limited by its high complex carbohydrate content and lignin. The feeding value of low quality forages may often be improved with some additives such as urea and sodium hydroxide (Schingoethe *et al.*, 1980). The aim of the present study was to evaluate the *in vitro* gas production parameters of sesame stover treated with NaOH, urea or sulphuric acid.

**Material and methods** Sesame stover was obtained from Iranian plant varieties adapted to grow in semi-arid conditions. The straw was manually chopped (5cm length) and used untreated, or treated with sulphuric acid (SSA, 2ml/100 g DM), urea (SSU, 3g/100g DM), NaOH (SSN, 4g/100g DM) or both NaOH and urea [SSUN, NaOH as 4g/100g DM was sprayed on the straw and kept for 48h, then urea (3g/100g of initial DM) was added). Treated SS was ensiled under anaerobic conditions for 4 weeks. Samples were then taken, and dried and ground (to pass a 2 mm sieve). *In vitro* gas production was determined according to the method of Menke and Steingass (1988). Rumen fluid was collected from three ruminally fistulated sheep (42±2.5kg, body weight) and strained through four layers of cheesecloth. The laboratory handling of rumen fluid was carried out under a continuous flow of CO<sub>2</sub>. Into each syringe was weighed 200mg of sample material (4 replicates per treatment sample). The syringe was then filled with 30ml of medium consisting of 10ml rumen fluid and 20ml buffer solution as described by Menke and Steingass (1988). The syringes were placed in an incubator (38.6°C). Gas production was recorded after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96h of incubation. Statistical analysis was conducted using SAS (1999). The gas production data were fitted using an exponential equation of  $P = b(1 - e^{-ct})$ , where b is the gas production from the quickly and slowly fermentable fraction, c is the fractional gas production rate (/h), t is the incubation time (h) and P is the volume of gas produced at time t.

**Results** Gas production parameters of the samples are presented in Table 1. The b fraction of gas production parameter was significantly ( $p < 0.05$ ) increased when SS was treated with NaOH. However, sulphuric acid caused a decrease in the b fraction compared with untreated sesame straw ( $p < 0.05$ ). The c fraction for SSN was lower than SS.

**Table 1** Gas production parameters of sesame stover treated with NaOH, urea or sulphuric acid (mean± SE)

Parameters	Treatment					Significance level
	SS	SSA	SSU	SSN	SSUN	
b (ml/200mg DM)	59.4±2.38 <sup>a</sup>	51.0±2.47 <sup>b</sup>	60.7±2.76 <sup>ac</sup>	72.3±2.88 <sup>cd</sup>	61.8±2.47 <sup>ac</sup>	< 0.05
c (/h)	0.04±0.004 <sup>a</sup>	0.05±0.006 <sup>ab</sup>	0.03±0.004 <sup>ac</sup>	0.03±0.003 <sup>bcd</sup>	0.03±0.003 <sup>cd</sup>	< 0.05

**Conclusions** The results of the current study indicated that both urea and NaOH had a potential to enhance sesame straw digestibility as indicated by the gas production parameters under the conditions of the present study. These results confirmed previous findings where NaOH used as chemical means of improving the digestibility of cereal straw. It was previously indicated that sodium hydroxide might enhance the digestibility when applied to rice straw (Liu *et al.*, 2002).

**Acknowledgements** The authors gratefully acknowledge funding from Excellence Centre for Animal Science, Ferdowsi University of Mashhad.

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## Comparison of different native barley varieties using an *in vitro* gas production technique using rumen fluid from fistulated and from slaughtered sheep as inocula

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**Introduction** Different barley varieties have been shown to affect *in vitro* dry-matter digestibility (IVDMD), ruminal starch digestion and animal performance (Boss and Bowman, 1996). Some of these differences may be due to differences in site and extent of nutrient digestion among barley varieties. Little information is available on digestive characteristics of different barley varieties and several native varieties are cultivated in eastern Azerbaijan province, Iran. An *in vitro* gas production technique was used to evaluate the effects of barley variety on gas production characteristics of barley grain. The treatments compared were 1) Sahand barley, 2) Abidar barley, 3) Sararud barley, and 4) Qarah barley. Also this study was carried out to compare the use of slaughtered sheep's rumen fluid as the source of microbial enzyme for the gas production technique with that of rumen fluid collected from fistulated sheep.

**Material and methods** Rumen fluid was provided by two fistulated sheep (2h after the morning feed), fed twice daily with alfalfa hay (400 g/kg) plus commercial sheep concentrate (600 g/kg). Different barley varieties were collected from several farms in the eastern Azerbaijan province, Iran. For the second method, rumen contents were removed from two healthy sheep immediately after their slaughter. Both rumen fluid collected from fistulates and from slaughtered animals were strained through four layers of cheesecloth before use. Gas production was measured using the method of Fedorak and Hudey (1983). Triplicate ground and dried samples of each barley variety (approximately 300mg) were weighed and placed in 50 ml capacity serum bottles; then bottles were incubated in 20 ml of buffered rumen fluid (buffer: rumen fluid, 2:1, v/v) for 48h. Gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36 and 48 h of incubation. The data at the different times was analysed using completely randomised design.

**Results** Results showed that gas production volume was greatest for the Sahand barley and lowest from the Qarah barley (Table 1). No significant difference between Abidar and Sararud varieties was observed. There was a relationship between *in vitro* gas production volume (ml/g DM), using fistulate rumen fluid (x), and *in vitro* gas production volume (ml/g DM) using slaughtered animal's rumen fluid (y) at different time intervals which suggests that rumen fluid from slaughtered animals can be used successfully for evaluating concentrate feedstuffs with the gas production technique (Table 2).

**Table 1** Effect of barley variety on *in vitro* gas production with fistulated sheep's rumen fluid (ml/g DM).

Time	Barley varieties				s.e
	Sahand	Qarah	Sararud	Abidar	
2h	27.5 <sup>ab</sup>	23.6 <sup>b</sup>	26.6 <sup>ab</sup>	30.3 <sup>a</sup>	0.94
12h	79.9 <sup>a</sup>	66.5 <sup>b</sup>	74.4 <sup>ab</sup>	81.8 <sup>a</sup>	1.82
24h	184.5 <sup>a</sup>	175.4 <sup>d</sup>	178.3 <sup>c</sup>	180.7 <sup>b</sup>	1.92
48h	206.5 <sup>a</sup>	198.2 <sup>c</sup>	203.1 <sup>b</sup>	203.2 <sup>b</sup>	1.33

Means in the same row with different letters (a, b, c, d) differ ( $P < 0.05$ ).

**Table 2** The relationship (simple x, y regression) between *in vitro* gas production volume (ml/g DM), using rumen fluid (x), and *in vitro* gas production volume (ml/g DM) using faecal fluid (y) in different time intervals.

Varieties	Incubation time	
	2h - 48h	2h, 12h, 24h, and 48h
	Variance accounted for ( $r^2$ )	
Sahand	0.92	0.88
Qarah	0.96	0.94
Sararud	0.96	0.93
Abidar	0.95	0.93

**Conclusions** Our results showed that there is a significant difference between barley varieties in *in vitro* gas production and they have different starch degradation characteristics. These differences may be important since barley is a source of rapidly degradable starch in the rumen and lower amounts of it would lead to decreased production level, while an excess of rapidly digestible starch may increase incidence of disorders such as acidosis or bloat. In addition, our results suggest that rumen fluid from slaughtered animals can be used to evaluate concentrate feedstuffs in the *in vitro* gas production technique, thus eliminating the need for fistulated animals, and possibly reducing the costs of the method. The total gas production volume obtained using by slaughtered animal's rumen fluid was is higher than that obtained from fistulate rumen fluid and so mathematical correction might be needed.

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## How binding compounds interfere in the *in vitro* rumen degradability results of the gas production bioassay for tannins

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**Introduction** The tannin bioassay is widely used to evaluate the effects of tannins on the rumen fermentation. To attenuate the tannin interference, polyethylene glycol (PEG) is used as binding compound. In presence of PEG, the complex formed by tannins and feed nutrients is broken and a new complex, more stable, is formed by PEG and tannins. Another compound used as binding compound is the polyvinyl polypyrrolidone (PVPP). The aim of this work was to evaluate the effect of addition of binding compounds on the degradability results of the gas production bioassay for tannins.

**Material and methods** The bioassay was carried out according to Bueno *et al.* (2008) using five substrates (feeds) and three inocula prepared from sheep rumen liquor. The obtained gas production profiles are published in Bueno *et al.* (2008). The tested feeds were: feed A - aroeira (*Astronion urundeuva*), feed B - jurema preta (*Mimosa hostilis*), feed C - sorghum grain (*Sorghum bicolor*), and two mixtures with 45% of sorghum leaves and 45% of concentrate (maize and soybean meal) plus 10% of either quebracho (*Schinopsis lorentzii*) extract (feed D) or Acacia (*Acacia mollissima*) tannin extract (feed E). For each feed, six bottles (with 1g of substrate each) were incubated for each inoculum: two as control and four with binding compounds (two with PVPP and two with PEG). Eight Santa Inês adult wethers (about 45 kg LW) were used as inoculum donors. For each inoculum, liquid and solid phases of rumen digesta were collected separately and the inoculum was prepared using 0.50 of each phase. Animals were fed Tifton hay and concentrate (soybean meal and maize), at the rate 70:30. The binding compounds were added to the substrate directly into the fermentation flasks (1g of binding compound per g of substrate). The interference of binding compounds in degradability results was evaluated directly and using the partitioning factor (mg substrate truly degraded per ml gas produced). Feeds and treatments were incorporated in a randomised design, in a complete factorial arrangement. Treatments (binding compounds) were compared by Tukey test.

**Results** The addition of PEG and PVPP promoted the increase of gas production, indicating that some feed nutrients were bound by tannins (Bueno *et al.*, 2008). PEG showed higher affinity to the tannins than PVPP. When binding compound is added to the substrate, the complex tannin-feed nutrient is broken and a new complex is formed. The nutrients become available to fermentation (degradation), resulting in an effective increase of degradability. But the complex tannin-PEG or tannin-PVPP is insoluble and is recovered in the filtered residue. Thus either PEG or PVPP contaminate the residue and increase its weight, decreasing the degradability result (Table 1). Even free binding compounds can interfere in the results. Although PEG is water soluble, PVPP is not, and the contamination in this case is even higher. PEG promotes a great increase in degradability of tanniniferous feeds, and the results usually are higher than the control, the results would be really higher if the PEG contamination would be discounted.

**Table 1** *In vitro* dry matter degradability and partitioning factor of five feeds incubated for 24 and 96 h

Time of Incubation	Feed	DM degradability				Partitioning factor (mg/ml)			
		control	PVPP	PEG	mean	control	PVPP	PEG	mean
24 h	Feed A	0.411	0.307	0.410	0.376 <sup>B</sup>	7.54	4.97	4.74	5.75 <sup>B</sup>
	Feed B	0.268	0.227	0.214	0.236 <sup>C</sup>	11.73	5.52	4.88	7.38 <sup>A</sup>
	Feed C	0.535	0.494	0.591	0.540 <sup>A</sup>	3.86	3.28	4.01	3.71 <sup>C</sup>
	Feed D	0.463	0.527	0.642	0.544 <sup>A</sup>	5.75	4.43	5.39	5.19 <sup>B</sup>
	Feed E	0.512	0.526	0.660	0.566 <sup>A</sup>	6.06	4.50	6.11	5.56 <sup>B</sup>
	mean	0.437 <sup>b</sup>	0.416 <sup>b</sup>	0.503 <sup>a</sup>	-	6.98 <sup>a</sup>	4.54 <sup>b</sup>	5.02 <sup>b</sup>	-
96 h	Feed A	0.517	0.443	0.491	0.484 <sup>B</sup>	4.09	3.18	3.09	3.47 <sup>A</sup>
	Feed B	0.297	0.282	0.264	0.281 <sup>C</sup>	4.49	3.07	2.82	3.46 <sup>A</sup>
	Feed C	0.783	0.742	0.796	0.774 <sup>A</sup>	2.97	2.70	2.93	2.87 <sup>B</sup>
	Feed D	0.721	0.745	0.821	0.763 <sup>A</sup>	3.54	3.09	3.42	3.35 <sup>A</sup>
	Feed E	0.750	0.757	0.832	0.780 <sup>A</sup>	3.58	3.17	3.56	3.44 <sup>A</sup>
	mean	0.614 <sup>b</sup>	0.594 <sup>c</sup>	0.641 <sup>a</sup>	-	3.74 <sup>a</sup>	3.05 <sup>b</sup>	3.16 <sup>b</sup>	-

<sup>A,B,C</sup> means of feeds, for each time of incubation, followed by different letters, within columns, are significantly different (P<0.05)

<sup>a,b,c</sup> means of treatments, for each variable, followed by different letters, within rows, are significantly different (P<0.05)

**Conclusion** In bioassays with binding assays, it is not recommended to evaluate the alterations in degradability as a tool to evaluate feed quality because there is an unavoidable contamination of the non degraded residue.

**Acknowledgements** The authors gratefully acknowledge funding from CNPq and FAPESP.

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## Fermentation and *in vitro* gas production of high pressure steam treated sugarcane pith by rumen fungi

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**Introduction** Gas production technique is a useful procedure to assess digestible value of the ruminant feeds. Sugarcane pith is a by-product of the final stage of the processing of sugarcane as it passes through rotary sieves to separate fine particles. Steam explosion has shown considerable potential as a method for the cost-effective pre-treatment of lignocellulosic material. At the end of this process, a substantial proportion of the hemicellulose fraction is made water soluble and the lignin fraction is modified to improve enzymatic accessibility and digestibility. By applying the steam explosion process to sugarcane bagasse, Kling *et al.* (1987) demonstrated that about 60% of the hemicellulose fraction was hydrolysed and the susceptibility of cellulose to enzymatic hydrolysis was increased. For treatments by steam, harsh conditions are needed ( $t > 180^{\circ}\text{C}$ ), these conditions result in formation of furfural by secondary dehydration reactions of hemicellulosic pentoses and soluble phenolic compounds, which may inhibit the activity of rumen microbes and cell-free enzymes. If the high pressure steam treatment had negative effects on activity of rumen microbes particularly fungi (rumen fungi are as primary colonizers of fibrous plant materials that degrade lignin-containing plant cell walls, and the ability of anaerobic fungi to degrade and utilize lignin would be an important attribute for rumen micro-organisms), the steam-processing industries of sugarcane pith should correct their processing method. Therefore, the objective of this study was to evaluate the effect of high pressure steam on the gas production parameters of sugarcane pith by rumen fungi during *in vitro* fermentation.

**Material and methods** The experimental samples were: untreated sugarcane pith (USP) or steam treated sugarcane pith (SSP), (at 19 bar for 3 min, 70% moisture). About  $200 \pm 10$  mg of oven dried and milled sample (1.0 mm screen) was incubated with 35 ml buffered rumen fungi in 100 ml glass syringes, according to the method of Menke and Steingass (1988). To prepare anaerobic rumen fungi, rumen fluid was collected from two fistulated Holstein steers ( $400 \pm 12$  Kg, body weight) fed twice daily a diet containing 5.72 kg lucerne hay and 3.08 kg concentrate mixture, then centrifuged (1000 rpm, 10 min) and streptomycin sulphate, penicillin G and chloramphenicol added (0.1 mg/ml each). All samples were incubated in triplicate (one run) with three syringes containing only incubation medium (blank) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation  $Y = B(1 - e^{-ct})$ , where B is the gas production from the fermentable fraction (ml), C is the gas production rate constant for B, t is the incubation time (h) and Y is the gas produced at time t. *In vitro* digestibility of organic matter (OMD, g/kg OM) of samples was calculated by the equation of Menke and Steingass (1988). Microbial biomass (MB) production was estimated by method of Blummel *et al.* (1997). Data of gas production parameters, OMD and MB were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range tests was used to compare treatment means at  $P < 0.05$ .

**Results** *In vitro* gas production parameters [(B) and (C)], OMD and MB of the samples by rumen fungi are shown in Table 1. All items were significantly influenced by the treatment. Gas production parameters of SP were significantly higher than UP ( $P < 0.01$ ). Steam resulted in increase OMD compared with the untreated samples (175.5 vs. 158.2 ml per 200 mg DM). Value of MB was decreased when sugarcane pith was treated with steam.

**Table 1** *In vitro* gas production parameters, OMD and MB of steam treated (STP) sugarcane pith by rumen fungi

	USP	SSP	s.e.m	P
B (ml)	29.5 <sup>b</sup>	45.6 <sup>a</sup>	0.51	<.0001
C (ml/h)	0.02 <sup>b</sup>	0.03 <sup>a</sup>	0.003	<.0001
OMD (g/kg OM)	158.2 <sup>b</sup>	175.5 <sup>a</sup>	0.006	<.0001
MB (mg/g)	62.3 <sup>a</sup>	49.1 <sup>b</sup>	0.51	<.0001

UP: Sugarcane pith untreated; SP: Steam treated sugarcane pith, B: Gas production from the fermentable fraction; C: Rate constant of gas production; OMD: Organic matter digestibility; MB: Microbial biomass; s.e.m: Standard error of mean, Means with different letters within samples differed ( $P < 0.05$ )

**Conclusions** It was concluded that *in vitro* gas production parameters, OMD and MB of sugarcane pith treated with high-pressure steam by anaerobic rumen fungi were improved compared with the untreated samples. The results of the present study indicated that use of high-pressure steam for treatment of sugarcane pith, solubilised the hemicellulose fraction which resulted in improved fermentation and gas production, as well as had no negative effect on rumen fungi.

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## Inter-animal variation in aspects of ruminal digestion when cattle were offered a range of rations

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**Introduction** Knowledge of the variability within a group of animals for a particular measurement is a key requirement when estimating the power of an experiment and the number of replicates required to conclude that a particular scale of difference between treatments is statistically significant. Studies on ruminal digestion using surgically modified animals frequently use a small number of animals and change-over experimental designs, so estimation of variances “a priori” can be difficult. In addition, knowledge of inter-animal variability in aspects of ruminal digestion could facilitate identification of animals with a more desirable pattern and/or extent of digestion. The objective of this study was to document the variation in aspects of ruminal digestion within a group of genetically similar beef cattle when offered contrasting rations.

**Material and methods** A group of 15 Rotbunde x Friesian steers, (from the same sire) fitted with ruminal cannulae were individually offered in sequence, a concentrate/straw ration (C: 20 g dry matter (DM)/kg body weight(W)), this ration supplemented with fish/plant oil (CO: 17.8 g DM/kg W), zero-grazed grass (G: 18 g DM/kg W) and zero-grazed grass supplemented with fish/plant oil (GO: 15.5 g DM/kg W). During the final week of each period of 7 weeks duration, rumens were emptied on three consecutive days at 1 hour prior to feeding (Day 1), 5 hours post-feeding (Day 2) and 11 hours post-feeding (Day 3). Both liquid and solid fractions of ruminal digesta were weighed and sampled at each evacuation for chemical analysis. Pool sizes of various components were mathematically reconstructed. The rate of disappearance of selected components was calculated as the difference in pool size/time interval. Variances compared by a Levene’s test using SAS.

**Results** The variances for pools of volatile fatty acids at each sampling time were similar across the rations examined (Table 1). The variances for the ammonia pool were lower ( $P < 0.05$ ) for the oil-supplemented rations at 5 and 11 hours post-feeding. Across the rations examined, variances were homogenous at each sampling time for the pools of organic matter (OM) and neutral detergent fibre (NDF). The variance for OM disappearance was highest for C and lowest for GO but was homogenous for NDF disappearance.

**Table 1** Variances (mean<sup>1</sup>) of ruminal digesta pools at different times post-feeding and digesta disappearance in steers

Ration	Time	C	CO	G	GO	P
Acetate	0	0.249 (1.91)	0.137 (1.73)	0.251 (1.69)	0.677 (2.92)	NS
	5	0.392 (2.20)	0.261 (2.58)	0.765 (3.57)	0.372 (3.21)	NS
	11	0.449 (2.54)	0.263 (1.97)	0.262 (2.63)	0.184 (2.41)	NS
Propionate	0	0.011 (0.43)	0.006 (0.42)	0.011 (0.36)	0.028 (0.67)	NS
	5	0.048 (0.62)	0.019 (0.77)	0.056 (1.04)	0.029 (0.94)	NS
	11	0.055 (0.65)	0.014 (0.52)	0.016 (0.60)	0.011 (0.60)	NS
Butyrate	0	0.014 (0.43)	0.014 (0.32)	0.010 (0.26)	0.018 (0.47)	NS
	5	0.037 (0.53)	0.022 (0.50)	0.043 (0.76)	0.016 (0.70)	NS
	11	0.036 (0.52)	0.012 (0.37)	0.013 (0.44)	0.009 (0.43)	NS
Ammonia	0	0.010 (5.88)	0.002 (4.74)	0.001 (2.64)	0.002 (1.90)	NS
	5	0.008 (4.17)	0.003 (1.90)	0.009 (5.38)	0.003 (2.13)	*
	11	0.007 (3.76)	0.003 (2.98)	0.002 (3.64)	0.001 (1.73)	*
OM	0	1.4 (8.35)	1.3 (8.09)	1.0 (7.22)	1.0 (8.06)	NS
	5	1.3 (6.76)	0.8 (6.08)	0.7 (5.17)	0.5 (5.47)	NS
	11	1.0 (4.99)	0.9 (5.04)	0.7 (3.97)	0.6 (4.27)	NS
NDF	0	0.7 (4.60)	0.5 (4.09)	0.3 (3.70)	0.4 (4.02)	NS
	5	0.5 (4.11)	0.3 (3.53)	0.2 (2.73)	0.2 (3.17)	NS
	11	0.5 (2.9)	0.4 (3.22)	0.3 (2.37)	0.3 (2.60)	NS
OM disappearance	-	0.03 (0.29)	0.01 (0.17)	0.01 (0.20)	0.002 (0.19)	*
NDF disappearance	-	0.006 (0.14)	0.004 (0.07)	0.002 (0.07)	0.002 (0.09)	NS

<sup>1</sup>Units =mol for acetate, propionate and butyrate; g for ammonia; kg for OM and NDF and kg/h for OM and NDF disappearance

**Conclusions** This study provides baseline data on the variances around the means of several aspects of ruminal digestion. In general, variances were homogenous across diverse rations indicating that a statistical procedure such as analysis of variance can be applied with confidence.

**Acknowledgements** Financial support via the European Union 6<sup>th</sup> Framework Project ProSafeBeef (FOOD-CT-2006-36241) is gratefully acknowledged.

## Effect of polyethylene glycol and polyvinylpyrrolidone on *in vitro* gas production of raisin waste

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**Introduction** In the Middle East, animals suffer from under feeding and malnutrition in winter due to the shortage of locally produced feeds which are not sufficient to cover the nutritional requirements of the animals (Besharati and Taghizadeh, 2009). The annual amount of agricultural by-products produced in Iran is large. The production of grape exceeds 2.87 million tonnes/year, with a high proportion of the yield being used for production of dried grape products. In this process, large amounts of raisin waste are produced. Raisin waste contains pieces of raisin plus grape cluster stems. There is little information available on the nutritive value of raisin waste. However, inclusion of grape by-product in the diet reduced digestibilities of the diet (Baumgartel *et al.*, 2007). Yinrong Lu and Yeap Foo (1999) reported that grape pomace tannins have adverse effects on nutrient utilisation, and are toxic at high intake levels (Reed, 1995) due to their ability to bind proteins, minerals and carbohydrates (McSweeney *et al.*, 2001). Tannins are the most widely occurring anti-nutritional factor in non-conventional feeds. Polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP) have a high affinity for tannins. Addition of PEG results in the formation of PEG-tannin complexes which inactivates tannins. The aim of this study was to determine the effect of PEG and PVP on *in vitro* gas production kinetics of raisin waste.

**Material and methods** The chemical composition of raisin waste was determined using the methods recommended by AOAC (1999). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations were determined using the methods of Van Soest *et al.* (1991). Total phenolics (TP) were measured using the Folin Ciocalteu method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteu reagent (Makkar, 2000). Tannic acid was used as the standard to express the amount of TP and TT. Ruminal fluid was collected from two fistulated sheep, about 2 h after their morning feed. Gas production was measured by the method of Fedorak and Hruby (1983). Approximately 300 mg of dried and ground (2 mm) raisin waste sample was weighed and placed into serum bottles in the presence (300 mg) and in the absence of PEG and PVP. There were three replicates per treatments. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml/g DM. The data at the different times was analysed using completely randomised design by the GLM procedure of SAS Institute Inc (2002).

**Results** The chemical composition of raisin waste is presented in Table 1. Total gas production (ml/g DM) from raisin waste incubated with and without PEG or PVP at different incubation times are shown in Table 2. At the early incubation times (2 and 4 h), the control treatment (no PEG and PVP) had the highest gas production volume among treatments, but up to 6 h of incubation time the gas production volumes for PEG and PVP treatments (Table 2) were increased ( $p < 0.05$ ).

**Table 1** The chemical composition of raisin waste (g/kg DM)

Feed	DM	CP	NDF	ADF	OM	Total phenols	Total tannins
Raisin waste	916	62.4	280	276	927.7	96.3	72.1

**Table 2** Total gas production volume (ml/g DM) from raisin waste incubated with and without PEG or PVP.

Treatments	Incubation times (h)								
	2	4	6	8	12	16	24	36	48
Raisin waste	33.1 <sup>a</sup>	73.8 <sup>a</sup>	101.0 <sup>b</sup>	118.7 <sup>b</sup>	141.8 <sup>b</sup>	156.6 <sup>b</sup>	180.9 <sup>b</sup>	203.2 <sup>b</sup>	208.7 <sup>b</sup>
Raisin waste + PEG	12.7 <sup>b</sup>	62.0 <sup>b</sup>	109.6 <sup>a</sup>	134.3 <sup>a</sup>	158.4 <sup>a</sup>	180.3 <sup>a</sup>	204.4 <sup>a</sup>	229.4 <sup>a</sup>	247.1 <sup>a</sup>
Raisin waste + PVP	9.8 <sup>b</sup>	54.4 <sup>c</sup>	104.6 <sup>ab</sup>	132.8 <sup>a</sup>	159.9 <sup>a</sup>	182.3 <sup>a</sup>	206.5 <sup>a</sup>	229.7 <sup>a</sup>	247.9 <sup>a</sup>
SEM	1.41	1.49	1.85	2.51	3.51	3.74	3.87	3.64	3.54

The means within a column without a common letter differ ( $p < 0.05$ ).

**Conclusions** PEG and PVP, which are non-nutritive synthetic polymers, have high affinities to tannins and makes tannins inert by forming tannin complexes. The increase in the gas production in the presence of PEG and PVP is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen and PEG and PVP preventing the tannins from bonding with the protein, thus making the protein more digestible.

**Acknowledgement** This study was funded by University of Tabriz, Tabriz, Iran.

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## Use of real-time polymerase chain reaction assay for monitoring *in vitro* ruminal cellulolytic bacteria population as affected by non-structural carbohydrates

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**Introduction** Cellulose is the most abundant polymer in nature, but mammals do not synthesize enzymes to digest cellulose. Mosoni *et al* (2007) demonstrated that the dynamics of cellulolytic bacteria were in good correlation with the response to diet shift, particularly the changes of concentrate. The aim of the present study was to determine the effect of adding of non-structural carbohydrates (NSC) including sucrose, starch and equal mixture of them to an *in vitro* medium containing cellulose (Cell) as sole nutrient on both ruminal total anaerobic bacteria population and two major cellulolytic bacterial species (*Fibrobacter succinogenes* and *Ruminococcus Albus*) using SYBR Green real-time polymerase chain reaction (PCR) assay.

**Material and methods** Experimental treatments consisted of non-supplemented Cell (150 mg) and Cell plus NSC (70 mg) as sucrose (CellSu) or starch (CellSt) or a 1:1 mixture of sucrose and starch (CellSuSt). Treatments were incubated in a 40% rumen fluid medium prepared as described by Arroquy *et al.* (2005). Forty five ml of medium were distributed into a 100 ml bottle. Then, each bottle was inoculated with 5 ml of strained rumen fluid, taken from 3 sheep before the morning feeding, and finely bubbled with CO<sub>2</sub>. The bottles (three bottles per each treatment) were incubated under anaerobic conditions for 48 h at 39 °C. Then, each bottle contents were filtered through a 22 µm filter, and liquid phase was used for DNA extraction. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. The designed 16s rRNA gene-targeted primer sets used for the real-time PCR are described in Table 1. Cellulolytic bacterial rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). PCR conditions for all species were as follows: 15 s at 95°C for denaturing, 15 s at 61°C for annealing and 30 s at 72°C for extension (40 cycles), except for 5 min denaturation in the first cycle. Data are expressed relative to quantification of the total bacterial population. Data were analyzed as a complete randomized design using GLM procedure of SAS (2003). Model was: Y = Mean + Treatment + residual.

**Table 1** PCR primers for real-time PCR assay

Target species	Forward primer	Reverse primer	Size (bp)
Total bacteria	5'-GTGSTGCAYGGYTGTCTGCA-3'	5'-ACGTCRTCCMCACCTTCCTC-3'	120
<i>Fibrobacter succinogenes</i>	5'-GTTCGGAATTACTGGGCGTAAA-3'	5'-CGCCTGCCCCTGAACTATC-3'	175
<i>Ruminococcus Albus</i>	5'-CCCTAAAAGCAGTCTTAGTTCG-3'	5'-CCTCCTTGCGGTTAGAACA-3'	122

**Results** Table 2 shows the population of the *in vitro* target cellulolytic and total bacteria in the medium containing cellulose while their responses to supplementing NSC enumerated by the real-time PCR assays. The results of the present study showed that adding different types of NSC did not have any significant effect on *F. succinogenes* and *R. Albus* as representatives of cellulolytic bacteria. However, it tended (P=0.09) to increase the total bacteria population.

**Table 2** *In vitro* DNA concentration of total bacteria and the population of the cellulolytic bacteria relative to total bacteria in the medium containing cellulose and different types of non-structural carbohydrates

Bacteria	Treatments				s.e.m	P
	Cell	CellSu	CellSt	CellSuSt		
Total bacteria (ng/µl)	0.0200	0.0226	0.0297	0.0557	0.0094	0.09
<i>Fibrobacter succinogenes</i>	0.3992	0.5691	0.5612	0.5152	0.0940	0.58
<i>Ruminococcus Albus</i>	0.0191	0.0218	0.0216	0.0118	0.0099	0.87

**Conclusions** Generally, treatments used in the present study had no significant effect on ruminal cellulolytic bacteria population relative to the total bacteria. Similarly, the use of competitive PCR to quantify cellulolytic bacterial species did not clearly demonstrate that high energy diets could result in a decrease in the cellulolytic bacterial populations (Koike and Kobayashi 2001). Martin *et al.* (2002) argued that this type of diet affected the fibrolytic enzyme activities more than the number of cellulolytic bacteria. However, there is a need to evaluate the effect of type of NSC on these bacteria under *in vivo* condition.

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## Effect of various Iranian native medicinal herbs or spices on *in vitro* ruminal disappearance of lucerne hay

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**Introduction** Recently, some studies have been conducted to determine the effects of medicinal herbs and spices on rumen microbial fermentation and nutrient disappearance (Busquet *et al.*, 2006; Hart *et al.*, 2008). However, a wide range of different results have been obtained when various medicinal herbs and spices have been added to different feed sources. The aim of the present study was to determine the effect of various Iranian native medicinal herbs or spices on *in vitro* disappearance of dry matter (DM), crude protein (CP) and neutral detergent fibre (NDF) of lucerne hay incubated with buffered rumen fluid.

**Material and methods** *In vitro* incubation was carried out as proposed by Menke and Steingass (1986). Approximately 300mg of dried and ground (through 2mm screen) lucerne hay (control, NDF = 537 and CP = 150g/kg DM) or lucerne hay plus 18mg DM of either garlic, nutmeg, cinnamon, cumin, or rosemary were placed in 100 ml glass syringes (5 replicates per each sample). Each syringe contained 40ml of buffered rumen fluid (ratio of buffer to rumen fluid, 2:1). Rumen fluid was obtained from three adult ruminally fistulated sheep ( $49.5 \pm 2.5$ kg, body weight), before their morning feed; the rumen fluid was immediately strained through four layers of cheesecloth. Syringes were incubated under a CO<sub>2</sub> atmosphere at 38.5°C. After 24h of the incubation, the syringe contents were filtered (48µm pore size) and residues were dried at 60 °C for 48h. Dry matter, CP and NDF concentrations of the residues were determined. Data were analysed using SAS (V. 9/1) and the Dennett's test used to compare the means ( $P < 0.05$ ).

**Results** The effect of medicinal herbs and spices on *in vitro* DM, CP and NDF disappearances of lucerne hay are shown in Table 1. Results of the present study indicated that turmeric and garlic caused a significant ( $P < 0.05$ ) increase in DM disappearance of lucerne hay. Under the conditions of the present study, medicinal herbs and spices caused also a significant ( $P < 0.05$ ) increase in the ruminal disappearances from lucerne hay, of CP and also, with the exception of rosemary, NDF.

**Table 1** *In vitro* disappearance of dry matter, crude protein and neutral detergent fibre from lucerne hay, alone (control) or with Iranian native herbs or spices, following 24h incubation with buffered rumen fluid

Treatments	Nutrients		
	Dry matter	Crude protein	Neutral detergent fibre
Lucerne hay (control)	0.542	0.617	0.369
Lucerne hay + Garlic	0.603 *	0.696*	0.533*
Lucerne hay + Nutmeg	0.572	0.720*	0.468*
Lucerne hay + Cinnamon	0.574	0.722*	0.471 *
Lucerne hay + Cumin	0.584	0.677*	0.452*
Lucerne hay + Turmeric	0.623*	0.756*	0.489*
Lucerne hay + Rosemary	0.556	0.705*	0.417
s.e.m	0.003	0.002	0.004

In each column an asterisk (\*) indicates  $P < 0.05$  compared with the control using Dennett's test.

**Conclusions** Results suggested that the medicinal herbs or spices used under the experimental conditions of this study (24h *in vitro* incubation) might alter ruminal disappearance of the lucerne hay nutrients. It was previously demonstrated that some medicinal herbs or spices may improve the cellulolytic and proteolytic activities of rumen microbiota (Khan and Chaudhry, 2008). These natural additives have the potential to alter the ruminal digestibility of ruminant feeds when used at appropriate concentrations. However, there is a need to test these herbs and spices under *in vivo* conditions using a wide range of different feedstuffs.

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## Effect of adding polyethylene glycol and polyvinylpyrrolidone on organic matter digestibility, metabolizable energy and net energy for lactation of grape pomace using *in vitro* gas production technique

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**Introduction** A major constraint to increasing livestock productivity in developing countries is the scarcity and fluctuating quantity and quality of the year-round supply of conventional feeds. In order to meet the projected high demand of livestock products and to fulfill the future hopes of feeding the millions and safeguarding their food security, the better utilization of non-conventional feed resources which do not compete with human food is imperative (Besharati *et al.*, 2008). The annual amount of produced agro-by-products in Iran is generous, whereas, production of grape exceeds 2.87 billion tonnes/year, that proportion of grape yield is used for production of grape juice. In this process, grape pomace is produced in high level (Besharati and Taghizadeh, 2009). There is little information available on the nutritive value of grape pomace. Although grape pomace is low in ME, it has been used in diets of ruminants fed close to maintenance ME levels, especially in sheep (Abel & Icking, 1984). However, inclusion of grape pomace in the diet reduced digestibilities of the diet (Baumgartel *et al.*, 2007). Yinrong Lu & Yeap Foo (1999) reported that grape pomace tannins have adverse effects on nutrient utilization, and are toxic at high intake levels (Reed, 1995) due to their ability to bind proteins, minerals and carbohydrates (McSweeney *et al.*, 2001). Tannins are the most widely occurring anti-nutritional factor in non-conventional feeds. The aim of this study was to determine the effect of adding polyethylene glycol and polyvinylpyrrolidone on organic matter digestibility, metabolizable energy and net energy for lactation of grape pomace using *in vitro* gas production technique.

**Material and methods** The chemical composition of grape pomace was determined using the methods recommended by AOAC (1999). The NDF and ADF concentrations were determined using the methods of Van Soest *et al.* (1991). Total phenolics (TP) were measured using the Folin Ciocalteu method (Makkar, 2000). Total tannin (TT) was determined after adding insoluble polyvinylpyrrolidone and reacting with Folin Ciocalteu reagent (Makkar, 2000). Tannic acid was used as the standard to express the amount of TP and TT. Ruminal fluid was collected approximately 2 h after morning feeding from 2 cannulated sheep. Gas production was measured by Fedorak and Hruby (1983) method. Approximately 300 mg of dried and ground (2 mm) grape pomace sample was weighed and placed into serum bottles in the absence and presence (300 mg) of PEG or PVP. The gas production was recorded after 24 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 0.02 gram of DM. The organic matter digestibility (OMD), ME and NE<sub>L</sub> contents of forages were estimated by the method of Menke and Steingass (1988). The short chain fatty acid content calculated using equation  $SCFA_{(mmol)} = 0.0222GP - 0.00425$ . The data at the different times was analyzed using completely randomized design by the GLM procedure of SAS Institute Inc (2002).

**Results** The chemical compositions of grape pomace are presented in Table 1. Effect of adding PEG and PVP on OMD, ME, GP, SCFA and NE<sub>L</sub> are shown in Table 2. Within the treatments, treatments with PEG or PVP had the highest OMD, ME, GP, SCFA and NE<sub>L</sub> values ( $P < 0.05$ ). Adding polyethylene glycol and polyvinylpyrrolidone to grape pomace increased the organic matter digestibility, metabolizable energy, short chain fatty acid and net energy for lactation of grape pomace.

**Table 1** The chemical composition of grape pomace (%DM)

Feed	DM	CP	NDF	ADF	Crude fat	OM	Total phenols	Total tannins
Grape pomace	93.3	6.62	18.7	18.4	1.41	87.7	3.01	2.27

**Table 2** Effect of adding PEG and PVP on OMD, ME, GP, SCFA and NE<sub>L</sub> \*

Treatments	Estimated parameters				
	GP <sub>(ml/0.2 g DM)</sub>	OMD <sub>(%)</sub>	ME <sub>(MJ/kg DM)</sub>	NE <sub>L</sub> <sub>(Mcal/lb)</sub>	SCFA <sub>(mmol)</sub>
Grape pomace	46.3 <sup>b</sup>	56.1 <sup>b</sup>	8.54 <sup>b</sup>	0.237 <sup>b</sup>	1.024 <sup>b</sup>
Grape pomace + PEG	55.6 <sup>a</sup>	64.3 <sup>a</sup>	9.79 <sup>a</sup>	0.254 <sup>a</sup>	1.229 <sup>a</sup>
Grape pomace + PVP	55.3 <sup>a</sup>	64.1 <sup>a</sup>	9.76 <sup>a</sup>	0.253 <sup>a</sup>	1.223 <sup>a</sup>
SEM	0.491	0.067	0.436	0.0009	0.0109

\* The means within a column without common letter differ ( $p < 0.05$ ).

**Conclusions** The increase in OMD, ME, GP, SCFA and NE<sub>L</sub> in the presence of PEG and PVP is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen.

**Acknowledgement** This study was funded by University of Tabriz, Tabriz, Iran.

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## Continuous monitoring of ruminal pH and redox-potential in dry cows using a novel wireless ruminal probe

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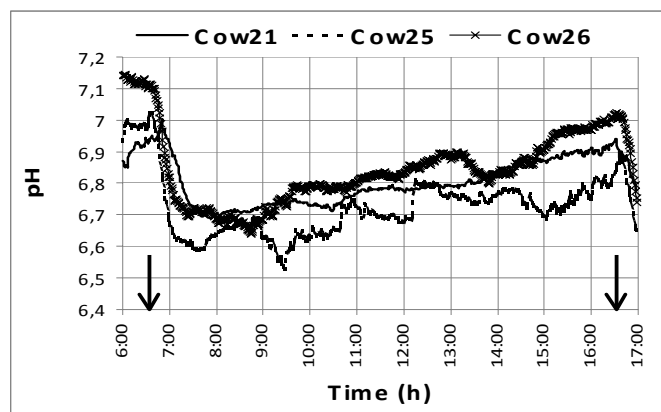
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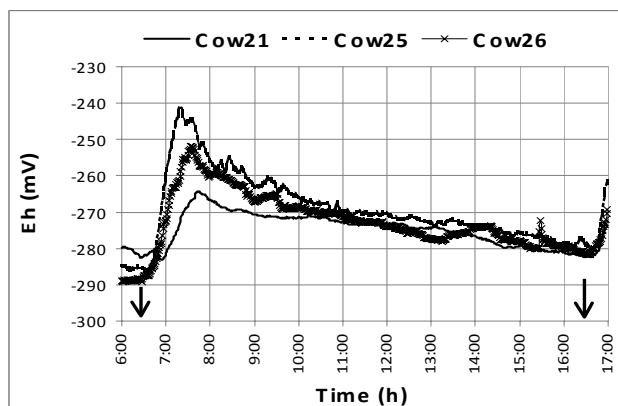
**Introduction** The metabolic activity of ruminal bacteria depends largely upon pH and redox-potential (Eh, Marden *et al.*, 2005). If these are not measured correctly, i.e. under strictly anaerobic conditions, they can be a source of a considerable error. These measures have been previously performed using (i) potentiometry on rumen fluid samples collected by an oral probe, (ii) by rumenocentesis, (iii) from a rumen cannulae using suction-strainer devices (Duffield *et al.*, 2004) or measured directly in the rumen through a sealed rumen fistula (Barry *et al.*, 1977). In the recent study of Marden *et al.* (2005), pH and Eh values were measured by means of an adapted *ex vivo* method of sampling and measurement. To our knowledge, there is no study with direct long-term measurement of Eh within the rumen. Thus, the aim of this study was to continuously monitor ruminal pH and redox-potential of individual dry cows using a newly developed wireless device.

**Material and methods** Three dry Holstein cows fitted with rumen cannulas were used for the measurement of ruminal pH and Eh using a newly developed wireless device. The measurement was carried out over a period of 14 d (10-d preliminary period and a 4-d experimental period). Cows were fed on a diet of maize silage (477 g/kg), lucerne hay (416 g/kg) and concentrate (107 g/kg). The diet was fed as a TMR in two equal portions (6:30 and 16:30 h). Ruminal pH and Eh was monitored using a novel wireless device consisting of a measuring probe anchored to the cannula lid via an antenna cable. The probe was composed of a hermetically sealed cylindrical stainless steel enclosure with the front and end plate covered (Antico, Pohodělce, Czech Republic). At the end cover there was a cable grommet to allow the passage of an antenna cable which was exteriorised from the rumen through the cannula lid and attached to a transmitter. In the front cover there were cable grommets for passage of a combined glass electrode with a reference gel electrode and redox-potential platinum electrode (Elektrochemické detektory, Ltd. Turnov, Czech Republic). The data measured within the rumen was wirelessly transmitted from the probe antenna to a receiver via an interface and USB port connected to computer. Ruminal pH and Eh were measured every 20 s and averaged over 15-min intervals. The probe was inserted into the ventral sac of the rumen of each cow through the cannula on day 10 of the preliminary period. Ruminal pH and Eh were measured continuously during between-feeding interval (i.e. for 11 h, starting at 06:00 h). Data were analysed using model:  $Y_{ij} = \mu + C_i + D_j + T_k + \varepsilon_{ijk}$ , where  $\mu$  = general mean,  $C_i$  = effect of cow ( $i = 3$ ),  $D_j$  = effect of day ( $j = 4$ ),  $T_k$  = effect of time ( $k = 44$ ),  $\varepsilon_{ijk}$  = residual error.

**Results** The mean ruminal pH was almost identical in Cows 21 and 26 being 6.80 and 6.83, respectively. The pattern of ruminal pH as presented in Figure 1 showed similar trend in all cows, with the rapid drop in pH value during 3 h postfeeding. The mean Eh of the ruminal fluid in Cow 21 was -275 mV and was lower than measured in Cow 25 (-268 mV,  $P > 0.05$ ). The mean Eh in Cow 26 was -272 mV and did not differ from the other Cows. Diurnal pattern of ruminal Eh is presented in Figure 2. The Eh values of the rumen fluid showed similar trend in all Cows and were low before feeding and then increased, reaching a maximum 1 h after the feeding, after which they decreased until the subsequent meal.



**Figure 1** Effect of cow on the diurnal pattern of ruminal pH



**Figure 2** Effect of cow on the diurnal pattern of ruminal Eh

**Conclusion** This device allows the continuous, long-term measurement of ruminal pH and redox-potential remotely under strictly anaerobic conditions.

**Acknowledgements** Supported by the MSMT, Project No. LA 08040.

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## The effect of non-fibre carbohydrates supplementation on methanogenesis bacteria and protozoa populations in rumen fluid as determined by real-time polymerase chain reaction

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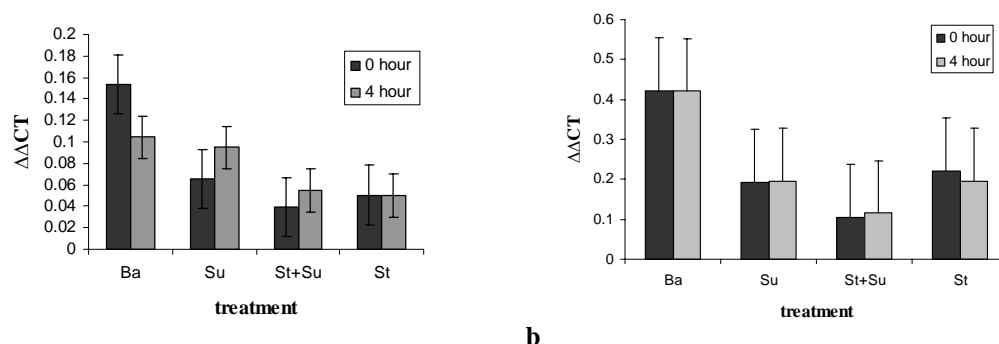
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**Introduction** Methane produced during ruminal fermentation represents a loss of 2–12% of the gross energy consumed by ruminants, and it is also a greenhouse gas that has been implicated as a contributor to the global warming (Johnson and Johnson, 1995). Thus, much research has been carried out on manipulation of the rumen fermentation to inhibit rumen methanogenesis with a view to increase energetic efficiency. The present experiment was conducted to determine the effects of diets containing different non-fibre carbohydrates (NFC, sucrose or starch) on rumen methanogenesis bacteria and protozoa populations in Holstein steers by real time PCR.

**Material and methods** Four Holstein steers (280± 15 kg, body weight) with rumen fistulae were assigned to a 4×4 Latin square design with 21 day periods; 17 days diet adjustment and 4 days sample collection. The basal diet contained lucerne hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/ kg, respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+Su) was added to the basal diet at the rate of 70g/kg DM. Diets were offered at 2–2.5 times maintenance requirements (7kg DM/day). The samples of rumen fluid taken before the morning feed, and 4 h post feeding were stored in liquid N<sub>2</sub> until used for bacterial and protozoa quantitation by qPCR. DNA was extracted from the samples using the QIAamp® DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer's instructions. Methanogenesis and protozoa rDNA concentrations were measured by real time PCR relative to total bacteria amplification ( $\Delta\Delta Ct$ ). The 16s rRNA gene-targeted primer sets used in the present study for methanogenesis bacteria were forward: TTCGGTGGATCDCARAGRGC and reverse: GBARGTCGAWCCGTAGAATCC. Cycling conditions were 95 °C for 5 min, forty six cycles of 95 °C for 15 s, 61 °C for 15 s and 72 °C for 15 s. The 18s rRNA gene-targeted primer sets used in the present study for protozoa were forward: GCTTTCGWTGGTAGTGTATT and reverse: CTTGCCCTCYAATCGTWCT. Cycling conditions were 95 °C for 5 min, fifty cycles of 95 °C for 15 s, 55 °C for 20 s and 72 °C for 30 s. fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1 °C/s increment from 65 to 95 °C, with fluorescence collection at 0.1 °C at intervals. Data are express relative to quantification of the total bacterial population using the primers described by Maeda *et al* (2003). Data were analyzed using the GLM procedure of SAS ( $y = \text{Mean} + \text{Treatment} + \text{Animal} + \text{Period} + \text{Time} + \text{Time} \times \text{Treatment} + \text{residual}$ ) and the means compared by the Duncan test ( $P < 0.05$ ).

**Results** Ribosomal DNA (rDNA) concentration of methanogenesis bacteria and protozoa in rumen fluid is shown in Figure1. Population of methanogenesis bacteria and protozoa in the ruminal fluid decreased, when basal diet was supplemented by either Sucrose or Starch ( $P < 0.05$ ). In addition St+Su has a higher significant decreasing effect on ruminal protozoa populations.



**Figure 1** Methanogenesis bacteria(a) and protozoa (b) (mean ± SD) in rumen fluid before and 4 h after morning feeding

**Conclusions** The results of the present study demonstrated that supplementation of ruminant diets with NFC caused a decrease on population of methanogenesis bacteria and protozoa in the free rumen fluid. In addition, results concluded that NFC types have different effect on rumen methanogenesis bacteria and protozoa populations. Type of supplemental carbohydrate provided in ruminant diets has been suggested to be a factor that may impact on ruminal microbiota populations. Among the NFC types, starch has the greatest potential to suppress rumen methanogenesis. Several factors seem to influence the concentration and composition of the protozoal fauna in the rumen, these include composition of diet, pH, turnover rate, frequency of feeding, and feed level (Franzolin and Dehority, 1996).

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## Fermentation kinetics and protein digestibility estimation of sheep diets containing different levels of Babaçu meal and cake *in vitro*

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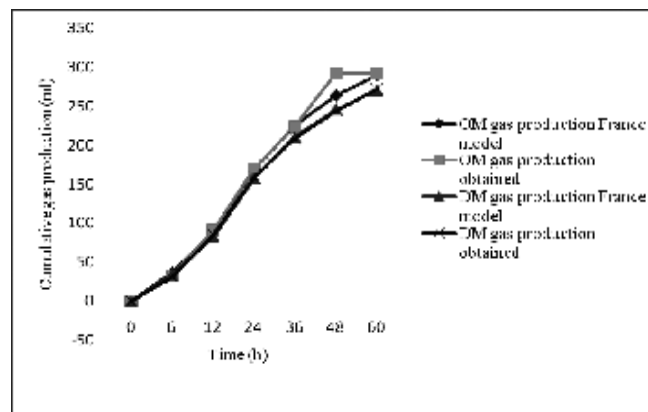
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**Introduction** The Brazilian production of Babaçu (*Orbignya sp*) is about 12 tons per year in an area of 10 million hectares. Babaçu meal and cake can be produced after the extraction of the oil from the nuts by solvent and mechanical pressure (respectively), which have been empirically used in the Brazilian Northeast in many ruminant production systems because of its low cost and availability in periods of feed shortage. This study was conducted to evaluate the fermentation parameters and protein digestibility of sheep diets containing different levels of Babaçu meal and cake.

**Material and methods** Diets (CP % and NDF were 17.3 % and 58.5%, respectively) with four levels of Babaçu meal and cake were investigated (0% - control, 10%, 20% and 30%). Three adult rumen cannulated sheep grazing on tropical grass pasture and feed diets based on 75% corn and 25% soybean meal were used as inoculum donor for *in vitro* gas production according to Mauricio *et al.*, 1999. Babaçu meal and cake samples preparation included pre-dried at 60 ° C for 48 hours and grinding. Both solid and liquid rumen fractions (1: 1) were collected before the morning feeding through the cannula using a stainless steel probe attached to a large capacity syringe. Ground samples (0.5g) were incubated in 25ml of mixed rumen fluid, 50ml of Menker's buffered medium in 160 ml serum bottles (Longo *et al.*, 2006). Once filed, all the bottles were closed with rubber stoppers shaken and placed in the incubator at 39°C and removed for recording of the gas headspace pressure at 6, 12, 24, 36, 48 and 60h incubation using a pressure transducer and data logger (LANA/CENA-USP, Piracicaba, SP, Brazil). Gas volume (V) was estimated by the equation:  $V = 7.365 \times \text{pressure (psi)}$ . After incubation the contents of bottles were used for determine the *in vitro* organic matter degradability (OMD). The kinetics of gas production was fitted to the exponential model proposed by France *et al.* (1993): A (ml) is the asymptotic gas production and L (h) is the lag time. Ruminal degradable protein (RDP) and intestinal protein digestibility (IPD) were estimated using the technique of Casalmiglia and Stern (1995). Two bags per sample with 2.0g of sample were incubated *in vitro* for 16h. After that, the bags were washed and dried at 60°C for 48 h. Sample residues (0,1g) were incubated for 1h in 10 ml of pepsin solution at 39°C. At the end of the incubation period, 13.5 ml of pancreatin solution were added and incubated at 39°C. After a 24 h incubation period, 3 ml of 100% (w/v) trichloroacetic acid (TCA) solution was add to precipitate the undigested proteins and supernatant used for measure the N content. Data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure (SAS, 1999).

**Results** There was no significant effect ( $P>0.05$ ) of the different levels of Babaçu meal and cake on A (327mL/g DM) and L (1.6h) parameters of gas production. Similarly, there was no effect ( $P>0.05$ ) for the average of the 3 experimental levels in OMD (65.4%), RPD (51.6%) and IPD (75.2%). Gas production kinetics during 60 h of incubation period is shown in Figure 1. No differences observed ( $P=0.10$  and  $P=0.09$ , respectively) for dry matter (DM) cumulative gas production and organic matter (OM) cumulative gas production.



**Figure 1** Cumulative gas production kinetics during 60 h of incubation period

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**Conclusion** This study suggested that the Babaçu meal and cake have no harmful effect on fermentation kinetics and protein digestibility *in vitro*. Once such properties have also been demonstrated *in vivo*, Babaçu meal and cake may be used as an alternative feed source in ruminant nutrition in Brazilian Northeast.

**Acknowledgement** This work was supported by CAPES, a Brazilian Government Agency.

## Characterisation of the fibre composition of common grass species under varying management conditions

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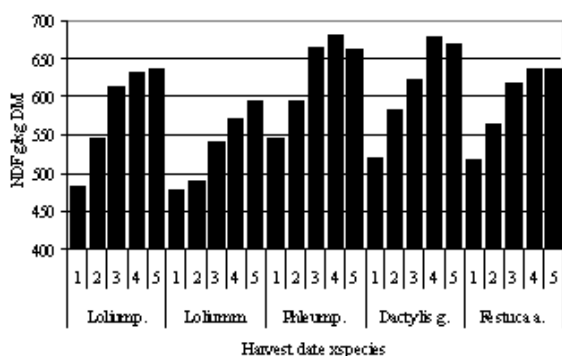
**Introduction** Ninety-one percent of agricultural land in Ireland is devoted to grass, making it the most important agricultural crop. Traditionally, grass and grass silage have formed the backbone of most beef and dairy production systems. However, in recent years interest has developed in alternative uses for grasslands and the potential of grass as a value added material (Kromus *et al.*, 2004). The fibre fraction represents the largest proportion of the grass plant, so that if grass is to be used for value added material the fibre composition needs to be quantified. The purpose of this study was to determine the chemical fibre of a range of common grasses, harvested at different stages of maturity and under different nitrogen fertiliser regimes.

**Material and methods** Five common grass species (*Lolium perenne* L. var. Gandalf, *Lolium multiflorum* var. Prospect, *Dactylis glomerata* var. Pizza, *Phleum pratense* var. Erecta, *Festuca arundinacea* var. Fuego) were grown in field plots (each 20 m<sup>2</sup>; with triplicate replication; n = 150) under two nitrogen fertiliser inputs (low = 0 kg/ha, high = 125 kg/ha) and harvested at five dates (fortnightly from 12 May – 7 July; Harvests 1-5) in the primary growth. On each harvest date the plots were harvested with a Haldrup forage plot harvester and weighed to estimate herbage yield. A representative herbage sample was then taken from each plot for chemical analysis. Samples were oven dried at 40°C for 48 hours, milled through a 1 mm screen and was then analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations using an ANKOM fibre analyser according to the method of Van Soest (1963). Data were analysed as a split-split plot design using the MIXED procedure of SAS, Version 9.1.2 (SAS, 2004).

**Results and Discussion** The NDF and ADF concentrations increased ( $P < 0.001$ ) with advancing plant maturity. On average, NDF concentration was highest ( $P < 0.001$ ) for *Phleum pratense* and lowest ( $P < 0.001$ ) for *Lolium multiflorum* (Figure 1). The ADF concentration was also highest ( $P < 0.001$ ) for *Phleum pratense*, followed by *Dactylis glomerata*, with values for *Lolium perenne* and *Festuca arundinacea* being similar (Figure 2). As harvest date advanced chemical fibre concentration increased ( $P < 0.001$ ) for all grass species, with the exception of *Phleum pratense* and *Dactylis glomerata* where a decrease in NDF concentration was observed from harvest period 4 to 5. On average, ADF concentration was higher ( $P < 0.05$ ) for the high N fertiliser treatment, however, the difference was negligible. No further significant interactions were observed.

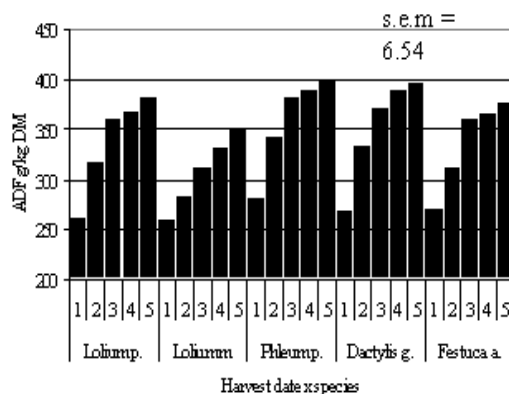
s.e.m = 7.01

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**Figure 1** NDF concentration of five grass species across five harvest dates

s.e.m. = 6.54 \*\*\*



**Figure 2** ADF concentration of five grass species across five harvest dates

**Conclusion** Grass fibre concentration increased with increasing plant maturity. Of the five grasses examined, *Phleum pratense* had the highest fibre concentration. Further study will involve the determination of the lignin concentration of each grass and the physical characterisation of the grass fibre.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland #RSF 07 557

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## Validation of the sulphur hexafluoride tracer technique for estimating methane emissions from dairy cows using respiration chambers: preliminary data

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**Introduction** Accurate methane (CH<sub>4</sub>) emissions have traditionally been measured using indirect calorimeters. However, respiration chambers restrict the number of cows that can be evaluated simultaneously and their results might not be extrapolated to grazing animals. A technique that makes use of an inert tracer gas (sulphur hexafluoride, SF<sub>6</sub>) has been developed for determining CH<sub>4</sub> emissions under production conditions (Johnson *et al.*, 2004). This technique, accounts only for CH<sub>4</sub> exiting through the mouth and nostrils. The objective of this study was to validate the SF<sub>6</sub> tracer technique for measuring CH<sub>4</sub> emissions from dairy cows using respiration chambers and to determine the proportion of CH<sub>4</sub> that is excreted through the mouth and nostrils compared to that excreted by the rectum.

**Material and methods** Twenty dairy cows of 3 breeds (4 Norwegians (N), 4 N X Holstein-Friesian (HF) and 12 HF) were used in this study: 4 primiparous and 16 multiparous cows with a mean body weight ( $\pm$  SD) at the start of the study of 515  $\pm$  17 and 642  $\pm$  79 kg, respectively. This cross-over study had a 2 X 2 factorial arrangement consisting of 2 levels of concentrates (300 and 600 g/kg DM), with or without yeast supplement. All diets were based on grass silage and concentrates offered *ad libitum* once daily as total mixed rations. Intakes and refusals were weighed and recorded. A permeation tube containing SF<sub>6</sub> was placed in the rumen of each cow. Four 6 week experimental periods were evaluated from early to late lactation. A 3 week washout phase was used between periods (concentrate level: 300 g/kg DM). On the last 12 days of each period, CH<sub>4</sub> emissions were measured using the SF<sub>6</sub> technique in 3 locations: before chamber measurements (Byre 1), in respiration chambers (Chamber) and after chamber measurements (Byre 2). Pairs of cows were placed in individual stalls and fitted with a halter and evacuated canister (adapted from Johnson *et al.*, 2004) on the last 3 days in each location. For the Chamber location, each pair was taken to open-circuit respiration chambers, with CH<sub>4</sub> output being measured simultaneously by both the calorimetry and SF<sub>6</sub> techniques. The canister was located in the back of each chamber with the nosepiece placed inside an air duct through which air was circulated towards gas analysers. Measurements done in the chambers by both techniques account for all CH<sub>4</sub> emissions produced by the animals, including those respired, eructated and released through the rectum. Data collected in the chambers were analysed by repeated measures using GenStat REML examining the effects of technique, period, day, concentrate level and yeast supplementation, while adjusting for breed, parity, chamber, bolus release rate, pair and individual cow. The SF<sub>6</sub> data collected in the three locations were similarly analysed but included the effect of location instead of technique and excluded chamber. The data presented correspond to the first two periods of the ongoing study. Results for concentrate level and yeast supplementation are not included.

**Results** There were no interactions between treatments; therefore, only main effects are presented. There were small but significant differences between CH<sub>4</sub> measurement techniques over the 2 periods, with the total CH<sub>4</sub> and CH<sub>4</sub> per kg of DM intake (DMI) and milk yield (MY) measured using the SF<sub>6</sub> technique being higher than those using respiration chambers (Table 1). There was no effect of period on the ratio of the CH<sub>4</sub> emissions measured using the SF<sub>6</sub> technique to the emission measured using the respiration chambers with mean values of 103 for period 1 and 108 for period 2 (SED 3.5;  $P > 0.05$ ). Similarly, there was no effect of day of measurement on methane SF<sub>6</sub> to calorimeter ratio (day 1 = 106, day 2 = 105 and day 3 = 105; SED 2.4;  $P < 0.05$ ), which highlights the relatively low variation of the SF<sub>6</sub> and calorimetry techniques between days. Using the SF<sub>6</sub> technique only, CH<sub>4</sub> output measured in the byre tended to be lower than in the calorimetry chambers for total CH<sub>4</sub> and for CH<sub>4</sub> per kg of DMI, while CH<sub>4</sub> per kg of MY was significantly lower in the byre (Table 2). This gives an indication of the proportion of methane being excreted through the rectum. Methane emissions per kg of DMI measured by the SF<sub>6</sub> technique were lower in period 1 than in period 2 (23.8 vs. 26.4; SED 0.56;  $P < 0.001$ ) and were not affected by day of measurement (day 1 = 25.4, day 2 = 25.0 and day 3 = 25.0; SED 0.45;  $P > 0.05$ ).

**Table 1** Effect of measurement technique on methane (CH<sub>4</sub>) emissions collected in respiration chambers

	Technique		SE	P	Ratio
	SF <sub>6</sub>	Chamber			
CH <sub>4</sub> g/d	455	415	14.9	<0.00	110
CH <sub>4</sub> g/kg	25.	23.9	0.65	0.026	107
CH <sub>4</sub> g/kg MY <sup>2</sup>	19.	17.9	0.66	<0.00	109

<sup>1</sup>Dry matter intake; <sup>2</sup>Milk yield

**Table 2** Effect of location on methane (CH<sub>4</sub>) emissions collected by the SF<sub>6</sub> technique

	Location		SED	P	Ratio
	Byre	Chamber			
CH <sub>4</sub> g/d	423	441	9.8	0.082	96
CH <sub>4</sub> g/kg DMI <sup>1</sup>	24.6	25.6	0.58	0.122	96
CH <sub>4</sub> g/kg MY <sup>2</sup>	18.2	19.2	0.45	0.043	95

<sup>1</sup>Dry matter intake; <sup>2</sup>Milk yield

**Conclusion** Over the 2 periods, the SF<sub>6</sub> technique slightly overestimated CH<sub>4</sub> emissions compared with respiration chambers, with relatively low variation within cows between days. Methane output measured by the SF<sub>6</sub> technique only, tended to be slightly higher in the chambers (accounting for all CH<sub>4</sub> sources) compared with the byre (CH<sub>4</sub> excreted by the mouth and nostrils only).

**Acknowledgements** This study was funded by Department of Agriculture and Food (ROI) through the Research Stimulus Fund Programme (RSF 07-517)

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## The effect of offering grass silage alone or in combination with legume: cereal wholecrop silage on methane emissions of Holstein steers

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**Introduction** The UK target under the Kyoto Agreement is to reduce greenhouse gas emissions (GHG) by 80% by 2050. With specific reference to agriculture, the UK Climate Change Committee indicate an 8.5% reduction in GHG emissions from agriculture by 2020. The main GHG's that are of concern in agriculture are methane (CH<sub>4</sub>) from enteric fermentation and manure storage and N<sub>2</sub>O from agricultural soils and fertiliser use. Diet has been shown to effect methane emissions. Waghorn (2002) demonstrated that ram lambs offered the legume crop lotus had almost a 55% decrease in methane emissions relative to ram lambs offered ryegrass/white clover (Waghorn, 2002). Increasing the proportion of concentrates in the diet have also be observed to decrease methane production per kg dry matter intake (DMI) relative to forage-based diets due to the intake and higher starch levels in the concentrate proportion favouring propionate production (McAllister *et al.*, 2000). However, there are no known research data available on the methane emissions from finishing beef cattle offered legume/cereal wholecrop silages. On this basis the objective of the current study is to measure methane emissions of Holstein steers offered legume/cereal wholecrop silages.

**Material and methods** Fifteen Holstein steers (491 ± 24.1 kg) were allocated to one of 5 forage treatments in a partially balanced change-over design. The five forage diets offered included perennial ryegrass-based grass silage (PGS), fescue/perennial ryegrass-based grass silage (FGS) and lupins/triticale, lupins/wheat and peas/oat wholecrop silages offered in combination with PGS at a ratio of 50:50 legume/cereal wholecrop: PGS on a dry matter (DM) basis. The forages were offered *ad libitum* and supplemented with 4 kg concentrates/head/day. The Holstein steers were housed in slatted accommodation and offered experimental diets for a minimum of 20 days. Three Holstein steers per treatment were then transferred to individual metabolism crates for 8 days to become acclimatised to being restrained individually. The animals were then transferred to indirect respiration calorimeter chambers for 3 days where methane (CH<sub>4</sub>) emissions were measured during the final 2 days. The process was then repeated with animals allocated to different diets. Data from the current study was used to calculate methane emissions from a parallel study (Kennedy and Dawson 2009) where continental finishing beef steers were offered the same forage diets as the current study. Results were analysed using one way ANOVA with start weight and animal tag number used as covariates.

### Results

Forage had no significant effect on forage DMI, total DMI, CH<sub>4</sub>, CH<sub>4</sub>/DMI and CH<sub>4</sub>/live weight (LWT) (Table 1). The results presented in Table 1 demonstrate forage treatment had no significant effect on methane emissions expressed as carbon dioxide equivalents (CO<sub>2</sub>e) per head or per kg carcass gain.

**Table 1** Effect of forage type on methane emissions from Holstein steers.

	Forage					sed	Sig.
	PGS	FGS	Lupins/ triticale <sup>‡</sup>	Lupins/ wheat <sup>‡</sup>	Pea/ Oats <sup>‡</sup>		
Forage DMI (kg/day)	5.4	6.3	6.1	6.5	6.2	1.08	NS
Total DMI (kg/day)	8.4	9.3	9.1	9.5	6.1	1.08	NS
CH <sub>4</sub> (litres/day)	358	342	312	338	369	30.3	NS
CH <sub>4</sub> /DMI (litres/kg)	43.1	37.2	34.8	35.1	40.4	3.74	NS
CH <sub>4</sub> /LWT (litres/day per kg)	0.71	0.68	0.62	0.67	0.73	0.061	NS
<i>Data from Kennedy and Dawson (2009)</i>							
CO <sub>2</sub> e (kg/head)	922	706	746	748	815	52.8	NS
CO <sub>2</sub> e/carcass gain (kg/kg) <sup>†</sup>	12.7	9.3	14.0	12.8	13.7	2.97	NS

<sup>‡</sup>: Legume/cereal wholecrop offered on 50:50 DM ratio with PGS; PGS: Perennial ryegrass-based grass silage.

FGS: Fescue/perennial ryegrass-based grass silage. DMI: Dry matter Intake. LWT: Live weight

CO<sub>2</sub>e : Carbon dioxide equivalents (((CH<sub>4</sub> Litres / 22.4)\*16 )\*(25)

**Conclusion** Offering Holstein steers legume/cereal wholecrop silage had no beneficial effect on intake or methane emissions relative to offering grass silage based diets. However, these conclusions need to be considered enlight of the major variability within the data which affects the ability to detect significant differences.

**Acknowledgements** Funding from EBLEX, HYBU CIG CYMRU, QMS, AgriSearch and DARDNI is gratefully acknowledged.

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## The effect of electromagnetic water treatment on *in vitro* methane production

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**Introduction** It has been reported that when water is treated with magnetic or electromagnetic fields it can enhance the growth rate of plants and animals (Colic and Morse 1998). The mechanism by which it acts is subject to on-going research, but one theory is that it is the gas-water interface (either intrinsically present gas or gas produced in response to electromagnetic and magnetic fields (EMF)) or water around non-polar species, which are the primary targets of EMF action. Production of free radicals and other reactive oxygen species such as ozone and hydrogen peroxide ( $H_2O_2$ ) seem to be responsible for some of the observed effects after EMF water treatment (Colic and Moose 1999). The properties of EMF treated water could be used as a potential methane mitigating strategy in livestock production. One hypothesis is that the treated water may inhibit methanogens through the production of  $H_2O_2$ ; methanogens lack catalases to breakdown  $H_2O_2$ . Additionally, owing to the purported higher oxygen content in EMF treated water, it could firstly make the rumen less desirable to methanogens and secondly, the additional oxygen should provide an additional sink for hydrogen thereby reducing methane formation. This study examined the potential of EMF treated water to suppress methane production in contrasting livestock feeds using the *in vitro* rumen total gas production (TGP) assay.

**Material and methods** The methane output for four contrasting livestock feeds (i.e. ryegrass, grass silage (S), barley grain (B) and S + B (50:50 on a dry matter basis)) was assessed using EMF treated and non-EMF treated water (control). The water in each case was the soluble component used to prepare the McDougall buffered medium. The buffer was EMF treated using the Energy Interface Delivery System (ZPM Europe Ltd, Ireland). In preparation for the *in vitro* TGP fermentations, each feed was dried (40°C; 48 h) and milled (1mm sieve) and a sample of each was weighed (0.5 g) into an incubation vessel (160 ml) and inoculated with 10 ml rumen liquor and 40 ml of McDougall buffered medium. There were three replicates of each feed per treatment and each replicate was inoculated with a different rumen liquor (RL) source. The RL was collected prior to morning feeding from three fistulated steers (60:40 silage to concentrate diet). Vessels were flushed with  $CO_2$ , sealed and incubated at 39°C. Dispensing the EMF treated and non-EMF treated buffer was undertaken simultaneously but in two different laboratories 100 meters apart to prevent cross contamination. Gas pressure was measured 24 h after inoculation and a 0.8 ml gas sample was used to determine  $CH_4$  concentration by GC. Data were analyzed by a two-way ANOVA accounting for feed, water treatment and their interaction, using GLM procedure in SPSS.

**Results** Electromagnetic treated water did not effect ( $P>0.05$ ) total gas production or methane output per gram DM incubated or DM digested in four contrasting livestock feeds over a 24 h period. As there was no effect ( $P>0.05$ ) of electromagnetic treated water on the apparent DM disappearance of the feeds, rumen fermentation in general was also unaffected by the treatment.

**Table 1** The impact of electromagnetic treated water on methanogenesis assessed using the *in vitro* total gas production technique

Treatment	Feed	TGP inc ml/g DM	$CH_4$ inc ml/g DM	TGP dig ml/g DM	$CH_4$ dig ml/g DM	$CH_4$ ml/ml TGP	aDMd g/g
Non-treated water	Grass	208	30.7	277	40.9	0.15	0.75
	Silage (S)	176	27.0	288	44.1	0.15	0.61
	Barley (B)	253	39.0	309	47.8	0.15	0.82
	S + B	198	35.6	278	49.9	0.18	0.71
EMF treated water	Grass	203	30.6	274	40.7	0.15	0.74
	Silage	172	27.9	258	46.1	0.16	0.68
	Barley	258	34.4	344	45.5	0.13	0.77
	S + B	206	34.4	285	47.7	0.17	0.72
Non-EMF water		209	33.1	288	45.7	0.16	0.72
EMF water		209	32.3	290	45.3	0.15	0.72
SEM	Treat. x diet	7.1	1.72	20.7	2.75	0.006	0.044
P	Treatment	NS	NS	NS	NS	NS	NS
	Diet	<0.0001	<0.0001	NS	NS	0.001	0.027

TGP inc/dig, total gas production ml per g DM incubated/digested;  $CH_4$  inc/dig, ml of methane per g DM incubated/digested; aDMd, apparent dry matter disappearance. EMF, electromagnetic and magnetic fields. Not significant (NS),  $P>0.05$ . There were no significant treatment x diet interactions ( $P>0.05$ ).

**Conclusions** Electromagnetic water treatment did not influence either total gas production or methane output in four contrasting livestock feeds. Assessing the effect of EMF treated water over a longer timeframe in a continuous culture assay may be worthwhile.

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## Effect of legume and perennial ryegrass herbage on *in vitro* methane output using the total gas production technique

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**Introduction** In grass based beef production systems, the type and quality of herbage offered may have an influence on enteric methane (CH<sub>4</sub>) emissions. Methane not only contributes to global warming (McGettigan *et al.*, 2008), it also represents an energy loss from the diet. The current study aims to compare the methane output from contrasting herbage at various stages of maturity.

**Material and methods** Herbage samples were obtained from a randomized complete block (n=4) designed field plot experiment in each of two successive years (Y). The herbage treatments (Hb) were *Lolium perenne* that received 0 kg [G1] or 150 kg [G2] of inorganic N fertiliser/ha/cut and two cultivars of *Trifolium pratense* (Merviot [L1] and Ruttinova [L2]). Independent plots of each herbage were harvested (Hv) twice during the primary growth (late May [I] & mid June [II]) and during an autumn regrowth [III]. Dried (40°C; 48h) milled (1mm) herbage samples were incubated in rumen fluid according to Mauricio *et al.* (1999). Methane and total VFA concentrations were determined using gas chromatography, for gas and liquid samples taken 24h after inoculation. Data were analysed using the PROC MIXED procedure in SAS considering observation from different years as repeated measures.

**Results** Chemical and fermentation characteristics of herbage are shown in Table 1. Crude protein (CP) content of the herbage was higher in the autumn (III) than in the early summer harvests (I & II) and negatively correlated (P<0.001) with water soluble carbohydrates (WSC) and NDF content. Both Hb and Hv significantly influenced (P< 0.05) all variables displayed in Table 1. Red clovers (L1 & L2) had a lower CH<sub>4</sub> output per g DM incubated (P<0.05) compared to non-fertilised ryegrass (G1) and fertilised ryegrass (G2) had a lower output of CH<sub>4</sub> per g digested (P<0.05) than L1. Autumn harvest (III) had a lower output of CH<sub>4</sub> per g DM digested (P<0.05) than harvests I and II. There was a negative correlation (P<0.001) between CP content and CH<sub>4</sub> output (per g inc. and dig.).

**Table 1** Herbage chemical composition, *in vitro* methane output and fermentation characteristics

Herbage (Hb)	Harvest (Hv)	CP g/kg DM	WSC <sup>1</sup> g/kgDM	NDF g/kgDM	aDMd <sup>2</sup> g/g	CH <sub>4</sub> inc <sup>3</sup> mmol/g DM	CH <sub>4</sub> dig <sup>4</sup> mmol/g DM	tVFA <sup>5</sup> mmol/L	C2:C3 <sup>6</sup>
G1	I	98	236	475	0.65	1.82	2.78	53	2.85
	II	100	137	542	0.58	1.65	2.83	46	2.77
	III	186	131	474	0.67	1.66	2.50	48	3.02
G2	I	170	97	503	0.63	1.67	2.65	49	3.11
	II	163	41	563	0.54	1.52	2.84	44	3.01
	III	261	86	451	0.71	1.55	2.22	46	3.24
L1	I	175	65	404	0.54	1.64	3.05	45	3.64
	II	176	33	494	0.52	1.48	2.87	41	3.36
	III	284	56	341	0.63	1.50	2.45	43	3.40
L2	I	180	66	421	0.58	1.62	2.68	45	3.64
	II	186	53	439	0.49	1.50	3.06	42	3.46
	III	287	52	354	0.63	1.50	2.37	44	3.35
SEM (Hb x Hv x Y) <sup>7</sup>		7.3	12.8	15.1	0.026	0.034	0.111	1.7	0.084
Y		<0.001	<0.001	<0.001	<0.001	<0.001	0.329	<0.01	<0.001
Hb x Hv		0.309	<0.001	<0.01	0.155	0.569	0.064	0.534	<0.05
Hb x Y		0.063	<0.001	<0.05	0.522	0.285	0.587	0.087	<0.05
Hv x Y		<0.001	<0.01	<0.001	<0.001	<0.05	<.001	0.504	<0.001
Hb x Hv x Y		0.125	<0.001	0.052	<0.05	0.001	0.332	0.017	0.356

<sup>1</sup>WSC- Water soluble carbohydrates; <sup>2</sup> aDMd- apparent DM disappearance; <sup>3</sup>CH<sub>4</sub>inc- methane expressed per g of DM incubated; <sup>4</sup>CH<sub>4</sub>dig- methane expressed per g of DM digested; <sup>5</sup>tVFA-total fermentation VFA; <sup>6</sup>C2:C3- ratio acetate to propionate; <sup>7</sup>n = 4.

**Conclusions** In general, CH<sub>4</sub> output was lowest in herbage with higher CP content. Fertilised ryegrass had a lower CH<sub>4</sub> output per g DM digested than Merviot red clover. Autumn harvest showed a lower methanogenic potential than early summer harvests.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland.

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## ***In vitro* methane output of perennial ryegrass produced under four grazing management regimes and sampled throughout the growing season**

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**Introduction** Enteric methane (CH<sub>4</sub>) production represents a loss of 0.02 to 0.12 of gross energy intake by ruminants. It is also one of the main greenhouse gases (GHG's) contributing to global warming being responsible for approximately 0.2 of the GHG effect. Understanding the impact of diet on enteric CH<sub>4</sub> production can help identify viable GHG reduction strategies on dairy farms. This study determined the effects of level of herbage mass (HM), level of sward allowance (SA) and stage of the growing season on the *in vitro* CH<sub>4</sub> output of perennial ryegrass grown under a well managed Irish dairy production system.

**Material and methods** Perennial ryegrass was produced throughout the 2008 growing season under four management regimes- two levels of HM (high ~ 2400 and low ~ 1600 kg DM / ha) and two levels of SA (high ~ 20 and low ~ 15 kg DM / cow per day) in a 2 x 2 factorial arrangement of treatments. Dairy cows were rotationally grazed throughout the grazing season with samples taken during five 4-week grazing periods (GP; 10 April to 8 May, 22 May to 19 June, 3 July to 31 July, 15 Aug to 12 Sept and 25 Sept to 23 Oct). Inocula for the *in vitro* total gas production (TGP) assay was obtained from the solid and liquid portions of the rumen contents of four rumen fistulated steers. After being filtered, the inoculum was used in the assay as described by Theodorou *et al.* (1994) and modified by Mauricio *et al.* (1999). Methane and volatile fatty acid (VFA) concentrations were measured using gas chromatography. The data were analysed using a model that accounted for the effects of HM, SA, HM x SA, and stage of season (repeated measures), and accounted for the correlations over time. As the data were not equally-spaced in time a spatial covariance structure was used.

**Results and Discussion** Grass fibre content and *in vitro* rumen fermentation characteristics are summarised in Table 1. Both TGP /g DM incubated and digested were lower for the high compared to the low SA treatment (P<0.05). No other variables were affected by the level of SA. The effect of high HM on CH<sub>4</sub> output corresponded with a reduction in DM disappearance. As fibre content was unaffected by HM, it is likely that the effect on CH<sub>4</sub> output were due to changes in fibre digestibility. Although there were no clear relationships between TGP or CH<sub>4</sub> output and the carbohydrate fraction of the herbage, CP was highest (P < 0.01) during GP 3 and 4, when CH<sub>4</sub> output was at its lowest.

**Table 1** Grass NDF content and *in vitro* rumen fermentation characteristics

	NDF (g/kg DM)	ml TGP/g DM inc <sup>1</sup>	ml TGP/g DM dig <sup>2</sup>	ml CH <sub>4</sub> /g DM inc <sup>1</sup>	ml CH <sub>4</sub> /g DM dig <sup>2</sup>	ml CH <sub>4</sub> /l TGP	Total VFA	A:P <sup>3</sup> ratio	aDMD <sup>4</sup>	pH
HM										
Low	397	180	225 <sup>a</sup>	46 <sup>a</sup>	57 <sup>a</sup>	108 <sup>a</sup>	50	1.63	807 <sup>a</sup>	6.58 <sup>a</sup>
High	404	184	232 <sup>b</sup>	47 <sup>b</sup>	60 <sup>b</sup>	111 <sup>b</sup>	50	1.62	794 <sup>b</sup>	6.56 <sup>b</sup>
SEM <sup>5</sup>	3.8	1.5	1.8	0.4	0.7	0.9	0.2	0.015	4.2	0.006
P value	0.175	0.099	0.010	0.013	<0.005	0.046	0.893	0.454	0.031	0.013
GP										
1	351 <sup>c</sup>	197 <sup>a</sup>	236 <sup>a</sup>	50 <sup>a</sup>	59 <sup>ab</sup>	111 <sup>ab</sup>	58 <sup>a</sup>	1.46 <sup>d</sup>	838 <sup>a</sup>	6.54 <sup>b</sup>
2	412 <sup>ab</sup>	184 <sup>b</sup>	241 <sup>a</sup>	48 <sup>ab</sup>	62 <sup>a</sup>	113 <sup>a</sup>	50 <sup>b</sup>	1.63 <sup>bc</sup>	786 <sup>c</sup>	6.58 <sup>ab</sup>
3	403 <sup>b</sup>	182 <sup>b</sup>	223 <sup>b</sup>	44 <sup>c</sup>	54 <sup>c</sup>	105 <sup>c</sup>	50 <sup>b</sup>	1.62 <sup>c</sup>	817 <sup>b</sup>	6.60 <sup>a</sup>
4	426 <sup>a</sup>	171 <sup>c</sup>	221 <sup>b</sup>	44 <sup>c</sup>	57 <sup>bc</sup>	108 <sup>cb</sup>	46 <sup>c</sup>	1.68 <sup>ab</sup>	777 <sup>c</sup>	6.57 <sup>ab</sup>
5	409 <sup>ab</sup>	174 <sup>c</sup>	221 <sup>b</sup>	46 <sup>bc</sup>	59 <sup>ab</sup>	112 <sup>ab</sup>	47 <sup>c</sup>	1.73 <sup>a</sup>	786 <sup>c</sup>	6.57 <sup>ab</sup>
SEM <sup>5</sup>	6.3	2.6	2.9	0.8	1.1	1.6	0.7	0.020	6.8	0.012
P value	<0.001	<0.001	<0.001	<0.001	0.001	0.008	<0.001	<0.001	<0.001	0.030

<sup>a-d</sup> Means within a factor within a column with different superscripts differ (P < 0.05); <sup>1</sup> incubated; <sup>2</sup> digested; <sup>3</sup> acetate : propionate; <sup>4</sup> apparent dry matter disappearance (g / kg DM); <sup>5</sup> standard error of the mean;

**Conclusions** An increase in HM increased TGP and CH<sub>4</sub> output and reduced apparent DM disappearance. Both TGP /g DM incubated and digested were lower for the high compared to the low SA treatment. Stage of season had an effect on all dependant gas and methane variables, most likely due to the observed changes in the chemical composition of the grass samples.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland #RSF 07 517.

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## The effect of sward maturity on the *in vitro* digestibility and methane production of sward components

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**Introduction** Methane (CH<sub>4</sub>) from enteric fermentation accounts for 13.2% of Ireland's total greenhouse gas (GHG) emissions (McGettigan *et al.*, 2008). Grassland based production dominates Irish ruminant production systems with approximately 90% of agricultural land dedicated to grazing. Grass based mitigation strategies are required to assist Ireland meet its GHG reduction targets. Sward maturity and individual sward components may impact on enteric CH<sub>4</sub> fermentation in grazing ruminants. The aim of this experiment was to assess the impact of maturity and individual sward components on *in vitro* digestibility and CH<sub>4</sub> production.

**Material and methods** Perennial ryegrass plots (7m x 1.5m) were closed on May 1<sup>st</sup> 2009 and harvested sequentially at 10 day intervals to give 5 harvest dates T1 (11/05/09), T2 (22/05/09), T3 (31/05/09), T4 (09/06/09) and T5 (19/06/09). The grass cuts were separated into leaf, stem and dead material, manually prior to drying. Samples were oven dried at 40°C for 48 hours, then ground to pass through a 1mm screen. Individual CH<sub>4</sub> emissions were measured using the *in vitro* rumen gas production technique of Mauricio *et al.* (1999). Rumen fluid was collected from three fistulated Friesian steers at 9.30am prior to feeding (60:40 grass silage to concentrate ration). Concentrate composition was: (g/kg) 830 barley, 100 soya, 50 molasses, 20 mineral and vitamins mix. Statistical analysis was performed using the mixed procedure of SAS with terms included for sward component, harvest date and their interaction. Linear and quadratic effects of harvest date were assessed separately for each sward component

**Results** Stem content within the harvested samples ranged from 0.20 – 0.61 on a DM basis. There was a linear decrease in apparent DM digestibility (aDMd) of leaf (P<0.001), stem (P<0.001) and dead material (P<0.05) as the season progressed. There was a quadratic relationship between harvest date and CH<sub>4</sub> per g DM incubated and digested for the leaf component (P<0.05) with production declining from T1 to T3 and increasing hereafter. There was a tendency for increased CH<sub>4</sub> per g DM digested for the stem component as the season progressed (P=0.1). All sward components differed for all parameters measured (P<0.05) with the exception of CH<sub>4</sub> /g DM incubated, where there was no difference between leaf and stem components (P>0.05). An interaction was detected for aDMd (P<0.001).

**Table 1** Effect of sward components on *in vitro* digestibility and methane production

Treatment	Harvest					s.e.m	P-value	
	T1	T2	T3	T4	T5		Linear	Quadratic
Leaf								
aDMd	0.72 <sup>a</sup>	0.70 <sup>b</sup>	0.66 <sup>bc</sup>	0.64 <sup>c</sup>	0.59 <sup>d</sup>	0.027	***	ns
CH <sub>4</sub> /g DM incubated	28.9	24.9	19.6	25.2	24.7	1.85	ns	*
CH <sub>4</sub> /g DM digested	40.0	36.1	29.6	39.7	42.4	2.94	ns	*
Stem								
aDMd	0.58 <sup>a</sup>	0.47 <sup>b</sup>	0.45 <sup>b</sup>	0.46 <sup>b</sup>	0.45 <sup>b</sup>	0.022	***	***
CH <sub>4</sub> /g DM incubated	28.9	24.7	24.2	28.5	28.9	2.10	ns	ns
CH <sub>4</sub> /g DM digested	50.0	51.7	54.7	62.3	64.7	4.93	P=0.1	ns
Dead								
aDMd	0.40	0.38	0.37	0.36	0.37	0.016	*	ns
CH <sub>4</sub> /g DM incubated	17.3	14.7	15.4	20.2	16.5	1.65	ns	ns
CH <sub>4</sub> /g DM digested	43.3	38.7	42.0	56.7	45.5	5.10	ns	ns

<sup>a,b,c</sup> Means within rows with different superscripts are significantly different (P<0.05). \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, ns= non significant (P>0.05)

**Conclusions** Over the range of growth stages assessed, digestibility declined and CH<sub>4</sub> emissions remained largely constant. Changes in content of individual components in the sward, particularly the replacement of leaf tissue with stem would likely cause an increase in CH<sub>4</sub> output per unit of DM digested. The effect of swards differing in maturity on *in vitro* CH<sub>4</sub> production merits further study.

**Acknowledgements** Financial support for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland

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## Effect of sward maturity on the dry matter intake, enteric methane emission and milk solids production of pasture grazed dairy cows

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**Introduction** Methane (CH<sub>4</sub>) from ruminant digestion is the largest source of greenhouse gases (GHG) from Irish milk production systems and is estimated to represent 46% of the total GHG cost of milk production (Deighton *et al.*, 2009). Pasture management decisions, such as grazing rotation length, can enable farmers to improve the quality of pasture available to the grazing dairy herd. Any reduction in the CH<sub>4</sub> emission from grazed pasture arising from an improvement in quality could be exploited immediately to improve the GHG efficiency of pasture based milk production. This study provides a direct comparison of the pasture intake, CH<sub>4</sub> emission and milk production of Holstein Friesian (HF) dairy cows grazing permanent perennial ryegrass (*Lolium perenne*) pastures of contrasting maturity and pre-grazing herbage mass.

**Material and methods** The study was conducted in Co. Cork, Ireland during the 2009 grazing season. Spring calving HF cows (n = 46) were allocated to treatment via a completely randomised design. Dietary treatments applied were low maturity pasture (LMP) or high maturity pasture (HMP) with pre-grazing growth periods of 14 and 24d respectively. Treatments were imposed from mid-April while measurements of pasture intake, enteric CH<sub>4</sub> emission and milk production were replicated during June and July. Pre-grazing pasture height was measured via plate meter and samples were collected to determine sward mass and composition above 4cm. Individual pasture intake was determined via the *n*-alkane technique (Dillon and Stakelum, 1989). Simultaneously the CH<sub>4</sub> emission of each cow was measured via the sulphur hexafluoride (SF<sub>6</sub>) tracer technique as described by Johnson *et al.*, (2007), with equipment modifications for free ranging dairy cattle. SF<sub>6</sub> permeation tubes were calibrated over an 8 week period, blocked by emission rate (mean 7.22 mg/d, SD 0.50) and randomly allocated. Samples were collected continuously for 120h in each period and concentrations of CH<sub>4</sub> and SF<sub>6</sub> were determined via gas chromatography. Milk yield was recorded twice daily (07.00 and 16.00h), composition was calculated from two successive AM and PM samples during each period. Data were analysed using the MIXED procedure of SAS (SAS Inst., Cary, NC). The linear model included diet, month and diet x month as fixed effects with cow as a random repeated effect.

**Results** The extended re-growth period significantly increased herbage mass of HMP in both months (P < 0.001). Mean herbage accumulated was 1076 vs. 2045 and 1074 vs. 1941 kgDM/ha for LMP vs. HMP during June and July respectively. Swards comprised similar proportions of leaf:stem:dead matter during June (LMP 73:19:8 vs. HMP 65:21:14), however, in July HMP displayed greater reproductive composition with a 13% higher proportion of stem than LMP (LMP 79:12:9 vs. HMP 62:25:13). Cows fed LMP emitted 9.4% less CH<sub>4</sub>/d and 11.5% less CH<sub>4</sub>/kg intake than those fed HMP. CH<sub>4</sub> emission per kg intake increased by 20% between June and July across both treatments (Table 1). A significant diet x month interaction resulted in a greater effect of pasture maturity upon CH<sub>4</sub>/kg milk solids yield during July than during June due to a larger increase in daily CH<sub>4</sub> emission and decrease in MS yield of HMP cows compared to their LMP counterparts.

**Table 1** Effect of sward maturity on enteric CH<sub>4</sub> emission, intake and milk solids yield of pasture grazed cows.

	Diet				Month				Diet x Month
	LMP <sup>†</sup>	HMP <sup>†</sup>	SED <sup>‡</sup>	Sig.	June <sup>†</sup>	July <sup>†</sup>	SED <sup>‡</sup>	Sig.	Sig.
CH <sub>4</sub> emission (g/cow/d)	276	304	13.4	*	282	298	10.4	*	NS
Dry matter intake (kg/cow/d)	15.8	15.5	0.85	NS	16.6	14.7	0.63	***	NS
Milk solids yield (kg/cow/d)	1.43	1.38	0.08	NS	1.54	1.26	0.07	***	NS
CH <sub>4</sub> /kg dry matter intake (g/kg)	18.0	20.3	0.86	*	17.4	20.9	0.75	***	NS
CH <sub>4</sub> /kg milk solids yield (g/kg)	199	231	11.3	**	186	244	8.97	***	**

<sup>†</sup>Least squares means; <sup>‡</sup>Standard error of the difference between means; Significance: \*\*\* = P<0.001; \*\* = P<0.01; \* = P<0.05; NS = not significant.

**Conclusions** Significant relationships exist between pasture maturity and CH<sub>4</sub> emissions per cow and per unit intake. CH<sub>4</sub> emission per unit milk solids yield was also significantly effected by pasture maturity. This effect was greatest during July (mid summer), coinciding with increased reproductive composition of the HMP relative to LMP. Managing swards to maintain low herbage mass and high leaf:stem ratio represents a simple yet potentially important tool in optimising the GHG efficiency of milk production from pasture, particularly during periods of the grazing season when grass plants exhibit reproductive growth.

**Acknowledgements** Financial support from the DAF Research Stimulus Fund (RSF-07-517) is gratefully acknowledged.

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## Effect of adding different levels of probiotic on *in vitro* gas production of noodle waste

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**Introduction** Probiotics present an attractive alternative to the use of chemical and hormonal promoters in the livestock growth production industry. The preparations entail the safe production of micro-organisms and have been used for many years and thus are generally accepted by both the farmer and the final consumer. *Saccharomyces cerevisiae* supplementation in ruminant diets can increase DMI, production performance, cellulose degradation, and nutrient digestibility (Callaway and Martin, 1997). The gas measuring technique has been widely used for evaluation of the nutritive value of feeds. Gas measurement provides useful data on digestion kinetics of both soluble and insoluble fractions of feedstuffs (Getachew *et al.*, 1998). In the gas method, kinetics of fermentation can be studied on a single sample and a relatively small amount of sample is required or a larger number of samples can be evaluated at one time. Besharati *et al.*, (2009) showed that probiotics can increase *in vitro* gas production from feeds. The purpose of this study was to study the effect on the *in vitro* gas production of noodle waste when different amounts of *Saccharomyces cerevisiae* were added.

**Material and methods** Samples of rumen fluid were collected from two rumen cannulated sheep fed twice daily a diet containing forage (400 g/kg) plus concentrate (600 g/kg) after the morning feed, and were then strained through four layers of cheesecloth. Gas production in each serum bottles (vial) was measured using a water displacement apparatus (Fedorak and Hruday, 1983). Approximately 300 mg of dried and ground (2mm) noodle waste sample was weighed and placed into serum bottles. There were three replicates per treatment. The treatment contained 0, 2.5, 5 and 7.5 g yeast *Saccharomyces cerevisiae* (Sc) per kg of noodle waste based on DM, respectively. Buffered rumen fluid with McDougal buffer (20ml, ratio of buffer to rumen fluid was 2:1) was pipetted into each serum bottle (McDougal, 1948). The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 1 g of DM. The data at the different times was analysed using completely randomized design by the GLM procedure of SAS Institute Inc (2002). The organic matter digestibility (OMD), ME and NE<sub>L</sub> contents of feed were estimated by the method of Menke and Steingass (1988). Samples of noodle waste were collected from food factories in the country of Tabriz.

**Results** Total gas production volume (ml/ g DM) in incubation times are shown in Table 1. At the early incubation times (2, 4, 6 and 8 h), treatments 3 and 4 (treatment with Sc, 5 and 7.5 g/kg DM, respectively) had the highest gas production volume among treatments, but after 8 h the gas production volume in treatment 4 (treatment with Sc, 7 g/kg DM) was the highest ( $p < 0.05$ ). Treatment 2 had the lowest gas production volume. Adding 5 g/kg *Saccharomyces cerevisiae* or more to noodle waste increased the estimated amounts of OMD, ME and NE<sub>L</sub> ( $p < 0.05$ ).

**Table 1** Total gas production volume (ml/g DM) in incubation times and estimated parameters\*

Treatments	Incubation times (h)									Estimated parameters		
	2	4	6	8	12	16	24	36	48	OMD (%)	ME(MJ/kg DM)	NE <sub>L</sub> (MJ/Kg)
Control	13.9 <sup>b</sup>	37.5 <sup>b</sup>	61.8 <sup>b</sup>	83.1 <sup>b</sup>	135.1 <sup>c</sup>	210.1 <sup>bc</sup>	306.2 <sup>b</sup>	355.6 <sup>bc</sup>	393.6 <sup>b</sup>	69.4 <sup>b</sup>	10.5 <sup>b</sup>	2.443 <sup>b</sup>
Sc 2.5g/kg DM	10.6 <sup>c</sup>	25.9 <sup>c</sup>	45.4 <sup>c</sup>	66.2 <sup>c</sup>	121.7 <sup>c</sup>	191.7 <sup>c</sup>	280.9 <sup>c</sup>	333.9 <sup>c</sup>	372.2 <sup>c</sup>	64.9 <sup>c</sup>	9.9 <sup>c</sup>	2.351 <sup>c</sup>
Sc 5g/kg DM	26.6 <sup>a</sup>	53.7 <sup>a</sup>	77.7 <sup>a</sup>	99.9 <sup>a</sup>	153.8 <sup>b</sup>	231.6 <sup>b</sup>	316.4 <sup>b</sup>	369.8 <sup>ab</sup>	401.4 <sup>b</sup>	71.2 <sup>b</sup>	10.8 <sup>b</sup>	2.471 <sup>b</sup>
Sc 7.5g/kg DM	31.6 <sup>a</sup>	61.1 <sup>a</sup>	85.1 <sup>a</sup>	111.9 <sup>a</sup>	175.4 <sup>a</sup>	258.1 <sup>a</sup>	344.2 <sup>a</sup>	392.5 <sup>a</sup>	421.3 <sup>a</sup>	76.1 <sup>a</sup>	11.6 <sup>a</sup>	2.572 <sup>a</sup>
SEM	1.59	2.96	4.22	5.04	5.35	7.11	7.38	8.31	8.48	0.354	0.321	0.567

\* The means within a column without common letter differ ( $p < 0.05$ ).

**Conclusions** It was concluded that *in vitro* gas production parameters, OMD, ME and NE<sub>L</sub> of noodle waste were improved with addition of 7.5 g/kg DM yeast *Saccharomyces cerevisiae*, and yeast culture had potential of improving rumen fermentation characteristics.

**Acknowledgement** This study was funded by University of Tabriz, Tabriz, Iran.

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## Effect of peppermint (*Mentha piperita*) essential oil on *in vitro* gas production parameters of lucerne hay and cottonseed hulls

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**Introduction** In ruminants, ionophores have been used to enhance ruminal fermentation. However, because of human health concerns, plant extracts and essential oils are used as alternative natural feed additives in replacement of ionophores (Garcia-Gonzalez *et al.*, 2008). The amount of gas produced from a feed is used as an index of its fermentability potential (Menke and Steingass, 1988). The aim of the present study was to evaluate the effect of peppermint (*Mentha piperita*) essential oil on the fermentation potential of lucerne hay (LH) and cottonseed hulls (CH).

**Material and methods** Peppermint essential oil (PE) is produced by heating peppermint leaves and stems to 100 °C for 24 h and collecting the vapour as a distillate. Samples of LH and CH were ground through a 2 mm screen and dried in an oven at 66 °C for 48 h. Samples were LH, CH and both feed samples plus PE (14 and 40 µl/g DM) using a 2×3 factorial design. *In vitro* gas production parameters of the samples were determined using the Menke and Steingass (1988) procedure. Approximately 0.3 g of sample (n=4) was placed in a 100 ml glass syringe containing 40 ml of buffered rumen fluid (ratio of buffer to rumen fluid was 2: 1). Rumen fluid was obtained from two rumen cannulated sheep (45.5± 2 kg, body weight) before the morning feed and immediately strained through four layers of cheesecloth. Animals were fed with 1.5 kg DM lucerne hay and 0.4 kg DM concentrates (165 g CP/ kg DM) per head per day. Syringes were incubated at 39 °C and the volume of gas produced was recorded at 2, 4, 8, 12, 24, 36, 48, 72 and 96 h. Statistical analysis was conducted using SAS (1999) procedure. The gas production data were fitted to an exponential equation of  $P = b(1 - e^{-ct})$ , where  $b$  is the volume of gas produced,  $c$  is the fractional rate constant of gas production (/h),  $t$  is the incubation time (h) and  $P$  is the volume of gas produced at time  $t$ .

**Results** The effect of PE on gas production parameters of LH and CH are shown in Table 1. Supplementation of both LH and CH with PE reduced the volume of gas produced ( $P < 0.05$ ). However, PE had no significant effect on the fractional rate ( $c$ ) of gas production [except in LH supplemented with PE as 40 µl/g DM, in which  $c$  was increased ( $P < 0.05$ )].

**Table 1** Effect of peppermint (*Mentha piperita*) essential oil on gas production parameters of lucerne hay and cottonseed hulls (Mean ± s.e.m)

		Feed Samples						s.e.m.	P value
		Lucerne hay			Cottonseed hulls				
		Level of PE (µl/g DM)			Level of PE (µl/g DM)				
Gas production parameters	-	14	40	-	14	40			
<i>b</i>	72.000 <sup>a</sup>	61.040 <sup>b</sup>	45.300 <sup>c</sup>	99.000 <sup>d</sup>	74.540 <sup>a</sup>	21.800 <sup>e</sup>	3.450	< 0.05	
<i>c</i>	0.070 <sup>a</sup>	0.067 <sup>a</sup>	0.078 <sup>b</sup>	0.011 <sup>c</sup>	0.013 <sup>c</sup>	0.012 <sup>c</sup>	0.002	< 0.05	

<sup>a, b, c, d, e</sup> Means within each feed sample with a different letters in each row are significant

**Conclusions** The unique finding of the present study is the reduction of the gas produced from quickly and slowly degradable fractions when PE was applied in both LH and CH. However, the rate constant of gas produced from CH was not influenced by the adding of PE. Therefore, it was concluded that PE had a potential to reduce the fermentability of LH when applied as 40 µl/g DM.

**Acknowledgements** The authors gratefully acknowledge funding from Excellence Centre for Animal Science, Ferdowsi University of Mashhad.

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## ***In vitro* gas production parameters of chickpea (*Cicer arietinum* L.) by-product**

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**Introduction** Chickpea (*Cicer arietinum* L.) is a legume seed, which is mostly used for human food. Approximately 7500 ton wastes of chickpea including pre-screening seeds and chickpea bran (known as chickpea by-product) are produced annually in North West Iran. The aim of the present study was to determine chemical composition and *in vitro* gas production parameters of chickpea by-product.

**Material and methods** Samples of chickpea by-product including pre-screening seeds and chickpea bran were collected from chickpea sorting factories located in North West Iran during September to December, 2008. Chemical composition including organic matter (OM), crude protein (CP), ether extract (EE) and crude fibre (CF) were determined using standard procedures (AOAC, 1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the method of Van Soest *et al.* (1991). Total extractable phenolic compounds (TPC) and total tannin (TT) were determined using procedures of Julkunen-Titto (1985) and Makkar (1992), respectively. Three fistulated Balochi sheep (49.5±2.5 kg) were used as rumen liquor donor for gas production technique. The animals were fed 1 kg DM lucerne hay and 0.3 kg DM concentrate (165 g CP/kg of DM). Rumen fluid was collected before the morning feeding and strained through 4 layers of cheesecloth into a CO<sub>2</sub>-filled flask. *In vitro* incubation of the samples was done using calibrated glass syringes followed the procedures of Menke and Steingass (1988). Approximately 200 mg of each sample was weighed into four replicate calibrated glass syringes of 100 ml. The syringes were pre-warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39 °C. Readings of gas production were recorded at 2, 4, 8, 12, 24, 36, 48, 72, and 96 h after incubation. Cumulative gas production data were fitted to a model of  $Y = b(1 - e^{-ct})$ ; where: Y= potential of gas production at time t; b= gas produced from the soluble and insoluble fraction (ml); c= gas production constant rate (ml/h); t= incubation time (h). Data were statistically analyzed using SAS (1999) software.

**Results** Chemical composition of chickpea by-product is presented in Table 1. Results of the present study indicated the chemical composition, except OM, was significant different between the samples evaluated ( $P < 0.05$ ). Gas production parameter and calculated amount of organic matter digestibility (OMO) and metabolizable energy (ME) are presented in Table 2. The amount of b and value calculated for OMD and ME of chickpea pre-screening were significantly higher than chickpea bran ( $P < 0.01$ ). Gas production rate constant of chickpea bran was significantly higher than chickpea pre-screening ( $P < 0.01$ ).

**Table 1** Chemical composition of chickpea by-product (g/kg DM)

Chickpea by-product	OM	CP	EE	CF	NDF	ADF	TN	TPC
Chickpea pre-screening	940	279	78	72	351	96	1	3.4
Chickpea bran	927	44	87	178	323	224	6.5	7.5
s.e.d	6.2	5.4	1.4	2.4	6.6	10.1	0.55	0.75
P	>0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

**Table 2** Gas production parameters, organic matter digestibility (OMD)\* and metabolizable energy (ME)\*\* content of chickpea by-product

Item	Chickpea pre-screening	Chickpea bran	P
b (ml)	66.4±2.42	93.6±2.51	<0.01
c (ml/h)	0.056±0.0052	0.024±0.0023	<0.01
OMD (%)	59.1±0.42	42.1±1.5	<0.01
ME (MJ/kg)	8.95±0.074	6.5±0.2	<0.01

\*OMD = 0.9991 Gas + 0.0595 CP + 0.0181 CA + 9

\*\*ME = 0.157 Gas + 0.0084 CP + 0.022 EE – 0.0081 CA + 1.06

**Conclusions** Results obtained in the present study regarding the chemical composition of chickpea pre-screening confirmed the finding of Abdi and Danesh Mesgaran (2009). Values calculated for both ME and OMD of chickpea pre-screening were significantly higher than those of chickpea bran ( $P < 0.01$ ), which might be due to difference in chemical composition and volume of gas production in the first 24 h. It was concluded that the by-products evaluated in the present experiment had a potential to use as suitable feed in ruminant rations. However, future feeding trials will be proposed to evaluate the effect of this by-product in ruminant production.

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## Use of *in situ* technique to evaluate three weed forages

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**Introduction** weed forages are important feed resources for ruminant in pasture of Iran, but there has been limited research on their nutritive value. The appellations feed resources, has become commonly used to design those local feeds, which could replace partially or totally conventional feedstuffs either grass forages or concentrate feeds without reducing livestock performance but should decrease the feeding cost (Sallam, 2005). *Cardaria draba*, *Corcus sativus* and *Eruca sativa* are three common weed forages that grow from March to November in pastures of Iran and offered to animals. Empirically, the nutritive value of these forages was confirmed by ranchers, however little information is known about their nutritive values scientifically, thus making it difficult to assess their potential contribution to sustain animal production. So the present study was carried out to determine *in situ* dry matter degradability of three common weed forages in pastures of Iran.

**Material and methods** Samples of weed forage were collected in March 2009 from Kashmar in the North East of I.R.Iran. The leaves and stems were dried for 48 h at 60 °C. The procedure used to determine dry matter disappearance was based on the method described by Krskov and McDonald (1979). The bags (pore size of 50 µm) were filled with 5g of samples and put in to the rumen of three ruminal fistulated steers. Steers were fed at maintenance level. The bags were removed at 2, 4, 8, 12, 24, 48, 72 and 96 hours after the start of incubation and each bag was washed immediately with tap water until color disappeared. For the  $t_0$  incubation time, the bags were just washed in the water. *In vitro* disappearance of DM was measured relative to original feed. The rate and extent of DM degradation was estimated according to the equation:  $p = a + b(1 - e^{-ct})$ . Effective degradability (ED) was calculated according to equation:  $ED = a + (b \times c)/(c + k)$ , assuming an outflow rate ( $k$ ) of 0.03 h<sup>-1</sup>. All data obtained from the trial were subjected to the general linear models procedure of SAS according to a completely randomised design.

**Results** Obtained data from *in situ* dry matter degradability are given in Table 1. The mean values of dry matter degradation characteristics for three native weed forages are significantly different ( $P < 0.05$ ). Values obtained from the parameter of  $a$ , normally considered as a soluble fraction dry matter, of *Eruca sativa* was higher respectively ( $P < 0.05$ ), but the insoluble degradable fraction ( $b$ ) of *Setaria Spp* was higher than other weed forages. Among the studied weed forages, *Setaria Spp* had lower fraction of  $c$  compared to other treatments.

**Table 1** Degradation characteristics and disappearance of DM in three weed forages.

	Weed Forages			SEM
	Cardaria draba	Setaria Spp.	Eruca sativa	
Degradation parameters				
$a^1$	0.53 <sup>a</sup>	0.35 <sup>c</sup>	0.56 <sup>a</sup>	0.005
$b^2$	0.30 <sup>c</sup>	0.50 <sup>a</sup>	0.35 <sup>b</sup>	0.001
$c^3$ /hr	0.106 <sup>a</sup>	0.059 <sup>b</sup>	0.107 <sup>a</sup>	0.004
Effective degradability				
$k=0.03$	0.767 <sup>b</sup>	0.692 <sup>c</sup>	0.839 <sup>a</sup>	0.002
Disappearance				
24hrs	0.809 <sup>b</sup>	0.758 <sup>c</sup>	0.885 <sup>a</sup>	0.01
48hrs	0.832 <sup>b</sup>	0.831 <sup>b</sup>	0.915 <sup>a</sup>	0.003
72hrs	0.833 <sup>b</sup>	0.843 <sup>b</sup>	0.916 <sup>a</sup>	0.003
96hrs	0.835 <sup>c</sup>	0.870 <sup>b</sup>	0.918 <sup>a</sup>	0.001

<sup>a,b,c</sup> means along same rows bearing different superscripts are significantly different ( $P < 0.05$ ).

<sup>1, 2,3</sup> constants in the equation  $P=a+b(1-e^{-ct})$  where  $P$  = level of degradation at time  $t$ ;  $a$  = readily soluble fraction;  $b$  = insoluble fraction but degradable in rumen;  $c$  = rate of degradation of  $b$  per hour.

**Conclusions** The differences between dry matter degradability between different sources of weed forages may be reflected in their composition. The observed variations in dry matter degradability suggest that the potential of degradability ( $a+b$ ) and effective degradability of *Eruca sativa* were higher than others. Thus, pasture improvement through incorporation of high quality forages in native pasture (such as *Eruca sativa*) is a solution to ruminant livestock production.

**Acknowledgments** The authors thank the Ferdowsi University of Mashhad and Excellence Centre for Animal Science for financial and technical support.

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## Kinetic of *in vitro* gas production of high fat sunflower meal treated with sodium hydroxide and or formaldehyde by rumen bacteria+protozoa

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**Introduction** Gas production technique is a useful procedure to assess digestible value of the ruminant feeds. Digestion of plant cell walls is carried out in the rumen by a complex of bacteria, fungi and protozoa and the degradability of cell walls of samples by both groups was higher each microbial alone that shows synergistic interaction between rumen microbial groups (Schofield, 2000). The feeding value of the sunflower meal depends on the oil extraction process, variety of sunflower and the proportion of the hulls removed during the extraction. High fat of sunflower meal may have negative effects on rumen protozoa and some of bacteria, so decrease digestibility. Formaldehyde decreases protein degradability and sodium hydroxide (NaOH) (Chen *et al.*, 2007) increase digestibility, these treatment may influence interaction between protozoa and bacteria. The objective of this study was to investigate the effect of high fat sunflower meal (SFM, 165 g fat/kg DM) as untreated or treated with formaldehyde and or sodium hydroxide on rumen bacteria and protozoa and interaction between them for degrading of SFM by the *in vitro* gas production method.

**Material and methods** The samples (five replicates) were: untreated SFM (USFM), NaOH treated SFM (40 g/kg DM, NSF), formaldehyde treated SFM (30 g/kg DM, FSF). About 500±10 mg of oven dried and milled sample (1.0 mm screen) was incubated with 30 ml buffered rumen (bacteria +protozoa) fluid (20 ml medium and 10 ml rumen bacteria +protozoa). To preparing bacteria +protozoa, rumen fluid was collected from two fistulated Holstein steers (400±12 Kg, body weight) fed twice daily a diet containing 5.72 kg lucerne hay and 3.08 kg concentrate mixture, then benomyle (500 ppm/ml medium) and metalaxyle (10 mg/ml medium) were added to rumen fluid and used in *in vitro* gas production technique. All samples were incubated in triplicate with three syringes containing only incubation medium or blank (three run of gas production) and gas production from the sample was corrected for the blank. Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. Cumulative gas production data were fitted to the exponential equation  $Y=B(1-e^{-Ct})$ , where  $B$  is the gas production from the fermentable fraction (ml),  $C$  is the gas production rate constant for  $B$ ,  $t$  is the incubation time (h) and  $Y$  is the gas produced at time  $t$ . *In vitro* digestibility of organic matter (OMD, g/kg OM) and metabolisable energy (ME, MJ/kg DM) of samples were calculated by the equation of Menke and Steingass (1988). Short chain fatty acid concentration (SCFA, µmol) was measured by the equation as proposed by Getachew *et al.* (1999). Data of gas production, ME, OMD, and SCFA were subjected to analysis as a completely randomized design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare treatment means at  $P < 0.01$ .

**Results** *In vitro* gas production parameters, OMD, ME, and SCFA of the sunflower meal by rumen bacteria+protozoa are shown in Table 1. Gas production parameters of NaOH treated SFM were significantly higher than the other treatments ( $P < 0.01$ ). NaOH resulted in increase OMD, ME and SCFA, but formaldehyde decreased them in compared with the other samples.

**Table 1** *In vitro* gas production parameters, OMD, ME, and SCFA of high fat sunflower meal treated with formaldehyde and or sodium hydroxide by rumen bacteria+protozoa

	Treatments			s.e.d	P
	USFM	NSFM	FSFM		
$B$ (ml)	174.3 <sup>b</sup>	186.2 <sup>a</sup>	138.9 <sup>c</sup>	0.5	0.01
$C$ (ml/h)	0.016 <sup>b</sup>	0.022 <sup>a</sup>	0.012 <sup>c</sup>	0.001	0.01
OMD (g/kg OM)	171.9 <sup>b</sup>	189.5 <sup>a</sup>	166.7 <sup>c</sup>	0.2	0.01
ME (MJ/kg DM)	13.30 <sup>b</sup>	16.75 <sup>a</sup>	12.35 <sup>c</sup>	0.3	0.01
SCFA (µmol)	0.62 <sup>b</sup>	0.81 <sup>a</sup>	0.45 <sup>c</sup>	0.002	0.01

**Conclusions** It was concluded that *in vitro* gas production parameters, OMD, ME and SCFA of sunflower meal treated with sodium hydroxide by rumen bacteria+protozoa were improved in compared with the other samples, sodium hydroxide hydrolyses the ester linkages between lignin and the cell wall polysaccharides mainly hemicellulose (Chesson, 1981) and followed by significant improvements in organic matter digestibility (Nakashima and Orskov, 1989). Therefore using of sodium hydroxide for treatment of sunflower meal resulted in to improve fermentation by rumen bacteria and protozoa in compared with formaldehyde.

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## The kinetic of *in vitro* gas production of tannic acid treated sunflower meal with or without polyethylene glycol

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**Introduction** Tannins are polyphenolic compounds of plant which bind to proteins, by hydrogen bonding. At normal pH of the rumen, protein remains bound to the tannin, but at the low pH of abomasum, the protein is released, so protein becomes available for digestion in the small intestine (El-Waziry *et al.*, 2005). The researchers reported treating soybean meal with tannic acid (hydrolysable tannins) in *in vitro* gas production technique was reduced protein degradation of soybean meal and kinetics of gas production, primarily by hydrogen bonding (EL-Waziry *et al.*, 2005). Poly ethylene glycol (PEG) was used as a tannin binder for their ability to counteract the action of tannin on gas production. The aim of this study was to investigate effects tannic acid (with or without PEG) on gas production and rumen fermentation of sunflower meal (SM) in *in vitro* condition.

**Material and methods** Experimental treatments were; untreated SM (USM), 3% tannic acid treated SM (TSM), 3% tannic acid treated SM +10 % PEG (TSM+PEG). Rumen fluid was supplied from two fistulated sheep were fed a 40:60 concentrate: forage (3 kg concentrate: 2 kg alfalfa hay and 4.5 kg corn silage) in prior to the morning meal. Samples ground through a 1.0 mm screen and 500 mg were weighed and incubated in 100 ml glass syringes with 35 ml of incubation medium, according to the method of Menke and Steingass (1988). Gas production was measured at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. All samples were incubated in triplicate together with three syringes containing only incubation medium (blank), and gas production from the sample was corrected for the blank. Cumulative gas production data were fitted to the exponential equation  $Y=B(1-e^{-Ct})$ , where B is the gas production from the fermentable fraction (ml), C is the gas production rate constant for B, t is the incubation time (h) and Y is the gas produced at time t. Ammonia-N (NH<sub>3</sub>-N) concentration (mg/dl) was determined in supernatant samples at the end of the incubation time by macro Kjeltec System Tecator (Büchi 1030, Sweden). *In vitro* digestibility of organic matter (OMD, g/kg OM) of samples was calculated by the equation of Menke and Steingass (1988). Short chain fatty acids (SCFA) were determined by the equation reported by Getachew *et al.* (1999). Gas production parameters, OMD, NH<sub>3</sub>-N and SCFA were subjected to analysis as a completely randomized design using the general linear model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare treatment means at  $P < 0.05$ .

**Results** Gas production and rate of gas production, OMD, SCFA and NH<sub>3</sub>-N of USM was significantly higher than the other treatments and 3 % tannic acid decreased gas production, OMD, SCFA and NH<sub>3</sub>-N content ( $P < 0.05$ ), but inclusion of 10% PEG caused to increase gas production and the other fermentative parameters ( $P < 0.05$ ).

**Table 1** *In vitro* gas production parameters, OMD, SCFA and NH<sub>3</sub>-N of sunflower meal treated tannic acid (with or without PEG)

	Treatments			s.e.d	P
	USM	TSM	TSM+PEG		
B (ml)	147.3 <sup>a</sup>	124.4 <sup>c</sup>	136.5 <sup>b</sup>	0.56	<.0001
C (ml/h)	0.07 <sup>a</sup>	0.02 <sup>c</sup>	0.03 <sup>b</sup>	0.001	<.0001
OMD (g/kg OM)	199.6 <sup>a</sup>	178.8 <sup>c</sup>	189.2 <sup>b</sup>	2.1	<.0001
NH <sub>3</sub> -N (mg/dl)	46.2 <sup>a</sup>	38.7 <sup>c</sup>	43.5 <sup>b</sup>	0.23	<.0001
SCFA (μmol/l)	0.94 <sup>a</sup>	0.65 <sup>c</sup>	0.82 <sup>b</sup>	0.31	<.0001

**Conclusions** Tannic acid decreased amount and rate of gas production, OMD, SCFA, and ammonia releasing from degradation protein and these parameters was increased with elimination of tannin from fermentation medium by PEG. Therefore, this result recommends, tannins (tannic acid) protect protein from degradation in the rumen.

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## Nitrogen fractionations, *in situ* ruminal degradation and post-ruminal crude protein disappearance of overheated and overheated-xylose processed guar meal

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**Introduction** The by-product of guar gum industry consisting of the guar germ and hull materials is called guar meal (GM), valuable to include in both ruminant and mono-gastric diets (Rahman and Leighton, 1968). The aim of the present study was to evaluate the effect of overheated and overheated-xylose processing on nitrogen fractionations, *in situ* ruminal CP degradation, and *in situ/in vitro* ruminal and post-ruminal protein disappearance of guar meal.

**Material and methods** Samples were raw GM (GMr), overheated processed GM (GM<sub>hp</sub>, 100 °C for 45 min using air-forced oven) and overheated-xylose processed GM (GM<sub>xp</sub>, xylose was included to give a final concentration of 10 g/kg DM, then heated as described for GM<sub>hp</sub>). Nitrogen fractions including non-protein nitrogen (NPN), buffer insoluble nitrogen (BISN), neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined as proposed by Licitra *et al.* (1996). *In situ* rumen degradation of CP of the samples was determined using four ruminal fistulated sheep (49.6±2 kg body weight). The animals fed 1.5 kg DM lucerne hay and 0.4 kg DM concentrate (165 g CP/ kg of DM) per head per day. Approximately, 6 g DM of each sample were placed in a polyester bag (9 × 17 cm; pore size of 52 µm, n=10) and incubated in the rumen for 0.0 (bags were washed with cold tap water), 2, 4, 8, 12, 16, 24 and 48 h. Ruminal disappearance of CP was determined using the 16 h incubation samples (n=8). Post-ruminal disappearance of ruminal undegradable residue was determined using 3-step procedure (Calsamiglia and Stern, 1995). Ruminal degradation parameters were determined using an exponential equation of  $P=a+b(1-e^{-ct})$ ; where P= potential of degradability, a= quickly degradable fraction, b= slowly degradable fraction, c= constant rate of degradation and t= time (Ørskov and McDonald, 1979). Data of ruminal and post-ruminal CP disappearances were analyzed using the GLM procedure of SAS (SAS Institute, 1990). Tukey test was used to compare the means at P< 0.05.

**Results** Data of nitrogen fractionations, *in situ* CP ruminal degradation parameters, and *in situ/in vitro* CP disappearance of ruminal and post-ruminal of rumen undegradable of raw, overheated and overheated-xylose processed guar meals are presented in Table 1. The NPN values of GM<sub>xp</sub> was significantly (P< 0.01) lower than GMr and GM<sub>hp</sub>. In addition, overheated-xylose processing caused to significantly (P< 0.05) increase the BISN, NDIN and ADIN content of GM<sub>xp</sub> compared with GMr. The degradation rate (c) was significantly (P< 0.05) decreased as a result of overheated-xylose processing. Overheated-xylose processing decreased ruminal CP disappearance and increased Post-ruminal CP disappearance of ruminal undegradable residue of GM (P< 0.01).

**Table 1** Nitrogen fractionations, *in situ* CP ruminal degradation parameters, and *in situ/in vitro* CP disappearance of ruminal and post-ruminal of rumen undegradable of raw, overheated and overheated-xylose processed guar meal

Items	Feed samples			s.e.m	P
	GM <sub>r</sub>	GM <sub>hp</sub>	GM <sub>xp</sub>		
Crude protein (g/kg of DM)	566	580	594	-	-
Nonprotein nitrogen (g/kg N)	320 <sup>a</sup>	298 <sup>a</sup>	217 <sup>b</sup>	8.76	< 0.01
Buffer insoluble nitrogen (g/kg N)	666 <sup>a</sup>	731 <sup>b</sup>	774 <sup>c</sup>	9.00	< 0.05
Neutral detergent insoluble nitrogen (g/kg N)	56 <sup>a</sup>	104 <sup>b</sup>	118 <sup>b</sup>	1.88	< 0.05
Acid detergent insoluble nitrogen (g/kg N) <sup>2</sup>	10 <sup>a</sup>	11 <sup>ab</sup>	18 <sup>c</sup>	0.66	< 0.05
Quickly degradable fraction (a)	0.10	0.09	0.08	0.03	> 0.05
Slowly degradable fraction (b)	0.94	0.95	0.9	0.06	> 0.05
Fractional constant rate of degradation (c)	0.10 <sup>a</sup>	0.08 <sup>a</sup>	0.06 <sup>b</sup>	0.01	< 0.05
Ruminal disappearance (g/g)	0.996 <sup>a</sup>	0.997 <sup>a</sup>	0.989 <sup>b</sup>	0.004	< 0.05
Post-ruminal disappearance of rumen undegradable (g/g)	0.918 <sup>a</sup>	0.906 <sup>a</sup>	0.965 <sup>b</sup>	0.008	< 0.05

<sup>a, b, c</sup> Means with a different letters in each row are significantly different at P< 0.05.

**Conclusions** It was demonstrated that overheated-xylose processing might increase the intermediate (BISN) and slowly degradable fractions (NDIN) of GM. In addition, these are effective methods of altering the CP rumen degradation characteristics of GM. Therefore, both methods could be used to increase the proportion of the rumen non-degradable protein fraction in GM sources which would then reach the small intestines unaffected by ruminal fermentation. It was concluded that overheated and overheated-xylose processing has a benefit effect on GM protein as reducing the ruminal disappearance and enhancing the post-ruminal value.

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## ***In vitro* first order dry matter disappearance kinetics of chemically and physically treated cottonseed hulls**

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**Introduction** Limited supplies and or high costs of conventional roughages and concentrates dictate that alternate sources of feeds should be used in ruminant rations (Brown *et al.*, 1976). Cottonseed hulls (CH) are a by-product of cotton processing, containing a large proportion of neutral detergent fibre (NDF) and associated lignin, and have been considered as a useful non-forage fibre source in ruminant rations (Hall and Akinyode, 2000). However, low dry matter (DM) digestibility of CH is an inhibitory factor to include this feedstuff in the high performance dairy cow diets (Brown *et al.*, 1976). Results of previous *in vitro* studies have revealed that sodium hydroxide treatment of fibrous feedstuffs can improve DM or NDF digestibility of them. The objective of this study was to determine the effect of chemical, using sodium hydroxide, or physical treatment, using microwave irradiation, on *in vitro* DM disappearance kinetics of CH.

**Material and methods** For chemical treatment, CH were treated with NaOH as 20 g/kg DM [a 20% solution of NaOH was sprayed on CH and kept for 0.5 h (CH2S0.5) or 48 h (CH2S48)] or 40 g/kg DM [a 40% solution of NaOH was sprayed on CH and kept for 0.5 h (CH4S0.5) or 48 h (CH4S48) at room temperature]. Physical processing was done using microwave irradiation (900 W) for 4, 6 and 8 min (CHm4, CHm6, CHm8, respectively). Samples were incubated in a medium prepared as described by Arroquy *et al.* (2005). Forty-five ml of medium was supplied into a 100 ml bottle containing 0.45 g DM of each sample (Four replicates per each sample were run). Then, each bottle was inoculated under carbon dioxide with 5 ml of mixed rumen microbes. Rumen fluid was obtained from three sheep (49.5±2.5 kg body weight) fitted by rumen fistulae, before the morning feeding, and immediately strained through four layers of cheesecloth. The animals fed 1 kg/d of DM lucerne hay and 0.3 kg/d DM concentrate (165 g CP/kg DM). The bottles were incubated for 24, 48, 72 and 96 h at 38.6° C. After each time of incubation, bottle contents were filtered through a 42 µm filter, and DM of unfiltered medium was determined. Non-linear first order model was used to estimate the digestion kinetic parameters of DM. The model was  $D_{(t)} = D_{(i)} \cdot e^{-k_d \cdot t} + I$ ; where,  $D_{(t)}$  is residual DM at any time,  $D_{(i)}$  is potentially degradable fraction,  $k_d$  is fractional rate constant of digestion ( $h^{-1}$ ) and  $I$  is indigestible fraction.

**Results** Non-linear first order parameters of *in vitro* DM disappearance of the samples are presented in Table 1. Physical or chemical treatment had no significant effect on DM disappearance parameters of CH.

**Table1** Non-linear first order parameters of *in vitro* DM disappearance of non treated or chemically and physically treated cottonseed hulls

Parameters	Treatments								s.e.m	P
	CH	CH2S0.5	CH2S48	CH4S0.5	CH4S48	CHm4	CHm6	CHm8		
$D_i$	0.310	0.310	0.260	0.340	0.350	0.300	0.26	0.290	0.088	P> 0.05
$K_d$	0.007	0.016	0.011	0.006	0.009	0.008	0.011	0.010	0.005	P> 0.05
$I$	0.140	0.140	0.190	0.110	0.110	0.150	0.190	0.160	0.091	P> 0.05
$R^2$	0.97	0.93	0.98	0.96	0.96	0.98	0.97	0.97		

**Conclusions** Results of the present study indicate that non-linear first order parameters of *in vitro* DM disappearance of CH were not influenced by the chemical or physical processing. Present results did not confirm previous findings used NaOH to enhance the ruminal degradability of NDF sources (Ololade *et al.*, 1975). It was previously indicated that DM digestion of forages might be enhanced by NaOH treatment (Canale *et al.*, 1985). Sadeghi and Shawrang (2008) showed microwave irradiation caused a decrease in degradation potential of barley grain starch. However, present results indicate that it is not beneficial to use microwave irradiation for enhancing the DM digestibility of CH.

**Acknowledgements** The authors gratefully acknowledge funding from Excellence Centre for Animal Science, Ferdowsi University of Mashhad.

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## Use of white rot fungi to improve the feed value of rice straw

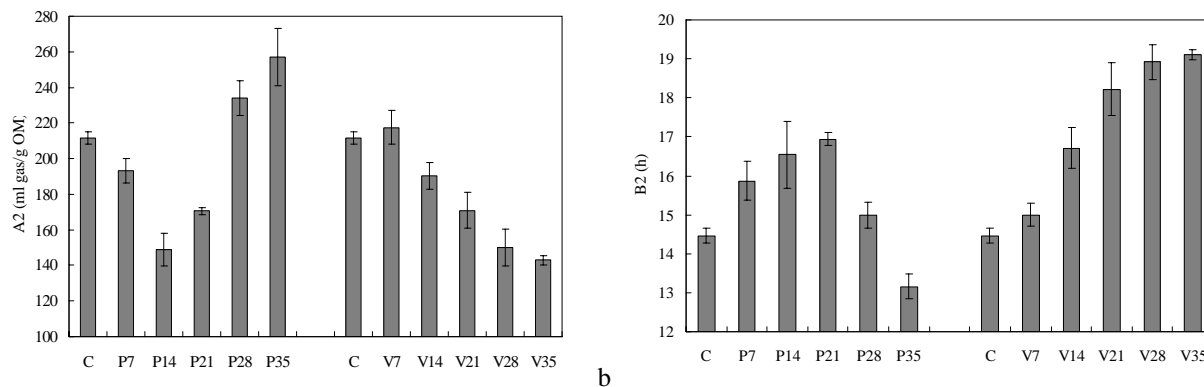
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**Introduction** In many countries straw is a main feed for cattle in the winter or in the dry season. However, the feed value of straw is too low to obtain high production levels. One of the main reason for the low feed value of straw is the high lignin level (for rice straw up to 5 %), preventing microbes to access the valuable carbohydrates and causing a low feed intake. In the past many attempts have been published to increase the feed value, using sodium hydroxide, urea and ammonia. Rodrigues *et al.* (2008) showed that enzymes of fungi are capable of increasing the digestibility of straw in rumen fluid. The aim of the present research was to investigate if growing fungi can be used to unseal the straw and to increase its feed value. Rice straw samples were incubated for different periods with two strains of white rot fungi before being fermented in rumen fluid, using the gas production technique.

**Material and methods** Rice straw from Thailand was chopped to pieces of 3 cm. The white rot fungi *Pleurotus ostreatus* and *Volvariella volvacea* were inoculated for 6 days on 2% malt agar disks (diameter, 7-8 mm) at 24 °C. Each 20 grams of chopped rice straw samples were put into 1000 ml Erlenmeyer flasks with 80 ml of distilled water. The flasks were autoclaved at 121 °C for 30 min and, after cooling, three agar disks of each fungus culture were added aseptically and incubated at 24 °C for 0 to 35 days. Three replications for each fungus culture of each incubation period were used. After incubation the rice straw samples were freeze dried, ground to 1 mm and analysed for fermentability in rumen fluid. Gas production incubations were performed as described by Cone *et al.* (1996), using rumen fluid from 2 non lactating Holstein Friesian cows receiving 1 kg of concentrate and *ad libitum* grass silage.



**Figure 1** (a) Gas production (A2, ml gas/g OM) of the non-soluble fraction and (b) time to reach half of A2 (B2, h) of untreated rice straw (C) and straw incubated for 7 to 35 days with the white rot fungi *Pleurotis ostreatus* (P) and *Volvariella volvacea* (V).

**Results** Figure 1a shows the gas production (ml gas/g OM), caused by fermentation of the non-soluble fraction (A2) of the straw samples after incubation with the white rot fungi *P. ostreatus* and *V. volvacea* for different periods (days). Figure 1b shows the half time to reach A2 (B2), which is an indication of the rate of fermentation of the non-soluble fraction, in this case NDF. A lower value of B2 means an increased rate of fermentation. The results show that prolonged incubation with *V. volvacea* results in a lower fermentation of the NDF (decreased A2) and a slower rate of fermentation (increased B2), indicating that during the incubation of the straw with the fungus, *V. volvacea* used the most easily digestible parts of the straw, remaining less for the rumen microbes. The same was seen for straw samples incubated with *P. ostreatus* up to 14 days. Incubating the straw for a prolonged period with this fungus resulted in an increased fermentation of the NDF in rumen fluid with an increased rate. This indicates that initially *P. ostreatus* also used easy assessable carbohydrates, but after 2 weeks the fungus used the difficult degradable components, most likely lignin, increasing the degradability of the straw in rumen fluid.

**Conclusions** The results show that a prolonged incubation of rice straw with the white rot fungus *P. ostreatus* increases its feed value for ruminants.

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## Cultivation of oyster mushrooms (*Pleurotus* species) to improve the *in vitro* dry matter digestibility of wheat straw for feeding to ruminants

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**Introduction** Wheat straw is an underutilized energy resource for animals: it is indigestible and has a low protein content. Most methods for improving the digestibility are not cost effective or have potential health and environmental hazards. This paper focuses on using a biological pre-treatment with *Pleurotus* spp, lignin degrading white-rot fungi, to improve wheat straw from low to high available energy feed. *Pleurotus* spp. are able to degrade the lignin in a selective way (Kerem & Hadar, 1993) thus making cellulose available for ruminants. *Pleurotus* spp. are widely cultivated worldwide to produce oyster mushrooms and have a high protein content, are free from cholesterol and are rich in carbohydrates, fibre, vitamins and minerals (Kues & Liu, 2000). *Pleurotus* spp. also have a medicinal potential. The aims of this study were to investigate the ability of different *Pleurotus* spp. to increase the protein content, degrade lignin and improve digestibility of the wheat straw. Production of edible mushrooms was also considered.

**Material and methods** Five *Pleurotus* spp. were screened for their ability to increase the protein content, degrade lignin and improve digestibility of wheat straw. Four of the *Pleurotus* strains used came from local resources (*P. ostreatus*: P. ost x TW, P. ost x TG, Po x K; *P. florida*) and 1 commercial strain of *P. pulmonarius* was purchased (Mycelia, Ghent, Belgium). Wheat grain was infected to inoculate straw. Straw was chopped into lengths of 3-5 cm and soaked in water for 20 mins and left to drain. Culture bags were each filled with 1 kg of soaked straw and a microelement solution was added (Per bag: ZnSO<sub>4</sub>·7H<sub>2</sub>O: 0.017 mg, MgSO<sub>4</sub>·7H<sub>2</sub>O: 0.32 mg and CuSO<sub>4</sub>: 0.025 mg). Bags were autoclaved sterilised and inoculated by adding 60 g of colonised grain. Bags were sealed and incubated for 19 or 42 days in total, in duplicate. Those which were incubated for 42 days in total were incubated for 19 days for colonisation at 22 °C, 65% relative humidity, 1 day cold shock at 10 °C and 22 days fruiting at 24 °C, 75% relative humidity with a 10 hours per day light cycle (470-700 nm). In the final stage pinholes were made in bags to support air exchange and this allowed emergence and proper development of fruit bodies. The other set of bags were incubated for 19 days as above. For each treatment, 2 bags without fungal inoculation were used as controls. Crude protein, and lignin content were determined (Halliday 1985), while the Modified Faeces Liquor technique (Omed *et al.* 1989) was used for digestibility estimation. The statistical analysis was performed using SPSS 14.2.

**Results** During the experiment all strains produced fruit bodies, but not all fruiting bodies reached the point of harvest at the experiment end. Increases in digestibility and crude protein were highly significant ( $p=0.000$ ), especially after 42 days incubation but lignin decreases were not ( $p=0.386$ ) (Tables 1-3).

**Table 1** Relative increase compared to control, \*statistically different from the control ( $p<0.01$ )

Strain	Digestibility (%)		Crude protein (%)		Lignin (%)	
	19 days	42 days	19 days	42 days	19 days	42 days
P. ostreatus x TG	23.92	103.77*	54.94 *	141.55*	7.61	20.93
P. ostreatus x TW	9.57	91.19*	55.64 *	128.97*	20.41	6.5
P. ostreatus x K	50.25 *	125.78 *	83.75*	121.38*	13.77	19.63
P. pulmonarius	57.41*	125.78*	66.80*	69.22*	13.30	13.72
P. florida	19.13	116.35 *	71.45*	160.81*	9.08	9.95

**Table 2** Pooled SEM

Treatment	Pooled SEM for IVDMD (%)	Pooled SEM for crude protein (%)	Polled SEM for Lignin (%)
19 days	1.0775	0.055	0.45
42 days	0.9601	0.064	0.78

**Table 3** P value for the effect of straw source, *Pleurotus* species, and interaction between straw source and *Pleurotus* species.

Analysis	Effect of straw source	Effect of <i>Pleurotus</i> spp.	Effect of straw source and <i>Pleurotus</i> spp.
Protein	$p=0.000$	$p=0.000$	$p=0.000$
Lignin	$p=0.03$	$p=0.94$	$p=0.356$
Digestibility	$p=0.000$	$p=0.000$	$p=0.000$

**Conclusions** This study showed that with fungal treatment it was possible to achieve increase in digestibility with small reductions in total lignin content (2-5% lignin). Combined with this were significant crude protein increases. These improvements increased with longer incubation periods. However the extent of the changes in digestibility, crude protein and lignin content was strain specific. The current research demonstrated that it is possible significantly increase digestibility, protein content of wheat straw and produce edible fruit bodies at the same time. It can be also assumed that feeding animals with protein enriched substrate and transformed substrate will not only constitute feed but may also be beneficial due to the immuno-stimulation properties of *Pleurotus*. The cultivation of *Pleurotus* species is also an economically sound strategy to convert agro-residues into nutritional foods and medicinal products.

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## Evaluation of condensed tannin content of some native tropical tanniniferous plants from semi-arid regions in Brazil

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**Introduction** During periods of feed shortage in the semi-arid regions, cattle and small ruminants consume leaves of trees and shrubs that have fallen to the ground. However these leaves, as with most tropical legumes, have significant levels of total tannins which can influence their nutritive value. Moreover, these plants may have a potentially high nutritional value and are well adapted to semi-arid conditions so they may be of use for feeding livestock. The objective of this study was to evaluate the condensed tannin content of some native tropical tanniniferous plants from semi-arid areas of Brazil.

**Material and methods** Samples of the plants were collected from the semi-arid regions of Pernambuco, located in northeast Brazil. Six browse species with potential use as forage were randomly selected in 450 ha natural grazing area: *Astronium urundiuva* Engl, *Sida cf. Cordifolia* L., *Caesalpinia pyramidalis* Tul, *Ipomoea* sp., *Desmanthus virgatus* L. and *Leucaena leucocephala*. The collections were carried out in the dry season (March) with five replicates for each plant. The plants were losing their leaves and starting the dormancy stage and this is the time the animals look for these plants to eat. The collected material was shade-dry. Total phenol (TP), total tannins (TT), and condensed tannins (CT) were analysed as described by Makkar (2003). Results were analysed using analysis of variance in a factorial design (six species and five repetitions). Data were compared by  $P < 0.05$  level using the SAS system (SAS, 2008).

**Results** Mean values of total phenols (TP), total tannins (TT) and condensed tannins (CT), are shown in Table 1. The results showed that there were significant differences between the experimental species in phenolic compounds concentrations. *Astronium urundiuva* Engl had the highest TP and TT concentrations (307.8 and 266.45 eq-g tannic acid/kg DM, respectively) followed with *Caesalpinia pyramidalis* Tul. (132.6 and 126.0 eq-g tannic acid/kg DM for TP and TT). Also the CT concentrations showed significant differences between the experimental plants. *Sida cf. cordifolia* L. had the highest content of CT (109.9 g-leucocyanidina g/kg DM) followed with *Desmanthus virgatus* L. and *Leucaena leucocephala* (82.85 and 53.35g-leucocyanidin /kg DM) respectively. On the other hand, *Astronium urundiuva* Engl, *Caesalpinia pyramidalis* Tul. and *Ipomoea* sp. had the lowest concentrations of CT (29.22, 5.63 and 4.61g-leucocyanidina /kg DM, respectively). These results indicated that CT concentrations of *Caesalpinia pyramidalis* Tul, *Ipomoea* sp. and *Astronium urundiuva* Engl were within the safe level (30 to 40 eq-g-leucocyanidina /kg DM) for ruminant nutrition as recommended by Barry, (2003).

**Table 1** Means of total phenol (TP), total tannins (TT) (eq-g TA /kg DM) and condensed tannins (CT) (eq-g leucocyanidin /kg DM) tropical tanniniferous plants from semi-arid region of Pernambuco, Brazil.

Plants	TP		TT		CT	
	Mean	SD	Mean	SD	Mean	SD
<i>Astronium urundiuva</i> Engl	307.80 <sup>a</sup>	39.01	266.45 <sup>a</sup>	38.60	29.22 <sup>d</sup>	20.52
<i>Sida cf. cordifolia</i> L.	88.83 <sup>cd</sup>	14.92	64.93 <sup>cd</sup>	10.36	109.90 <sup>a</sup>	24.05
<i>Caesalpinia pyramidalis</i> Tul.	132.60 <sup>b</sup>	17.17	126.00 <sup>b</sup>	16.38	5.63 <sup>e</sup>	4.30
<i>Ipomoea</i> sp.	133.90 <sup>d</sup>	16.99	112.20 <sup>d</sup>	19.15	4.61 <sup>e</sup>	2.77
<i>Desmanthus virgatus</i> L.	109.20 <sup>bc</sup>	16.78	59.75 <sup>d</sup>	13.81	82.85 <sup>b</sup>	15.14
<i>Leucaena leucocephala</i>	93.34 <sup>cd</sup>	17.69	76.36 <sup>d</sup>	13.25	53.35 <sup>c</sup>	16.59

<sup>a,b,c,d,e</sup> Means within the same column having different letters are significantly different ( $p \leq 0.05$ )

**Conclusion** The content of condensed tannins of some tropical tanniniferous plants from semi-arid region in Brazil (*Ipomoea* sp., *Caesalpinia pyramidalis* Tul. and *Astronium urundiuva* Engl ) are within the range of safe levels, and probably do not cause problems for ruminant nutrition. Further research is needed to study the effects of these plants on rumen fermentation and degradability in livestock.

**Acknowledgements** The authors would like to thank CAPES for financial support.

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## Chemical composition and dry matter degradability coefficients of Fennel seed

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**Introduction** Fennel (*Foeniculum vulgare*), is a plant species in the genus *Foeniculum* (treated as the sole species in the genus by most botanists). It is a member of the family *Apiaceae* (formerly the *Umbelliferae*). It is a hardy, perennial, umbelliferous herb, with yellow flowers and feathery leaves. Fennel is widely cultivated, both in its native range and elsewhere, for its edible, strongly-flavoured leaves and seeds. Its aniseed flavor comes from anethole, an aromatic compound also found in anise and star anise, and its taste and aroma are similar to theirs, though usually not as strong. Seeds of the fennel plant are widely used in many of the culinary traditions of the world. There are historical anecdotes that fennel is a galactagogue improving the milk supply of a breastfeeding mother (Crellin *et al.*, 1989). This use, although not supported by direct evidence, is sometimes justified by the fact that fennel is a source of phytoestrogens, which promote growth of breast tissue (Mark, 2000). The effects in animals is unclear, thus determination of these effects will be important for animals especially ruminants. The objective of this experiment was determination of DM degradability and chemical composition of Fennel seeds.

**Materials and methods** Samples of Fennel seed were obtained from Golchekan-Zamani factory in the Razavi Khorasan district. The samples were analyzed for dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and organic matter based on procedure described in AOAC methods (2000). Measurements of *in situ* DM were performed in 3 rumen fistulated bull cows (fed at maintenance) using the nylon bag technique (Krskov and McDonald, 1979). Nylon bags which were approximately (9×18 cm) containing 5 g (2mm screen) were incubated in the rumen of fistulated bull cows for 2, 4, 8, 16, 24, 48, 72, 96 and 120 h. There were 4 replications per treatment. The rate and extent of DM degradation were estimated according to the equation:  $P = a + b(1 - e^{-ct})$ . Effective degradability (ED) was calculated according to equation:  $ED = a + (b \times c)/(c + k)$ , assuming an outflow rate (k) of 0.02 h<sup>-1</sup>. The data were analyzed using the ANOVA procedure of SAS (SAS Institute, 2002).

**Results** Degradable coefficients and ruminal disappearance of DM are summarized in Table 1. The chemical composition of fennel seed is shown in Table 2.

**Table 1** *In situ* degradability coefficients disappearance (Mean with SEM) of DM of Fennel seed.

Fennel seed	
Degradation parameters	
a <sup>1</sup>	0.32±0.01
b <sup>2</sup>	0.46±0.01
c <sup>3</sup>	0.037±0.003
a+b <sup>4</sup>	0.58±0.02
Effective degradability	
k=0.02	0.62±0.03
k=0.03	0.58±0.03

<sup>1,2,3,4</sup> constants in the equation  $P=a+b(1-e^{-ct})$  where P = level of degradation at time t; a=readily soluble fraction; b=insoluble fraction but degradable in rumen; c=rate of degradation of b per hour and a+b = potentially degradable fraction.

**Table 2** Chemical composition(g/kg) of Fennel seed

Fennel seed	
Composition (%)	
DM	914±4
ADF	382±13
NDF	421±15
CP	182±11
ASH	117±2

**Conclusions** The crude protein and Ash contents of fennel seed, evaluated in the present study, were almost high. Rapidly and slowly degradable fractions of fennel seed for DM indicated that more than 0.58 of this feed could be digested in the rumen. High digestibility of fennel seed and high nutritional value of it shows that it can supply intermediate protein for animals.

**Acknowledgments** The authors thank the Ferdowsi University of Mashhad and Excellence Centre for Animal Science for financial and technical support.

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## Effect of urea and polyethylene glycol on chemical composition of pistachio by-products silage

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**Introduction** Increase in fossil fuel costs and shortage of water sources have increased animal feed costs in many countries. Use of agricultural by-products such as pistachio by-products (PB) as animal feeds is a means of recycling which reduces environmental pollution. Up to 400,000 tonnes/year of this by-product is produced in Iran. Because of its high moisture content and a high level of tannins, ensiling and treating PB with urea and polyethylene glycol (PEG) are potential ways to preserve it and overcome the anti-nutritional effects of tannins. The aim of this study was to evaluate the effect of urea and PEG treatments on deactivation of tannins in PB silage.

**Material and methods** Fresh PB was collected from pistachio de-hulling factories in Feyzabad (Iran), and samples of these by-products averaging 32% dry matter (DM) were ensiled for 60 days in 16 plastic buckets with 4 replicates. Four treatments were as follows: Pistachio by-products silage (PBS) as a control, PBS supplemented with urea at 0.1 and 0.5 % of DM, and PBS supplemented with PEG at 1% of DM. Silage pH was determined on expressed juice obtained by thorough mixing of 50g of fresh silage with 450 ml of distilled water and allowing to stand at 25°C for 30 min. This extract was also used for ammonia-N measurement. The pH of extract was measured using a portable digital pH meter (METROHM 691) and the silage crude protein was analysed by the AOAC (1990) method. Ensiled samples were dried in an oven at 40°C and then ground to pass a 2 mm sieve and 0.5 mm sieve for tannin assay. Total phenolics (TP) and total tannins (TT) were measured by using the Folin Ciocalteu method (Makkar *et al.*, 1993) and the results were expressed as tannic acid equivalent. Total condensed tannins were determined by the procedure of Porter *et al.* (1986). Data were analysed statistically using GLM procedure of SAS (9.1) as a completely randomised design. Means were separated by Duncan's multiple range test when a significant ( $P < 0.05$ ) treatment effect was observed.

**Results** Silage pH was increased ( $p < 0.05$ ) by urea (0.5%) addition, but was not influenced by PEG treatment. Adding urea significantly increased the crude protein and ammonia-N concentrations of silages when compared to the control treatment. Ammonia N concentration was increased ( $p < 0.05$ ) by adding PEG. The lowest content of total phenolics, total tannins and condensed tannins were observed in PEG treatment that was statistically significant for TP and TT when compared to other treatments. Addition of urea (0.5%) decreased ( $p < 0.05$ ) TP and TT as compared to the control. Condensed tannins were not influenced by treatments ( $p < 0.05$ ).

**Table 1** Chemical composition and contents of phenolics of ensiled and treated pistachio by-products (g/kg DM)

Item	Pistachio by-products silage treatment				SEM	P-value
	Control	Urea (0.1%)	Urea (0.5%)	PEG		
pH	4.25 <sup>b</sup>	4.31 <sup>ab</sup>	4.34 <sup>a</sup>	4.25 <sup>b</sup>	0.024	< 0.05
Crude protein (g/kg DM)	120.1 <sup>b</sup>	127.5 <sup>a</sup>	128.7 <sup>a</sup>	120.2 <sup>b</sup>	0.82	< 0.05
Ammonia-N (mg/dl)	5.27 <sup>c</sup>	8.90 <sup>b</sup>	12.45 <sup>a</sup>	8.03 <sup>b</sup>	0.273	< 0.05
Total phenolics (TP; g/kg DM)	99.9 <sup>a</sup>	94.5 <sup>b</sup>	93.9 <sup>b</sup>	89.0 <sup>c</sup>	1.53	< 0.05
Total tannins (TT; g/kg DM)	51.8 <sup>a</sup>	47.9 <sup>a</sup>	43.0 <sup>b</sup>	34.0 <sup>c</sup>	1.53	< 0.05
Condensed tannins (g/kg DM)	11.5	10.1	9.5	8.2	0.79	> 0.05

<sup>a, b, c</sup> Means within a row with different superscripts differ ( $p < 0.05$ ).

**Conclusions** These results show that pistachio by-products can be ensiled since it has a relatively low pH of 4.25 after ensiling. Ensiling and addition of urea and PEG can be effective in deactivation of PB tannins leading to an improvement in nutrient bioavailability.

**Acknowledgements** Financial support from the Agricultural Faculty of Ferdowsi University of Mashhad is gratefully appreciated.

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## Logistic regression analysis of some environmental factors affecting days open in Iranian primiparous Holstein cows

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**Introduction** In practical dairy cattle breeding schemes, both production and reproduction performance of animals are of particular economic importance. Over the past decades, most selection pressure has been on cow's milk yield resulting in increasing fertility problems. Profitability of a dairy farm enterprise is a function of several factors among which good reproduction performance of animals is of great importance. A cow with higher conception rate is expected to produce more income over her herd life for a breeder. Among different measures associated with cow fertility, days open has long been considered by researchers. Genetic and non-genetic factors influence days open. In much research, days open has been analysed by a linear model in which a normal distribution is assumed for the trait. However, few studies have been undertaken to evaluate factors affecting open days using a non-linear model. The main aim of this study was to analyse some environmental factors associated with days open in Iranian first lactation Holstein cows using a logistic regression approach.

**Material and methods** Records were collected from 5511 Iranian first lactation Holstein heifers distributed in 64 herds from Mashhad, Iran between 1994 and 2007. Average lactation milk yield, Holstein gene percentage, air temperature at calving day, age at first calving and days open were 7413 kg, 93.42%, 16.05 °C, 26.33 months and 114 days, respectively. Days open was not normally distributed. A logistic regression model (Fang, 2005) was fitted to the data (SAS Institute Inc. 2004). In the data file, as applied by Cole and Null (2009), days open lower than 50 days and greater than 250 days were set to 50 and 250 days, respectively. In the model, fixed environmental effects of herd, year and season of calving along with two-level effects of milk yield (MCODE), age at first calving (ACODE), Holstein gene percentage (HCODE) and air temperature at calving day (TCODE) were included. Two-level effect for each environmental factor was set based on its average. Days open was also defined as a dichotomous dependent variable based upon its average. For logistic regression model, the probability of days open greater than its average was modeled.

**Results** Herd, year and season of calving as well as milk production level were found to be highly ( $P < 0.001$ ) associated with the probability of days open being greater than average. Days open was not significantly affected by ACODE, HCODE and TCODE. In Table 1, odds ratio estimates obtained for season of calving and milk production level are presented.

**Table 1** Odds ratio estimates (95% confidence interval) for different levels of calving season and milk production

Effect	Summer	Autumn	Winter
Season of calving (as compared to spring calvers)	0.591 (0.501-0.697)	0.714 (0.579-0.880)	0.913 (0.736-1.133)
Milk production level (as compared to lower than its average)	2.457 (2.139-2.823)		

**Conclusions** Many studies (Kirkpatrick, 1999) have shown that days open is of low heritability indicating that most phenotypic variation is explained by environmental factors. Improving reproduction performance, therefore, could be achieved by herd management practices such as providing better nutrition and health. Furthermore, as pointed out by Kirkpatrick (1999), crossbreeding could be an effective tool for exploiting heterosis in a low heritability trait. The results obtained in this study revealed that as compared to spring calvers, cows calving in summer had a lower chance of days open greater than the average (114 days) and that winter calving cows are expected to experience more fertility difficulties as compared to summer and autumn calvers. As expected, high milk producing cows were found to have greater chance for longer open days suggesting that there is a need to take into account of open days as the genetic evaluation of dairy cows is undertaken.

**Acknowledgements** The authors would like to express especial thanks to the Agricultural Jihad Organisation, Mashhad, Iran for providing the data used in this research.

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## Prediction of live-weight from linear body measurements in Iranian buffaloes

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**Introduction** Determining the live weight of domestic animals is necessary for management practices such as ration formulation, drugs administration, recording for breeding and genetic improvement. Weighing is often difficult in large animals such as cattle and buffalo, where many farms do not have complete restraint and handling systems, and few have equipment to determine body weights. Therefore, equations to estimate body weight from other body measurements, which have a high correlation with body weight, are needed. Bassano *et. al.* (2001) reported the use of linear body measurements to estimate body weight of Alpine Ibex. These equations would be useful when weighing facilities are not available. The objective of this investigation was to find regression equations to predict live-weight from body measurements for Iranian buffaloes.

**Material and methods** Data were collected from two groups of animals. Most measurements were made on the livestock research farm of Safiabad from 80 buffaloes over 5 years and the remainder (100 buffalo) were collected from commercial farms in Khuzestan, Iran. Body weight (BW) was measured using a weighbridge and the following linear body measurements using a tape measure. Body length (BL) was measured as the distance from the external occipital protuberance to the tail base. Height-at-withers (HW) was measured as the distance from the ground level to the withers and heart girth (HG) represented the circumference of the chest just behind the forelimbs. Data collected were classified based on sex and age. Mean  $\pm$  SE for body weight and linear body measurements (BL, HW and HG) were calculated. The relationships between bodyweight and linear body measurements were estimated by Pearson correlation. Regressions of body weight on HG, HW and BL utilizing individual observations were performed. Linear, quadratic, power and cubic effects of the independent variables were considered. To indicate the accuracy of linear measurement of the predictive equation, we used the coefficient of determination ( $R^2$ ). Statistical analyses were performed by using SPSS statistics program.

**Results** The mean body weight of buffalo males and females were observed as  $281.5 \pm 4.9$  and  $295.4 \pm 4.7$  kg, respectively. The mean body length of males was found to be  $1.22 \pm 0.01$  m while that of females was  $1.21 \pm 0.01$  m. The mean height-at-withers in males was  $1.18 \pm 0.005$  m while that of females was  $1.17 \pm 0.004$  m. Mean heart girth in males was  $1.45 \pm 0.01$  m and in females  $1.49 \pm 0.01$  m. Body weight was correlated with body length (males and females: 0.95), height-at-withers (males: 0.92, females: 0.93) and heart girth (males and females: 0.97), respectively. The correlation coefficients between body weight and all body measurements were high but heart girth was found to be the measurement most closely related to live-weight. After comparing different regression equations of body weight on heart girth, the equations below estimated buffalo body weight (kg) from heart girth (cm) with the highest  $R^2$ . Suggested equations differed between buffalo males and females significantly ( $p < 0.0001$ ) using general linear model and contrast procedure of SAS.

### Males

Birth to 1 year old: Weight =  $80.352 (\text{Heart girth})^{2.959}$ ,  $R^2 = 99.1\%$

1 year old and above: Weight =  $-755.929 + 676.1 (\text{Heart girth})$ ,  $R^2 = 97\%$

### Females

Birth to 1 year old: Weight =  $79.627 (\text{Heart girth})^{2.942}$ ,  $R^2 = 99.1\%$

1 year old and above: Weight =  $-642.061 + 601.5 (\text{Heart girth})$ ,  $R^2 = 96.6\%$

### Both sex

Birth to 1 year old: Weight =  $79.984 (\text{Heart girth})^{2.949}$ ,  $R^2 = 99.1\%$

1 year old and above: Weight =  $-670.831 + 621.4 (\text{Heart girth})$ ,  $R^2 = 96.2\%$

**Conclusion** The high correlation between body weight and heart girth would imply that live weight could be predicted fairly accurately from heart girth. Heinrichs *et. al.* (1992), reported the highest  $R^2$  for the regression of body weight on heart girth ( $R^2 < 0.95$ ) in investigation of body measurement of Holstein heifers. Özluturk *et. al.* (2006) concluded that the models including heart girth alone could be used to predict precisely body weights of Eastern Anatolian Red cattle. On the basis of these equations, we can design and produce a weight tape which can be used as a simple tool to estimate buffalo weight by farmers, buffalo feed and breeding expertise, veterinarians.

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## The impact on groundwater of the land spreading of organic wastes onto biomass crops

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**Introduction** Land-spreading on bioenergy crops is a possible route for the disposal of some organic wastes. Issues related to water-pollution make research into the environmental impact of these practices a priority. Hazards include the possibility of a decline in surface- and ground-water (GW) quality from pollution by heavy metals (HMs) and nutrients contained within such wastes. The viability of land-spreading of bio-solids and brewer's waste was investigated from an overall water-quality perspective including runoff water, soil moisture, and GW. The interaction of spread waste, crop, rainfall, the soil matrix is very complex; however, and the focus of this presentation will only be on the GW component of the overall water-quality picture.

**Material and methods** Spreading was conducted on an annual basis on long-established plantations of *Miscanthus X Giganteus* located in Oak Park, Co. Carlow, Ireland. The plots were 0.12 ha in size and organic wastes were applied at 3 treatment levels: heavy (H=100%), light (L=50%) and control (C=0%). Three plots on the Barley Field (BF) were spread with Brewer's Waste (BW) and three on the nearby Near Avenue Meadow (NAM) plantation with biosolid (BS). The procedures for spreading and upper limit of spreading was based on regulatory limits and standards (DoE, 1998; EPA, 2006) and each year was considered as a replicate period for statistical purposes. Biosolid waste was spread on the NAM using a towed disc-spreader and BW on the BF using an irrigation system. Prior to spreading, GW wells were inserted into each plot; 3 wells were placed on treated plots and 1 well on each control plot. Monthly samples were taken for 24 months between Oct 2007 and Oct 2009. Samples collected from plots with multiple wells were bulked before analysis each month. All samples were collected in accordance with guidelines published by the US EPA (US EPA, 1996). Samples were filtered using a Sarstedt 0.45um micropore filter, sent to the Teagasc Water Lab in Johnstown Castle, (Co Wexford) and analyzed for NO<sub>3</sub>, P and K and heavy metals (HMs): Ni, Cd, Pb, Zn, Cu, and Cr using AA spectroscopy. Conductivity and pH analysis were conducted on all samples and monthly rainfall and GW well-water levels were recorded. Nutrient and HM levels were assessed in relation to waste-spreading quantities applied; mean concentrations recorded over the 24 month monitoring period, and the interim guideline values (IGVs) set by the EPA for each of the species in response to the EU Water Framework Directive (EC Directive, 2004).

**Results** Table 1 provides mean concentration levels of bulked monthly GW samples for each plot for the 22 month period from Oct 07 to Aug 09. Maximum concentrations are also included in each box (in italics on the right). Results are still pending for Sept-Oct 09 and statistical work on significance, variance, and treatment-correlation is currently being undertaken.

**Table 3** Mean/Max Nutrient and HM concentrations Oct 07-Sept 09 (H = heavy 100%) (L = light 50%) (C = control 0%).

Plot, Waste Type, and Treatment level	NAM 1, Biosolid (C)	NAM 2, Biosolid (L)	NAM 3, Biosolid (H)	BF 9, BW (C)	BF 8, BW (L)	BF 7, BW (H)	IGVs
Mean/Max NO <sub>3</sub> 07-09 (mg/l)	6.4/78.3	6.2/22.7	8.7/74.2	8.7/104.2	4.9/27.2	7.0/57.7	25 mg/l
Mean/Max K 07-09 (mg/l)	1.2/4.9	The 1.0/3.0	3.8/13.4	1.9/4.1	2.3/10.4	40.8/894.0	5 mg/l
Mean/Max P 07-09 (mg/l)	0.05/0.91	0.44/9.7	0.2/2.85	0.004/0.04	0.2/0.18	0.5/0.42	0.03 mg/l
Mean/Max Cu 07-09 (ug/l)	8.7/40.7	8.1/49.1	11.2/57.8	8.0/26.2	13.1/33.6	16.3/85.4	30 ug/l
Mean/Max Zn 07-09 (ug/l)	5.1/39.7	5.2/35.4	8.4/62.5	3.1/12	4.8/22.4	13.5/154.9	100 ug/l
Mean/Max Ni 07-09 (mg/l)	13.4./161.1	8.5/152.9	11.4/132.5	13.9/238.9	16.5/208.2	9.9/175.1	20 ug/l
Mean/Max Pb 07-09 (ug/l)	12.5/149.1	25.0/443.6	33.6/445.0	26.4/272.3	18.7/224.5	20.5/300.5	10 ug/l
Mean/Max Cd 07-09 (ug/l)	1.1/4.7	0.5/2.1	1.5/17.9	0.7/5.4	1.5/11.9	1.4/13.5	5 ug/l
Mean/Max Cr 07-09 (ug/l)	3.1/19.0	0.4/4.6	4.0/16.7	1.5/5.6	2.1/12.2	2.2/12.9	30 ug/l

**Conclusions** Maximum concentrations were recorded for several species that were well in excess of IGVs. However, mean values were generally within IGVs (with some exceptions) and treatment did not seem to significantly impact most species' concentrations outside of natural variability. Mean K, P, and Pb concentrations exceeded IGVs; however, this may be due to high background levels or high variability and any correlation with treatment is yet to be fully established.

**Acknowledgements** This author would like to acknowledge the funding received for this work from the Department of Agriculture's Stimulus Research Fund.

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## Characterisation of soiled water on Irish dairy farms

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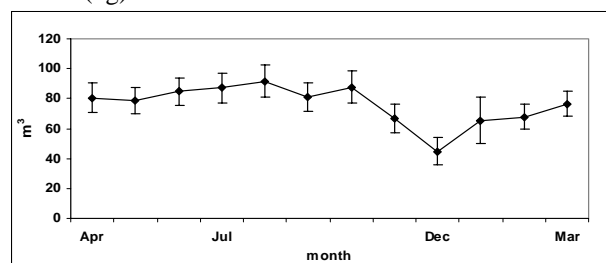
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**Introduction** Soiled water on dairy farms consisting mainly of parlour wash water and run-off from soiled yard areas and contains nutrients that are potentially plant-available but may also pose a risk of environmental pollution if not managed correctly. Effective management of soiled water may lead to cost savings in fertiliser use and address environmental concerns as legislated in the EU Nitrates Directive and Water Framework Directive. Knowledge of the volumes, contents and management practices on Irish farms is a first step towards effective management, but there is very little information on this to-date. The objective of this study was to characterise soiled water and soiled water management practices on Irish dairy farms.

**Material and methods** Sixty dairy farms were selected with a geographic distribution reflecting that of the national dairy herd. Farm selection was constrained by the requirement that all soiled water was collected in a single tank and that there were no other inputs to this tank. Farms were visited every 28 days for one year, commencing in April 2008, giving 13 sample dates per farm. A 500 ml sample was taken from below the surface crust and analysed for total N (TN), NH<sub>4</sub>-N, Total Oxidisable N, K, molybdate reactive P, Total P (TP), Biochemical Oxygen Demand (BOD) and dry matter (DM). Soiled water volumes were recorded by logging vacuum tanker loads or by flow meters on farms using a pumped irrigator system. Farm descriptions and management characteristics were recorded also. Descriptive statistics were used to characterise soiled water quantities and composition.

**Table 1** Mean soiled water concentrations (mg/l) and annual production (kg).

Nutrient	Mean concentration (mg/l)	s.d.	Annual production kg/cow/year (s.d)
TN	587	536	6.0 (3.96)
NH <sub>4</sub> -N	212	206	2.2 (1.2)
K	568	513	5.7 (3.0)
TP	80	68	0.8 (0.5)
DM	5000	5200	9784 l (5135)
BOD	2246	2112	



**Figure 1** Mean soiled water production (and standard errors)

**Table 2** Farm description and management details

Description	Mean	s.d	Description	%
farm size	74ha	31ha	spring calving	74
herd size	102	92	roofed collection yard	31
# milking units	12	7	single stage storage	90
area washed	98.35 m <sup>2</sup>	77.34 m <sup>2</sup>	other effluents mixed	17
tank size	66 m <sup>3</sup>	42m <sup>3</sup>	vacuum tanker	81

**Results and Discussion** Eighty one percent of farms used a vacuum tanker to spread soiled water while the remainder used an irrigation system. Average annual volume produced was 916 m<sup>3</sup> (SD, 723). Mean tank storage capacity for soiled water was 33 days (SD, 23) and 94 % of farms had at least the legally required storage capacity of 10 days. As mean tank size was 66 m<sup>3</sup> (SD, 42) there is limited capacity to store soiled water for long periods. This presents a challenge for nutrient management as soiled water cannot generally be stored until optimal application times. Production varied over the year with a winter minimum (Fig. 1), likely due to the decrease in cows milking in this period (74% of farms were spring calving) (Table 2). This minimum coincides with the period of highest risk of nitrate leaching from land application and likely decreases the risk of nutrient loss from soiled water. Seventy three percent of samples were below the legal definition of soiled water for BOD (2500 mg/l). This further increased to 86% during the winter period, most likely due to the decrease in cows milking, tanks becoming unused and stagnant and increased dilution from rainfall. Mean N, P and K concentrations were 587, 80 and 568 mg/l, respectively. NH<sub>4</sub>-N concentration was 212 mg/l, the balance consisting mostly of plant-unavailable organic N. Therefore, roughly a third of TN consists of rapidly plant-available NH<sub>4</sub>-N. Although nutrient concentrations are low, in comparison to slurry, the large volumes produced offer a potentially significant nutrient source, particularly given evidence for increased N availability at lower DM content. On average, 9784 l of soiled water were produced per cow per year, supplying 6.0 kg N, 5.7 kg K and 0.8 kg P. Nutrient quantities and concentrations vary widely and this presents a challenge for effective nutrient management.

## Conclusions

Production had a winter minimum coinciding with the period of highest risk of nitrate leaching from land application. Although nutrient concentrations are low in comparison to slurry, soiled water contains significant N, K and P. Nutrient quantities and concentrations varied widely and this presents a challenge for effective nutrient management.

**Acknowledgements** This Study was funded by the Department of Agriculture, Fisheries and Food Research Stimulus Fund.

## Use of geophysical techniques to conceptualise groundwater connectivity and sub-surface nutrient (NO<sub>3</sub>) fluxes in hydrogeologically complex terrain

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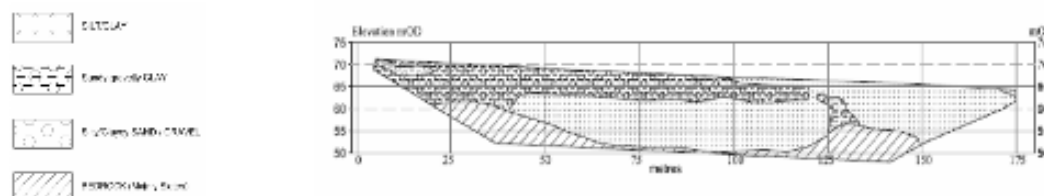
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**Introduction** In a previous study by Fenton *et al.* (2009), shallow groundwater nitrate (NO<sub>3</sub>) contamination was characterised using a methodology combining geochemistry data and hydrogeological testing from 17 piezometers on a 4.2 ha hydrogeologically complex site in SE Ireland over a 2 yr period. NO<sub>3</sub> contaminant mass flux was calculated across three control planes (rows of piezometers perpendicular to groundwater flow direction) showing natural attenuation but no differentiation between dilution and denitrification using this method was possible. Tobit regression, using a background concentration threshold of 2.6 mg NO<sub>3</sub>-N L<sup>-1</sup> showed when assessed individually in a step wise procedure hydraulic conductivity ( $K_{sat}$ ) measured in the screened intervals of each piezometer, was significantly related to groundwater NO<sub>3</sub> concentration. The model relationships indicated that areas with higher  $K_{sat}$  values have less time for denitrification to occur, whereas lower  $K_{sat}$  values allow denitrification to occur. When Cl<sup>-</sup> distribution was examined by the model,  $K_{sat}$  and ground elevation had the most explanatory power but  $K_{sat}$  was not significant, pointing to dilution. Denitrification was further supported with significant correlations between NO<sub>3</sub> occurrence and N<sub>2</sub>/Ar ratio, redox potential, dissolved O<sub>2</sub> and N<sub>2</sub> and was close to being significant with N<sub>2</sub>O. This method allows separation of areas of denitrification and dilution on site but is limited to piezometer drilling depths. Such techniques give no knowledge of bedrock thickness or connectivity of shallow contaminated groundwater with deeper groundwater in the aquifer.

**Material and methods** Six adjacent plots were surveyed using: 1) electromagnetic conductivity mapping (to 6 m depth) to map lateral variations in overburden type and thickness, 2) 2D resistivity profiling (to 25 m depth) to determine overburden and bedrock strata and investigate variations in depth of bedrock, overburden material, bedrock type, connectivity and presence of fracturing and faulting. Physical and chemical groundwater properties are then qualitatively compared with the geophysical results.

**Results** Subsurface conductivity was split into three categories: >26  $\mu S\ m^{-1}$ , 20-26 and <20, which are indicative of silt-clay, gravelly clay and gravelly clay with silt clay lenses over bedrock (Fig 1). Resistivity profiles interpreted depth to bedrock from 6.5 m to 16.3 m. In combination both methods show that NO<sub>3</sub> migration is likely to be impeded by low permeability clay in plot 4 -matching low  $K_{sat}$  areas identified by Fenton *et al.* 2009. NO<sub>3</sub> concentrations in this area remain low due to denitrification. NO<sub>3</sub> migration is present in the sandy gravelly clay layers and in places has good connectivity with generally unproductive except for local zones aquifer below - matching areas with higher  $K_{sat}$  and lower denitrification potential. Mean NO<sub>3</sub>-N concentrations of 12 mg L<sup>-1</sup> exist in these connected areas. Connectivity between the pollution source and the aquifer exists in plots 1, 2, and 5 at discrete locations, which could be important for contaminant mass flux losses. The low  $K_{sat}$  layers in the other plots inhibit this connectivity and natural attenuation should protect surface water and groundwater receptors at these locations. Low permeability zones on several plots (1, 5) may prevent infiltration, migration of lateral flow forcing water to the surface where the high  $K_{sat}$  zones pinch out and contribute to overland flow generation. Particle size distribution samples around the site at surface and 30 cm allow geophysical calibration of shallow sediments. Geophysical results identify areas where natural attenuation through denitrification is possible. Such techniques allow the development of a conceptual model of any site. It can also aid in vertical travel time calculation, as the depth to bedrock may be achieved. Such calculations are important in calculation lag time in catchments between implementing a programme of measures and first improvements in water quality of a waterbody. This techniques also allows accurate drilling of wells to various depths on site.



**Figure 1** Resistivity profiles for plot 5 showing connectivity and confined nature of the underlying aquifer

**Conclusions** Geophysical techniques allow greater insights into subsurface hydrology and contaminant migration pathways. Subsurface geological divisions in this study are indicative of differential  $K_{sat}$  zones, which can be used to infer flow pathways, denitrification and natural attenuation. Identifying the more permeable layers can aid in understanding the migration of NO<sub>3</sub> to surface and groundwater bodies. Such techniques can pinpoint areas of connectivity between highly permeable strata and so identify potential hotspots of contamination, potentially into the deeper aquifer, that may warrant future investigation. Such techniques could also identify areas for overland flow generation.

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## Maintaining biodiversity in intensive grassland: Ground beetle communities in watercourse margins

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**Introduction** Riparian 'buffer' strips, i.e. linear stretches of vegetation next to a watercourse, are now widely used in agri-environmental schemes as a means of reducing water pollution and increasing riparian biodiversity, however additional research into the effects they have on biodiversity is needed. Watercourse banks give the most protection from adjacent habitat conversion to semi-natural grassland plant species (Smart *et al* 2006). Watercourse margins are also important refuges for many invertebrate species (Matern *et al.* 2007). Currently, participants in REPS must fence all watercourse margins 1.5 metres from the stream edge. As no further management is required, these 'buffer' strips often succeed to later successional vegetation communities. A grazed watercourse margin, once fenced, and depending on a nearby source population, can quickly be colonised by bramble and/or gorse vegetation and subsequently by fast growing trees such as willow. The aim of this project is to study ground beetle assemblages within riparian 'buffer' zones in intensively managed grassland. We intend to identify the effects of riparian 'buffer' succession on ground beetle assemblages.

**Material and methods** Ten grassland-based REPS farms within Co. Wexford were selected for sampling. Within these farms a total of 30 fenced, riparian sampling locations were selected. Three sampling locations/plots 20m in length were established on each farm, with each location allocated into one of three categories: Grassy sites (i.e. low herbaceous vegetation); scrubby sites (i.e. over 1.5m vegetation, dominated by bramble and/or gorse) and woody sites (i.e. dominated by mature trees, willow, alder etc.). Pitfall traps were used to sample surface dwelling arthropods between early July and mid September within each vegetation category. Seven pitfalls were placed at each sampling location, giving 21 traps per farm and 210 in total. Traps were placed parallel to each stream, within 1m of the bank edge and with 2m between each trap, and invertebrates were removed every two weeks. Ground beetles from two 2-week sampling periods were identified to species level. Ground beetle assemblage data was analysed with Non-metric Multidimensional Scaling (NMDS) in PCORD and species richness and abundance data were analysed with ANOVA.

**Results** Over two 2-week sampling sessions from July and September 2008 a total of 1845 ground beetles consisting of 49 species were collected. Nineteen species were found in all three habitats. Seventeen species occurred in just one habitat, 14 of those in only one plot. Excluding species which occurred in only one plot (rare species), there were nine species which were not found in any wood plots and there were three species which were not found in grass plots. Only one species found in more than one plot was restricted exclusively to a particular habitat category, *Bembidion guttula* which was only found in scrub habitat on three farms. An ANOVA comparing trap mean species richness for plots in each habitat category did not reveal a significant difference between grass, scrub and wood plots (Table 1). Mean trap abundance was significantly different between different habitat categories (Table 1) with grass having the highest abundance. NMDS ordination showed no pattern of similarity for the same habitats on different farms. However on individual farms each habitat category provided species which were not recorded in either of the other two habitat categories (Table 1).

**Table 4** Differences in abundance, species richness and number of unique species in each habitat

	Total	F <sub>2,54</sub> ; P	Grass	Scrub	Wood
Abundance	1845	4.09; 0.022*	6.95 ± 1.176*	3.44 ± 0.505*	4.65 ± 0.830*
Species	49	0.984; 0.380*	1.19 ± 0.080*	1.13 ± 0.102*	1.02 ± 0.084*
Unique species		3.786; 0.029**	3.42 ± 0.428**	2.58 ± 0.467**	1.79 ± 0.355**

\* Trap Mean per plot ± SE, \*\* Mean no. of species ± SE found only that habitat

**Conclusions** Habitat categories did not show a significant difference in species richness. Plots from the same category on different farms did not show any pattern of similarity. Grass plots showed highest abundance but this was not unexpected, as these plots were part of a larger grass matrix i.e. within the grazed field. On average, each habitat category added unique species to the overall farm-scale riparian diversity of ground beetles. Lack of management subsequent to fencing can result in homogenisation of watercourse margin habitat with a dominance of one particular type of vegetation e.g. gorse scrub. It is important that agri-environment measures relating to watercourse margins take into account the need for habitat heterogeneity in order to provide a variety of habitat for species.

**Acknowledgments** This project was funded by the Department of Agriculture, Fisheries and Food under the National Development Plan 2006 Research Stimulus Fund.

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## Composting the solid fraction of separated pig manure with sawdust, chopped straw or shredded green waste

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**Introduction** Since August 2006, with the implementation of Statutory Instrument No. 378 (S.I. No. 378) the potential for application of organic fertiliser to land in Ireland has been greatly limited. Therefore, alternative management strategies for pig manure, such as composting, should be considered and explored. Composting can increase the fertilizer value of animal manure by binding the mineral nutrients into a stable organic structure. The objective of this study was to investigate the co-mixing and composting of the separated solid fraction of pig manure with sawdust, shredded green waste (a mixture of tree leaves, foliage and small twigs) or chopped straw and to determine the suitability of the resulting product as a compost. The hypothesis is that the addition of bulking agents to the manure will improve the compost process.

**Material and methods** A decanter centrifuge (GEA Westfallia Separator UCD 205, GEA WestfalliaSurge GmbH, Germany) was used to perform the mechanical separation of the liquid pig manure. Average dry matter (DM) of liquid pig manure before separation was 24 g/kg (SD = 0.17). The DM of the solid and liquid fraction after separation was 380 g/kg (SD = 3.19) and 3 g/kg (SD = 0.07), respectively. The manure to bulking agent ratio was *c.* 2:1 (by volume). There were 4 treatments each with 4 replicates: (T1) 38 kg of solid fraction of separated pig manure (SPM); (T2) 38 kg of SPM + 9.5 kg of sawdust; (T3) 38 kg of SPM + 9.5 kg of shredded green waste and (T4) 38 kg of SRM + 2.8 kg of chopped straw. Sixteen fully insulated compost tumblers (Jora JK270 Composter, Joraform AB, Sweden) were used to compost the mixtures. A replicate of each treatment was initiated each day for 4 days. Compost temperature was recorded daily with a long stemmed thermometer. Aeration was provided by turning the tumblers daily. The experiment lasted for 56 days and samples were collected from each tumbler on days 0, 3, 7, 14, 21, 28, 42 and 56 for DM and pH analyses

**Results** The temperature curves for all 4 treatments were typical of those found in other pig manure composting studies (Huang *et al.*, 2006, Szanto *et al.*, 2007), with three distinct phases observed. In the first initial mesophilic phase the temperature inside the compost piles began to rise immediately after piling, rapidly achieving the peak temperature. Initial temperature (Day 0) was 32.4, 34.8, 31.9 and 32.0°C for T1, T2, T3 and T4, respectively. By Day 2-3 temperatures had reached >60°C for all treatments. Maximum temperature for T1 (66.5°C), T2 (66.8°C), T3 (67.1°C) and T4 (64.3°C) were achieved on days 4, 2, 4 and 7, respectively. During the second phase, temperatures remained above 50°C for a period of *c.* 2 weeks in all 4 treatments. The final compost phase was characterized by a drop in temperature reaching ambient levels after *c.* 22 days for T3 and T4 and after 18 and 20 days for T1 and T2, respectively. The moisture content of a compost pile is generally recommended to be between 50 and 60%. At Day 0, moisture content of the compost piles were 64.1, 51.7, 61.0 and 58.2% for T1, T2, T3 and T4, respectively. From Day 3 onwards moisture content for T2, T3 and T4 remained below 60% and above 49%. The change in pH for the 4 treatments showed a similar pattern. Initial pH (Day 0) was 8.3, 8.2, 8.1 and 8.5 for T1, T2, T3 and T4, respectively. The pH for T1, T2, T3 and T4 increased slightly on Day 3 to 8.7, 8.5, 8.5 and 8.7, respectively; and it was still slightly higher than initial pH on Day 7 being 8.5, 8.8, 8.5 and 8.7 for T1, T2, T3 and T4, respectively. From Day 14 onwards pH started to decrease and from Day 21 it did not vary much until the end of the experimental period on Day 56 when pH was 7.1, 7.1, 7.0 and 7.4 for T1, T2, T3 and T4, respectively. One explanation for the increase in pH between Day 0 and 7 may be the production of ammonia (NH<sub>3</sub>) during ammonification and mineralization of organic Nitrogen as a result of high microbiological activity during this period.

**Conclusions** The pattern of temperature, moisture and pH showed that the separated solid fraction of pig manure was successfully composted with or without the addition of bulking agents (sawdust, shredded green waste or chopped straw). Currently a germination test and a respiration test using the compost samples are being performed to determine compost maturity and their suitability as growth media.

**Acknowledgements** This research was funded by the Irish Department of Agriculture and Food's Research Stimulus Fund Programme (RSFP) under the National Development Plan 2007-2013.

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## The use of Integrated Constructed Wetlands for the treatment of swine wastewaters

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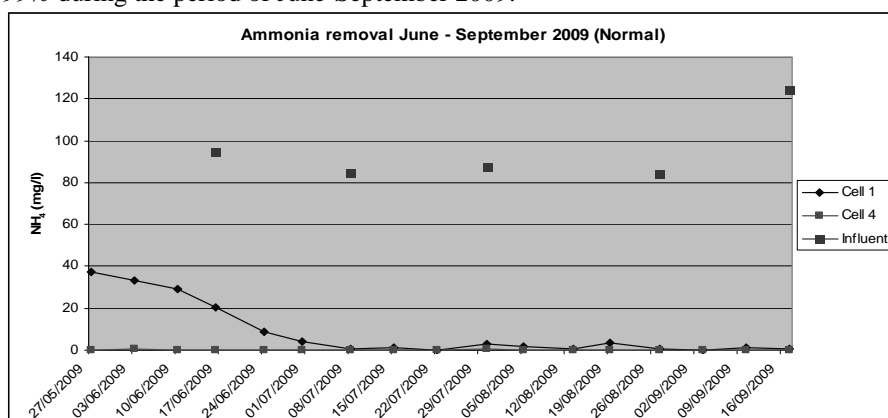
**Introduction** The treatment of agricultural wastewaters (yard runoff, dairy washings, slurries) by means of land-spreading is under scrutiny due to the EU Nitrates Directive, which limits the amount of nitrogen that can be applied to land. These limits have particular challenges for Piggery management, because they have limited access to spread-lands compared to other farming enterprises. This makes the nutrient content of pig slurries a potentially costly issue to address. The use of alternative methods to supplement or even replace land-spreading have been investigated for several decades with Constructed Wetlands showing potential for the treatment of agricultural wastewaters, including that of swine wastewater (slurry). The objective here was to examine the treatment efficiency of an Integrated Constructed Wetland (ICW) approach for the treatment of anaerobically digested swine wastewater. ICW systems are surface-flow constructed wetlands that treat wastewaters through means of natural processes, sedimentation, mineralization, plant and microbial uptake, denitrification, nitrification and atmospheric releases.

**Material and methods** A series of Meso-scale wetlands was developed in Teagasc Centre, Moorepark, Fermoy, Co. Cork to examine their treatment efficiency of swine wastewater from an anaerobic digester. After an extensive examination of the literature the use of the ICW concept (Harrington and Ryder, 2002) was decided upon and 4 key treatment operations were identified; high and low hydraulic loading, nutrient loading and effluent recycling. Polyethylene containers were used to construct 16 systems to examine the 4 treatments, with each treatment having 4 replicates. Each system comprises of 4 cells with a total system area of 0.788m<sup>2</sup>. The influent to the cells is separated swine wastewater post-anaerobic digestion. This liquid is diluted down to set ammonia (NH<sub>4</sub>) concentrations. These systems were identified as normal, recycling, high nutrient loading and high flow rate. The parameters of these systems is outlined below;

- 1) Normal: 37m<sup>3</sup>/ha/day loading rate @ 100mg/l NH<sub>4</sub>
- 2) Recycling: 37m<sup>3</sup>/ha/day loading rate @ 100mg/l NH<sub>4</sub> with 100% effluent recycled through the system weekly.
- 3) High Nutrient Loading: 37m<sup>3</sup>/ha/day loading rate @ 200mg/l NH<sub>4</sub>.
- 4) High Flow Rate: 74m<sup>3</sup>/ha/day loading rate @ 100mg/l NH<sub>4</sub>.

These systems run continually under automated pumping mechanisms adhering to the application rates above. Sampling of the Meso-scale systems is performed weekly. Storage tanks containing the influent, cells 1 and 4 of each system are sampled. Each sample is analysed for ammonia, molybdate reactive phosphorus, nitrate, nitrite, total oxidised nitrogen and chloride. BOD<sub>5</sub> is analysed fortnightly.

**Results** The initial removal rates in January of 2009 showed an average ammonia removal rate of 99.5%. During March-May the removal rates dropped to 75% during freezing temperatures. The removal rates of ammonia have averaged over 99% during the period of June-September 2009.



**Conclusion** The treatment of swine wastewaters by Constructed Wetlands has been demonstrated over several decades. The use of ICW as demonstrated in this Meso-scale study has shown that ICW could be a very effective approach to swine wastewater management. The Meso-scale study has shown removal rates of up to 99.5% for ammonia and phosphorus. They require a large land area for adequate construction and implementation in comparison to the land requirements of storage lagoons, filtration systems or anaerobic digesters. However, based on loading and sizing, it is significantly less than that which is required for sufficient land-spreading. They have also been shown to have greater treatment efficiency than those methods listed. There are also numerous additional benefits to the use of such systems, including the removal of non-point pollution sources, economic benefits, carbon sequestration, the potential for residual energies from dried accumulated organic matter than could be used as a fertilizer, biodiversity and habitat creation.

**Acknowledgements** The authors gratefully acknowledge research funding from Department of Agriculture, Food and Fisheries.

## Predicting beef cut composition and meat quality traits by spiral computed tomography

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**Introduction** Carcass composition and meat traits are relevant for the definition of beef quality. Direct assessments of both groups of traits require the slaughter of the animal and are costly and time-consuming, which has limited the inclusion of quality traits in breeding programmes. X-ray computed tomography (CT) is a non-invasive technique that provides accurate predictions of beef carcass composition (Navajas *et al.*, 2009). Studies in sheep showed that average CT muscle density is correlated with intramuscular fat content, fatty acid profile and eating quality (Bishop and Karamichou, 2009). The aim of this study was to assess the potential of CT tissue density values, analysed using a multivariate approach, as predictors of beef cut composition and meat quality in Aberdeen Angus and Limousin crossbred cattle.

**Material and methods** Data was recorded on 88 Aberdeen Angus (AAx) and 106 Limousin (LIMx) crossbred heifers and steers that were slaughtered with average live weights of 582 and 609 kg and ages at slaughter of 546 and 544 days for AAx and LIMx, respectively. After slaughter, the left carcass sides were kept and chilled for 48 h. After quartering, 11-12th rib and lumbar sirloins were vacuum packed in the abattoir and transported at temperatures of 1-2 °C to the SAC-BioSS CT unit. Spiral CT scans (SCTS) were collected of each cut using a Siemens Somatom Esprit scanner. They were subsequently transported to the University of Bristol for dissection into subcutaneous and intermuscular fat and muscle, and meat quality analysis (colour, instrumental texture and sensory traits; Prieto *et al.*, 2009a) (fatty acid and intramuscular fat content; Prieto *et al.*, 2009b). Multivariate calibration method (partial least square regression, PLSR) was used to predict beef cut composition and meat quality parameters using muscle and fat CT densities of cross-sectional images from either the 11-12th rib or lumbar sirloins as independent variables. Internal full leave-one-out cross-validation was performed in order to avoid over-fitting the PLSR equations using The Unscrambler program (version 8.5.0, Camo, Trondheim, Norway). The predictive ability of the PLS calibration models was evaluated in terms of coefficient of determination ( $R^2$ ) and Root Mean Square Error of Cross-Validation (RMSECV).

**Results** Accuracy of CT-PLSR calibrations for cut composition and meat quality traits are presented in Table 1. Cut composition was predicted with high accuracy ( $R^2$ : 0.81 to 0.99). Accurate CT predictions were found for most fatty acids ( $R^2$ : 0.61 to 0.75) and intramuscular fat content (IMF,  $R^2$ : 0.71 to 0.76) in both breeds. However, low to very low accuracies were found for colour, instrumental texture and sensory traits with  $R^2$  ranging from 0.01 to 0.26.

**Table 1** Prediction statistics of cut composition and meat quality traits of beef

	Aberdeen Angus crossbred		Limousin crossbred	
	$R^2$	RMSECV	$R^2$	RMSECV
Subcutaneous fat/intermuscular fat/muscle (g)	0.94/0.81/0.99	34.6/161.5/58.5	0.92/0.86/0.97	34.5/42.2/57.4
L*/a*/b* colour	0.12/0.04/0.05	2.4/2.2/1.8	0.19/0.18/0.19	2.38/1.92/1.82
SSF3/SSF14/ Volodkevitch shear force (N)	0.06/0.13/0.26	67.4/34.2/11.8	0.16/0.10/0.03	70.9/33.3/14.6
Tenderness/juiciness/flavour	0.01/0.04/0.05	0.8/0.4/0.6	0.03/0.02/0.17	0.7/0.5/0.5
Palmitic/stearic/oleic acid (mg.100 g <sup>-1</sup> muscle)	0.74/0.65/0.75	167.6/83.8/245.2	0.69/0.61/0.66	158.0/79.4/235.5
SFA/MUFA/PUFA (mg.100 g <sup>-1</sup> muscle)	0.71/0.72/0.26	281.6/318.4/21.5	0.67/0.66/0.09	253.1/279.3/25.9
Intramuscular fat (mg FA.100 g <sup>-1</sup> muscle)	0.76	567.4	0.71	539.1

SSF(3, 14): slice shear force 3 and 14 days pm, FA: fatty acid, SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

**Conclusions** The multivariate analysis of SCTS of beef cuts provided very accurate estimations of tissue weights in both AAx and LIMx and yielded accurate predictions of the IMF content and fatty acid composition in both breeds, without damaging or devaluing the cuts. The accuracies of these predictions were higher in AAx than in LIMx beef samples, probably due to a higher concentration of IMF in the former. No reliable CT predictions were found for colour, instrumental texture and sensory traits. The CT prediction of beef meat and carcass quality traits simultaneously may be of valuable information for beef cattle breeding programmes.

**Acknowledgements** We are grateful to the Scottish Government for funding the research and Scotbeef, QMS, BCF and Signet for their substantial support.

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## Prediction of beef carcass and selected primal subcutaneous fat yields using data from images captured on-line in abattoir

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**Introduction** Current grading methods for fat yield in UK abattoirs are limited to subjective fat classification score based on the EUROP based system. Video image analyses (VIA) methods have previously been employed commercially to estimate fat classification and carcass yield in other EU countries. Recently, fat trim yield has been strongly predicted ( $R^2 = 0.8$ ) by VIA system (Vote *et al.*, 2009) and fat thickness measured manually has shown to correlate with intramuscular fat content (Indurain *et al.*, 2009). Carcass and primal fat-yield information would be commercially useful in determining the quality and value of primal cuts. The aim of this study was to assess the potential of predicting half carcass and selected primal-cut subcutaneous fat yield using features extracted from high-resolution carcass digital images taken on-line at chain speed.

**Material and methods** Cross-bred steers and heifers ( $n = 29$ ; sired by either Aberdeen Angus or Limousin bulls) were used in this study. The animals were slaughtered at an average carcass weight and age at slaughter of 340 (SD = 33.7) kg and 584 (SD = 60.0) days, respectively. Images were captured of left exterior and right interior carcass side, both orthogonal to carcass split dissection plane adjacent to grading area of the line using a high resolution digital camera (2592 by 1944 pixel) capturing colour images. All images were spatially corrected for distortion to enable dimensional extraction. Software processing algorithms were developed using Inspector analysis package (v8, Matrox Electronic. Systems. Ltd, Quebec) to automatically segregate and estimate measure of total subcutaneous fat covering over left half carcass as a planar projected area (CARFA – mm<sup>2</sup>). Linear dimensions of subcutaneous fat depths were measured manually on images, adjacent to carcass landmark areas 4<sup>th</sup> rib (4RFD), 8<sup>th</sup> rib (8RFD), 13<sup>th</sup> rib (13RFD) and 3<sup>rd</sup> lumbar (3LFD) on right half carcass, measured as mm. At 48 h after slaughter, left carcass sides were split into primal cuts that were vacuum packed and subsequently dissected to components. Total weight (g) of subcutaneous fat of half carcass and sample primals: forerib and sirloin (subdivided into 11<sup>th</sup>-12<sup>th</sup> rib section, 13<sup>th</sup> rib section and lumbar section) were collected. Half carcass and selected primal-cuts' subcutaneous fat yield were predicted using as independent variables: 1) CARFA, by means of Simple Linear Regression and 2) 4RFD, 8RFD, 13RFD, 3LFD and CARFA, using Partial Least Square Regression. Internal full leave-one-out cross-validation was performed and the predictive ability of the calibration models was evaluated in terms of coefficient of determination ( $R^2$ ) and Root Mean Square Error of Cross-Validation (RMSECV) using The Unscrambler program (v8.5.0, Camo, Trondheim, Norway).

**Results** Calibrations for subcutaneous fat yield of half carcass and selected primal-cuts are presented in Table 1. Estimated projected carcass subcutaneous fat area did not seem to offer any prediction for subcutaneous fat yield of both half carcass and all primal components ( $R^2$  range: 0.01 to 0.02). Adding 4 linear measures of subcutaneous fat depths as independent variables significantly increased the accuracy of prediction in all cases with  $R^2$  ranging from 0.48 to 0.62.

**Table 1** Descriptive and prediction statistics for subcutaneous fat yield of half carcass and selected primal-cuts of beef

Y variable				X variables		Prediction		
Subcutaneous fat (g)	Range	Mean	SD			ns	R <sup>2</sup>	RMSECV
Half carcass	6370-16935	10844	2557	CARFA		1	0.02	2798.7
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		3	0.48	2224.9
Forerib	345-1450	854	287.1	CARFA		1	0.01	302.3
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		3	0.62	206.7
Sirloin	565-1930	1160	406.2	CARFA		1	0.01	411.5
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		3	0.55	329.7
11-12 <sup>th</sup> rib sirloin	145-560	306	118.4	CARFA		1	0.02	123.5
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		3	0.37	112.5
13 <sup>th</sup> rib sirloin	85-325	170	59.8	CARFA		1	0.01	62.8
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		4	0.58	47.3
Lumbar Sirloin	310-1275	684	258.6	CARFA		1	0.01	272.4
				4RFD + 8 RFD+ 13RFD+ 3LFD + CARFA		3	0.53	215.1

**Conclusions** Data extracted from high resolution digital images (higher than typical commercial VIA systems) can predict with moderate accuracy subcutaneous fat yield of both half carcass and high value primal cuts. With further development, such as high resolution imagery over whole carcass to accurately assess fat thicknesses in addition to the subcutaneous fat area, this technique could estimate with higher accuracy fat yields relating to carcass quality.

**Acknowledgements** Financial support for this research was received from the Scottish Government.

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## Use of biodiesel co-product from *Jatropha curcas* as ingredient for animal feed

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**Introduction** Studies about new sources of renewed energy have been intensified in recent years, motivated especially for the high prices and the scarcity of the petroleum as well as the concerns on global climatic changes. One of these sources is *Jatropha curcas*, Euphorbiaceae family, widely distributed in all continents (Cano-Asseleih *et al.*, 1989) as well in Brazil. Different research with *Jatropha* seeds found some toxic or irritant compounds including curcin, flavonoids, vitexine, isovitexine and the major toxic principle 12-deoxyl-16-hydroxyphorbol, a phorbol ester which caused clinical signs such as diarrhoea, dyspnoea and dehydration (Aregheore *et al.* 2003) in different species of animals. Despite this, seeds are an excellent oil source (60% of oil in the kernels) and after the full removed of its oil, it provides a meal with a highly nutritious and economic protein supplement (53-58% of crude protein) for animal diets if the toxins are removed (Becker and Makkar, 1998). This is the first of a full study about the utilization of *Jatropha curcas* meal in swine diets. So, the purpose of this work was to evaluate the utilization of detoxified *Jatropha curcas* meal from Brazilian sources in animal diets.

**Material and methods** Twenty-four Wistar rats (*Rattus norvegicus*), 21 to 25-day-old and 69.9 g  $\pm$  5.40 initial live weight (LW), were kept for a 28 day experimental period and housed in 24 metabolism cages according to initial weight in a randomized complete block (LW) design with six replications per treatment. The *Jatropha curcas* seeds were collected from different producers of biodiesel from different toxic sources in the southern part of Brazil. The detoxified meal was produced by LABORE Ind. The animals were divided in four isoproteic and isoenergetic diets (treatments), T1) Basal casein diet described by Reeves *et al.* (1993); T2) Basal casein diet with replacement of casein with 5% of detoxified *Jatropha curcas* meal; T3) Basal casein diet with replacement of casein with 10% of detoxified *Jatropha curcas* meal. All animals received water and diets *ad libitum* throughout the experimental period. At the beginning and at the end of the experiment all rats were weighed for initial and final body weight (IBW and FBD) (g), respectively. The average daily gain (ADG) (g/day) and the average feed intake (AFI) (g/day) were measured three times per week and the mortality was checked daily. To calculate the feed conversion (FC) the ADG were divided by AFI. After 28 days, after fasting for 12 hours, all animals were slaughtered in a halothane saturated chamber. After this, blood samples were collected and analyzed for complete blood assay: red blood cells count (RBC) ( $\times 10^6/\text{mm}^3$ ), haemoglobin (HB) (g/dl), packed cell volume (PCV) (%), mean cell volume (MCV) (fl), mean corpuscular haemoglobin concentration (MCHC) (%), total plasma (TP) (g/dl), white blood cells count (WBC) ( $\times 10^3/\text{mm}^3$ ) and the hepatic transaminases enzymes (alanine aminotransferase (ALT) (IU/l) and aspartate aminotransferase (AST) (IU/l)). The data were analyzed using GLM of SAS package (SAS Institute, 2001) and the analysis of variance and treatment means were compared by Tukey test ( $P < 0.05$ ). This study was carried under the accordance of the Commission for Ethics in Experimentation with Animals of Center of Nuclear Energy in Agriculture, University of Sao Paulo.

**Results** The performance, blood parameters and hepatic transaminases enzymes were not affected ( $P > 0.05$ ) by the level of inclusion of detoxified *Jatropha curcas* meal.

**Table 1** Performance, blood count and hepatic enzymes of rats fed with dietary levels of detoxicated *Jatropha curcas* meal

	Performance					Blood parameters								
	IBW	FBW	ADG	AFI	FC	RBC	HB	PCV	MCV	MCHC	TP	WBC	ALT	AST
Control	69.4	214.2	5.4	16.0	3.0	6.1	12.9	39.8	66.0	32.7	6.6	7.0	18.9	139.5
5% DJCM	71.4	217.0	5.4	15.6	2.9	6.8	13.9	41.2	60.	34.6	6.4	7.9	17.8	107.8
10%DJCM	68.9	217.3	5.5	16.0	2.9	6.2	12.7	38.8	62.1	33.3	6.3	6.6	20.4	141.3
s.e.d	5.4	9.6	0.4	0.8	0.2	1.1	1.9	9.0	5.0	3.8	0.2	2.9	3.8	33.34
P	-	0.71	0.70	0.52	0.24	0.55	0.61	0.91	0.28	0.77	0.28	0.64	0.57	0.11

**Conclusions** The results showed that the detoxified *Jatropha curcas* meal may be a promising protein source, but more investigations are necessary to check the efficacy of detoxification in different animal species and higher level of inclusion.

**Acknowledgements** The authors would like to thank FAPESP for the scholarship for the first author, LABORE Ind. and Mr. José Luiz de Paula Eduardo for the detoxification of JC meal, Mr. Antonio Roberto de Godoi and Mr. Joaquim Everaldo dos Santos for help during the experiment.

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## The effect of nutritional management of dairy cows post-calving and yeast supplementation on milk fatty acid profiles in the first eight weeks of lactation

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**Introduction** There is increased focus on earlier turnout of cows to pasture post-calving. However, nutritional management of cows in early lactation can influence milk production and milk composition throughout lactation. Through effects on rumen pH (Dawson and Hopkins, 1991), yeast supplementation (YS) may influence biohydrogenation of fatty acids (FA) in the rumen and therefore, milk FA profiles. The objective of this experiment was to evaluate the impact of nutritional management of the dairy cow in early lactation on subsequent milk FA profiles. This research is part of a larger study investigating the effect of abrupt introduction of cows to pasture immediately at calving with a more delayed introduction to pasture, and the effect of dietary YS (Yea-Sacc<sup>1026</sup> TS, Alltech, INC., Nashville, KY, USA) on rumen physiology at this time.

**Material and methods** The experiment was designed as a 2x2 factorial (grass vs TMR in early lactation +/- YS). The yeast was offered at 10g/hd/d x 10<sup>8</sup> CFU of live strain *S. cerevisiae*<sup>1026</sup>/g, Yea-Sacc<sup>1026</sup> TS. Treatments were: 1) Abrupt introduction to pasture post-calving (AP) + YS; 2) AP - YS; 3) TMR for 21 days post-calving (TMR21) + YS and 4) TMR21 - YS. In the dry period (60 days pre-calving) all cows were fed grass silage (GS) ad-libitum. The TMR was provided *ad libitum* and consisted of 50% maize silage, 45% GS, and 5% wheat straw, and 8 kg concentrate +/- YS. At day 22 post calving, TMR21 cows (+/- YS) received the same diets as AP (+/- YS) cows. Pasture allowance of 20 kg DM/hd/d (measured at a cutting high of 4 cm) was maintained across the trial period. Grazing cows were supplemented with concentrate (+/- YS) at rate of 4 kg/hd/d. Cows were milked x2 daily, and milk yield was recorded at each milking. Milk samples were collected weekly for an 8 week period and were mixed according to their am and pm yield. Feed and milk FA were analyzed by GC according to Palladino *et al.* (2009). Data were analyzed using PROC MIXED of SAS.

**Results** Cows fed TMR21 had higher short chain FA (SFA) (P<0.01) and medium chain FA (MCFA) (P<0.05) whilst unsaturated FA(UFA) (P<0.05), UFA:SFA (P<0.01), and long chain FA (LCFA) (P<0.05) were lower compared to AP cows (Table 1). *Cis-9* C18:1 was in also higher in milk from AP cows. Yeast supplementation had no effect on milk FA profiles in the current experiment and there was no interaction between diet and YS.

**Table 1** Effect of early lactation diet and YS on milk FA composition in the first 8 weeks of lactation

Item (g/kg of Total FA)	Yeast			Diet			Significance <sup>2</sup>		
	+	-	SED <sup>1</sup>	Grass	TMR	SED	Yeast	Diet	YeastxDiet
<i>cis-9</i> C18:1	219.1	236.5	10.80	254.4 <sup>a</sup>	201.2 <sup>b</sup>	16.11	NS	**	NS
<i>cis-9 trans-11</i> C18:2 (CLA)	8.1	7.6	0.87	7.9	7.7	1.30	NS	NS	NS
SFA <sup>3</sup>	642.2	621.5	13.34	600.9 <sup>b</sup>	662.8 <sup>a</sup>	19.91	NS	**	NS
UFA	283.0	300.4	13.77	321.4 <sup>a</sup>	262.1 <sup>b</sup>	20.56	NS	*	NS
UFA:SFA	0.46	0.50	0.029	0.55 <sup>a</sup>	0.41 <sup>b</sup>	0.043	NS	**	NS
SCFA <sup>4</sup>	147.3	133.1	12.93	132.5	147.9	19.30	NS	NS	NS
MCFA <sup>5</sup>	452.9	445.8	7.03	423.3 <sup>b</sup>	471.4 <sup>a</sup>	18.97	NS	*	NS
LCFA <sup>6</sup>	330.7	348.3	14.97	372.1 <sup>a</sup>	306.9 <sup>b</sup>	22.36	NS	*	NS

<sup>a, b</sup>Means within a row with different superscripts differs (P<0.05); <sup>1</sup>Standard error of the differences; <sup>2</sup>NS = not significant; \* = P<0.05; \*\* = P<0.01; <sup>3</sup>Level of significance correspond to transformed variable; <sup>4</sup>SCFA; C4:0 to C8:0; <sup>5</sup>MCFA; C10:0 to C16:1; <sup>6</sup>LCFA; C17:0 to C22:6

**Conclusions** Dietary management in early lactation influenced milk fatty acid profiles. Cows offered grass immediately post-calving had higher LCFA, lower SFA, MCFA and UFA:SFA compared to cows offered TMR for the first 3 weeks post-calving. Yeast supplementation had no effect on milk FA profiles in the current experiment.

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## Effect of cobalt extra-supplementation on milk production and composition of heat stressed lactating Holstein dairy cows

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**Introduction** In ruminants, Vitamin B<sub>12</sub> is produced with cobalt by rumen microbes. Complete replacement of cobalt by Vitamin B<sub>12</sub> in ruminant diets is not possible due to the positive effect of cobalt on the feed digestibility. The aim of this study was to assess the effect of different levels of inorganic cobalt supplementation on milk production and composition of lactating Holstein cows in Iran under heat stress condition. Cobalt supplementation may improve feed digestion when heat stress causes significant depression in feed digestibility, fat yield and milk yield.

**Material and methods** In this experiment the response to cobalt supplementation was evaluated. Twelve multiparous Holstein cows were assigned to one of four diets in a completely randomized 3×3 Latin square design with four 24 d periods. Cows experienced heat stress conditions throughout the experiment (37 ± 6 °C). The pre-experimental average milk yield of cows was 30.2 ± 4.98 and all of cows were in mid lactation (134 ± 27 DIM). The experiment involved in two phases, the first phase was 10 days, the adaptation period. During this stage cows were fed treatment diets and there was no sampling or milk recording. In the second stage, the experimental phase, in addition to feeding treatment diets, sampling was carried out. Cows were milked three times daily. During the experimental phase, in each period, total milk yield was recorded and milk samples were taken. Animals received diets containing 53.28% concentrate and 46.72% forage DM basis. The base diet contained 15.3% CP, 1.55 Mcal/Kg NEL. Animals were fed 3 times a day, and had free access to water. Treatments were C0) control, C1) control + 30 mg/d cobalt, C2) control + 40 mg/d cobalt and C3) control + 50 mg/d cobalt. Milk samples were analyzed for milk lactose, fat, protein, total solid (TS), solid non-fat (SNF) by Milkoscan system. The data was analysed by multiple regression analysis. The statistical model was:  $Y_{ijklmn} = \mu + R_i + C_j + T_k + M_l + RE_m + e_{ijklmn}$ . In this model,  $Y_{ijklmn}$  is the amount of each observation;  $\mu$  Total average;  $R_i$  effect of time period;  $C_j$  effect of diet;  $T_k$  effect of cow;  $M_l$  effect of square (replication of experiment);  $RE_m$  residual effect of previously diet and  $e_{ijklmn}$  effect of factors that were not controlled. Data were analyzed by SAS (version 8) statistical software and residual effect of last ration was corrected. Comparison between means was done by Duncan's multiple range test.

**Results** are presented in Table1. There was no significant difference in milk fat, protein, lactose, TS and SNF percent between treatments. There was a significant difference in milk yield and FCM 3.5% between all treatments. In addition, there were significant differences between all of treatments for milk fat yield and milk protein yield.

**Table 1** Means of milk yield and composition in experimental diets

	Control	Control diet			SEM
		+ 30mg Cobalt	+40mg Cobalt	+ 50mg Cobalt	
Milk yield, (kg/d)	27.15 <sup>d</sup>	28.22 <sup>a</sup>	28.09 <sup>b</sup>	27.51 <sup>c</sup>	0.74
FCM 3.5%, (kg/d)	23.10 <sup>d</sup>	23.96 <sup>c</sup>	24.11 <sup>b</sup>	24.21 <sup>a</sup>	0.60
Fat, %	2.37	2.35	2.37	2.50	0.04
Protein, %	3.11	3.08	2.92	3.05	0.06
Lactose, %	5.26	5.24	5.05	5.25	0.06
Total solids, %	11.34	11.27	11.17	11.40	0.07
Solid non fat, %	8.97	8.68	8.80	8.90	0.09
Fat yield, (kg/d)	0.632 <sup>d</sup>	0.653 <sup>c</sup>	0.664 <sup>b</sup>	0.688 <sup>a</sup>	0.02
Protein yield, (kg/d)	0.833 <sup>b</sup>	0.853 <sup>a</sup>	0.818 <sup>d</sup>	0.831 <sup>c</sup>	0.02

Within rows, means with different superscripts differ (P<0.05).

**Conclusions** Observed significant improvements in FCM %3.5 and milk fat production could be attributed to improvement of ruminal B<sub>12</sub> vitamin that has a major role in milk fat synthesis (Kincaid, *et al.*, 2003) or increase in cellulose digestion (Allen, 1986) which can improve energy intake. Therefore, cobalt supplementation up to 50 mg/d is recommendable, but studies with liver and serum cobalamin status or ruminal fiber digestion under these conditions might be useful.

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## The effect of high fat sunflower meal treated with formaldehyde and sodium hydroxide on milk yield and composition of Holstein dairy cows

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**Introduction** Sunflower meal is entirely adequate as the sole source of supplemental protein in dairy rations. Owing to the relatively high protein degradability of sunflower meal, dietary inclusion levels are limited in dairy cattle diets (Erasmus *et al.*, 1988). Treating proteins have been used to reduce their degradation in the rumen, formaldehyde that combined with proteins and sodium hydroxide denatures protein (Cozzi *et al.*, 1995). The objective of this study was to determine the influence of high fat sunflower meal (containing 165 g fat /kg DM) as untreated or treated with formaldehyde or NaOH on dry matter intake (DMI) and milk yield and composition.

**Material and methods** Twenty-one multiparous early lactation Holstein cows averaging 620 kg of body weight were allotted at day 25 to 35 of lactation to three groups of seven cows. Cows were fed individually a total mixed diet based on corn silage and supplements for *ad libitum* intake over a 7-weeks period (1-week adaptation). Treatments included untreated sunflower meal (USM), sunflower meal treated with 30 g/kg DM formaldehyde (FSM), or sunflower meal treated with 40 g/kg DM sodium hydroxide (SHSM). The composition of concentrate of experimental diets was 10.69 % untreated and treated sunflower meal, 2.76% soy bean meal, 4.88 % canola meal, 6.96 % cotton seed, 14.07 % barley grain, 15.66 % corn grain, 6.2 % wheat bran, 0.24 % D calcium phosphate, 0.54 % NaHCO<sub>3</sub> and 0.78 % vitamin-mineral supplement (DM basis). Diets were fed twice daily at 0900 and 2100 h for 10%orts. Milk yield and DMI was recorded daily. Milk samples were obtained weekly from each cow for three consecutive milkings and after mixed and get a sample were analyzed to determine fat, protein, lactose and SNF percentage. All results were subjected to least squares ANOVA for a completely randomized design. Data were analysed using the general linear models procedure of SAS (1991) as repeated measurements using covariance analysis.

**Result** The effect of treatment on milk yield and composition and dry matter intake (DMI) of cows was significant (Table 1). Milk yield and DMI for treatments containing formaldehyde and NaOH was the highest. Formaldehyde and NaOH treatment significantly increased protein and lactose percentage of milk in compared with untreated sample ( $P < 0.05$ ).

**Table 1** Dry matter intake, milk yield and compositions of lactating dairy cows fed diets containing sunflower meal

	Experimental diets			s.e.d	P
	USM	FSM	SHSM		
Milk yield (kg/d)	41.0 <sup>b</sup>	42.8 <sup>a</sup>	42.4 <sup>a</sup>	0.04	0.04
Milk protein (%)	2.48 <sup>b</sup>	2.67 <sup>a</sup>	2.62 <sup>a</sup>	0.03	0.003
Milk fat (%)	2.85	2.9	2.85	0.06	0.71
Milk lactose (%)	4.49 <sup>c</sup>	4.65 <sup>b</sup>	4.58 <sup>a</sup>	0.02	0.002
SNF (%)	7.73 <sup>b</sup>	7.88 <sup>a</sup>	7.78 <sup>b</sup>	0.04	0.03
DMI kg/d	27.92 <sup>b</sup>	28.69 <sup>a</sup>	28.52 <sup>a</sup>	0.02	0.02

**Conclusions** It appears formaldehyde and NaOH increased DMI, milk yield, percentage of fat, protein and lactose in cows fed with high fat sunflower meal, and the effect of formaldehyde was more than sodium hydroxide. Wilson (1970) reported that increase milk protein percentage by formaldehyde due to the protection of the protein from rumen microbial degradation.

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## Fatty acid composition of milk from Holstein cows fed fish oil, canola oil, or their combination in early lactation

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**Introduction** Supplementing the diet of cows with long chain unsaturated lipid decreased the medium chain fatty acids (10:0, 12:0, and 14:0) and increased the 18:0 and 18:1 content of milk fat with the greatest decrease when a high linoleic acid source was fed. Fish oil which contains relatively high concentrations of two polyunsaturated fatty acids of the n-3 family: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), is an effective means of increasing milk fat CLA content, due to an inhibition of *trans* C18:1 reduction in the rumen that results in an increased supply of *trans*-11 C18:1 available for endogenous conversion in the mammary gland. The combination of marine lipids with plant oils is an established strategy for increasing milk fat CLA content (Palmquist and Grinari, 2006). Lipids from canola seeds contain about 6% C16:0 and as high as 58% *cis*-C18:1, which have potentially interesting characteristics to change fatty acids profile of milk fat. Our objective was to evaluate the effects of feeding fish oil and canola oil separately and in combination on the fatty acid profile of milk fat and on the yield and composition of milk from dairy.

**Material and methods** Eight multiparous early lactation Holstein cows (42±12 DIM, 40±6 kg daily milk yield) were fed a total mixed ration supplemented with either 0% oil (Control), 2% canola oil (CO), 2% fish oil (FO), or 1% canola oil + 1% fish oil (COFO), according to a double 4 × 4 Latin square design. Oils were added at a level of about 2% of dietary DM, resulting in a dietary ether extract content of 4.7%. Milk samples were collected at the regular milking time over a 48-h period at the last two days of each period and stored at -20°C until analysis for fatty acid composition using gas liquid chromatography (GC Younglin Acme 6000 column bpx70 100\*0.25\*250 micron SGE company). Using hexane, milk lipid extracted, then following extraction fatty acid methyl esters were synthesised using methanolic sodium. Methyl esters of fatty acids were analyzed by gas chromatography equipped with a flamed ionized detector and silica capillary column [30 m × 0.32 (internal diameter) with 0.25-µm film thickness] with hydrogen gas as the carrier gas. Data were analyzed as a replicated 4×4 Latin square using generalized linear model (PROC GLM, SAS Inst, Inc., Cary, NC).

**Results** The proportions of *trans* 18:1, *cis*-11 18:1, *trans*-10,*cis*-12 18:2, *cis*-9,*trans*-11 18:2 (CLA), 18:3, 20:5(EPA) and 22:6 (DHA) were affected significantly by oil supplemented diets (Table 1, P<0.05).

**Table 1** Milk fatty acid content in cows fed control, fish oil (FO), fish oil with canola oil (FOCO), or canola oil (CO) diet

Fatty Acids (g/100g fatty acids)	Treatments <sup>1</sup>				SE	p Value
	Control	FO	FOCO	CO		
18:1 <i>trans</i>	0.38 <sup>a</sup>	1.57 <sup>b</sup>	0.57 <sup>a</sup>	0.58 <sup>a</sup>	0.05	0.001
18:1 <i>cis</i> -9	22.2	23.53	22.75	25.53	1.06	0.22
18:1 <i>cis</i> -11	0.78 <sup>a</sup>	0.84 <sup>a</sup>	1.81 <sup>b</sup>	1.12 <sup>b</sup>	0.08	0.006
18:2 <i>trans</i> -9, <i>trans</i> -12	0.25	0.27	0.29	0.26	0.04	0.93
18:2 <i>cis</i> -9, <i>cis</i> -12	2.56	2.74	2.98	2.84	0.06	0.11
18:2 <i>trans</i> -10, <i>cis</i> -12	0.05 <sup>a</sup>	0.3 <sup>b</sup>	0.15 <sup>a</sup>	0.06 <sup>a</sup>	0.02	0.03
18:2 <i>cis</i> -9, <i>trans</i> -11 CLA	0.47 <sup>a</sup>	1.05 <sup>b</sup>	1.26 <sup>b</sup>	0.88 <sup>ab</sup>	0.09	0.03
18:3	0.44 <sup>a</sup>	0.69 <sup>b</sup>	0.60 <sup>b</sup>	0.48 <sup>a</sup>	0.01	0.009
20:5 EPA	0.05 <sup>a</sup>	0.26 <sup>b</sup>	0.17 <sup>b</sup>	0.04 <sup>a</sup>	0.001	0.03
22:6 DHA	0.04 <sup>a</sup>	0.28 <sup>b</sup>	0.14 <sup>b</sup>	0.06 <sup>a</sup>	0.01	0.04

**Conclusion** Milk fatty acid profile was significantly affected by diet containing fish oil and canola oil. AbuGhazaleh *et al* (2003) reported that the concentrations of *cis*-9, *trans*-11 CLA in milk fat increased when cows were fed the high oleic and high linoleic sunflower seed with fish oil. The results of the current experiment reveal that combining fish oil with canola oil positively improves fatty acid composition of milk fat.

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## Effect of different levels of fish oil and canola oil on productive performance of early lactating Holstein dairy cows

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**Introduction** Lactation in the dairy cow is characterized by a dramatic increase in the nutrient demands for milk synthesis that coincides with a prepartum decline in dry matter intake (DMI) which leads to negative energy balance in early lactation. Supplemental fat sources are utilized in rations for dairy cows as a common method to increase the energy density of the diet or to modify milk production, milk fat content and milk fatty acids profile (Juchem *et al.*, 2008); However, its effects depend on the digestibility of the fat sources and effects of supplemented fat on other diet component digestibility. It is well recognized that feeding vegetable oils containing unsaturated fatty acids inhibit ruminal fermentation, decreased dry matter intake (Harvatine and Allen, 2006b) and fibre digestibility especially in high concentrate diets. The current study was designed to evaluate the effect of fish oil and canola oil supplemented diets on DMI, nutrient digestibility and nutrient intake in high producing dairy cows in early lactation.

**Material and methods** Eight multiparous early lactation Holstein cows ( $42 \pm 12$  DIM,  $40 \pm 6$  kg daily milk yield) were fed a total mixed ration supplemented with either 0% oil (Control), 2% canola oil (CO), 2% fish oil (FO), or 1% canola oil + 1% fish oil (COFO), according to a double  $4 \times 4$  Latin square design with four treatments, four periods, and two cows per treatment as the main plot, and the four sampling periods as the subplot. Each period lasted 21 d, which included a 14-d diet adjustment period followed by a sampling period. Oils were added at a level of about 2% of dietary DM, resulting in a dietary ether extract content of 4.7%. TMR mixture and faeces were sampled on first 5 days of each sampling period and were stored at  $-20^\circ\text{C}$ . At the end of each period feed and faeces samples were mixed to get the final sample and were stored at  $-20^\circ\text{C}$  up to the end of experiment. Finally all the feed and faeces samples were dried in a forced-air oven at  $60^\circ\text{C}$ , and stored in sealed plastic containers at room temperature until analyzed. In preparation for analyses, dried feed and faeces were ground first through a 2-mm screen and were analyzed for fat, ADF (Robertson and Van Soest, 1981), NDF (Van Soest *et al.*, 1991), and CP (AOAC, 1990; method no. 988.05), acid-insoluble ash (AIA) (Van Keulen and Young, 1977). AIA content of feed and faeces was used as a natural marker in ruminant to determine apparent digestibility of some nutrient, using following formula:

$$\text{Apparent digestibility (\%)} = 100 - [100 \times (\text{feed AIA (\%)} / \text{faeces AIA (\%)}) \times (\text{feed nutrient (\%)} / \text{faeces nutrient (\%)})]$$

Data were analyzed as a replicated  $4 \times 4$  Latin square using generalized linear model (PROC GLM, SAS Inst, Inc., Cary, NC).

**Results** The effects of supplementing diets with fish oil and canola oil on nutrient intake and digestibility are presented in Table 1. DMI and organic matter (OM) intake decreased in FO diet and fat intake increased in all oil supplemented diets ( $P < 0.05$ ). OM and NDF digestibility decreased in FO diets ( $P < 0.05$ ).

**Table 1** The effect of diets on nutrient intake, nutrient digestibility for lactating dairy cows

Parameter	Treatments <sup>1</sup>				SEM	p	<b>Conclusion</b> Results of the current experiment reveals that supplementing diet with fish oil had significant effects on intake and digestibility of some nutrient, but combining fish oil with plant oil will reduce their adverse effects. Strong negative effects of linseed oil on ruminal fibre digestibility with high proportion of concentrate in diet (67% in DM basis) were reported previously (Ueda <i>et al.</i> , 2003).
	Control	FO	FOCO	CO			
Intake, Kg/d							
DM	24.92 <sup>a</sup>	22.21 <sup>b</sup>	24.61 <sup>a</sup>	24.86 <sup>a</sup>	0.61	0.04	
OM	23.19 <sup>a</sup>	19.89 <sup>b</sup>	21.07 <sup>a</sup>	21.91 <sup>a</sup>	0.57	0.05	
NDF	7.98	7.06	7.45	7.96	0.45	0.43	
ADF	4.76	4.05	4.30	4.43	0.24	0.33	
Fat	0.79 <sup>a</sup>	0.98 <sup>b</sup>	1.18 <sup>b</sup>	1.12 <sup>b</sup>	0.01	<0.0001	
Digestability							
OM	65.58 <sup>a</sup>	60.62 <sup>b</sup>	62.98 <sup>a</sup>	62.33 <sup>a</sup>	1.05	0.04	
NDF	61.81 <sup>a</sup>	51.55 <sup>b</sup>	52.22 <sup>a</sup>	53.89 <sup>a</sup>	2.52	0.04	
ADF	43.63	42.11	42.42	43.72	1.05	0.19	
Fat	65.43	77.76	69.57	74.9	3.83	0.16	

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## Evaluation of nutritional values of caraway-seed pulp fed to Holstein dairy cattle

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**Introduction** Caraway seed pulp (CSP) is an agro-industrial by-product extracted during herb processing and manufacture (Raina, 1990, Syed *et al*, 1986). Annually 6000 ton caraway is produced in I.R. of Iran and after extracting its juice, 93% of it is CSP. Due to shortage of feedstuff, CSP can be used as a feed source for ruminant nutrition. The CSP are high in protein and fat with reported ether extract (EE) of 6%, CP of 15.2%, ADF of 51% and NDF of 55%. Because of the nutrient content, CSP appear to be a desirable feed ingredient in animal nutrition. Unfortunately, there is no data available on the nutritional value of CSP, especially in dairy cattle. The main objective of present experiment was to evaluate the effects substitution of CSP with wheat bran on dairy cattle performance, milk composition and some blood metabolites.

**Material and methods** Eight lactating Holstein cows (50±10 d. postpartum 600±20 kg) were randomly allocated to the 4 treatments based on calving date, lactating number and daily milk production (based on 10 days before the start of the experiment) in a 4×4 Latin square design. All cattle were housed in a tie-stall barn, fed individually, and were milked three times a day. Four dietary treatments offered to animals were different levels of CSP which were substituted with wheat bran (WB) in concentrate. The TMR ration was 15% WB and CSP substituted with WB in the following order: 1- WB (100% WB, 0% CSP as a control group), 2- 33.3% CSP + 66.6% WB, 3- 66.6% CSP+33.3% WB and 4- 100% CSP+0%WB. Diets were formulated based on NRC 2001. Nutrient requirement of dairy cattle and TMR diet were offered to animals as ad-libitum. Each experimental period was 21 days including a 14 day adaptation period and 7 days for collection of samples. Milk samples were analysed for protein, lactose and fat. Blood samples were drawn from the jugular vein into evacuated tubes on the last day of each experimental period at about 3-4 post feeding. The collected serum was frozen at the -20°C after centrifuging for the future analysis for glucose and cholesterol concentrations, Data were analysed by using the GLM procedure of SAS (9.1).

**Results** The dry matter intake (DMI), milk yield and its composition are presented in the Table 1. Differences between treatments for DMI and milk yield and composition were not statistically significant ( $p>0.05$ ). The substitution of wheat bran by the CSP tended to increase milk fat concentration compared to the control, However, the difference between them was not statistically significant ( $p>0.05$ ). Cholesterol increased with increasing dietary CSP and was greatest for cows offered the 100% CSP diets.

**Table 1** Dry matter intake, milk production, and composition and blood metabolites of experimental dairy cow<sup>1</sup>

Item	Treatment (caraway seed pulp level % substituted with WB)				SEM
	0	33	66	100	
DMI(kg day <sup>-1</sup> )	22.22	22.93	22.33	23.53	0.17
Milk yield(kg day <sup>-1</sup> )	36.28	37.24	36.30	37.00	0.35
Milk composition (%)					
Protein	2.67	2.63	2.59	2.61	0.02
Lactose	4.54	4.54	4.44	4.52	0.02
Fat	2.84	2.94	3.06	3.15	0.06
Blood metabolites (mg/dl)					
Glucose	56.88 <sup>a</sup>	44.76 <sup>ab</sup>	42.86 <sup>b</sup>	46.81 <sup>ab</sup>	1.45
Cholesterol	143.23 <sup>b</sup>	166.66 <sup>ab</sup>	170.39 <sup>a</sup>	186.68 <sup>a</sup>	2.82

<sup>1</sup> Different superscripts in each row designate significant differences ( $P<0.05$ )

**Conclusion** Results indicated that substitution of wheat bran by CSP tended to increase milk fat content although the differences between treatments were not statistically significant. However, results indicated that substitution of CSP at 100 % level with wheat bran (dry mater basis) may improve farm income by decreasing the cost of milk production without any adverse effect on animal health and performance. This Agro-Industrial by-product can be regarded as a source of effective fibre for the ruminant animal. However more experiments are needed for confirmation of these finding and suggestions.

**Acknowledgments** Financial support from Animal Science, Ferdowsi University is acknowledged also Mr M.M Moheghi gratefully acknowledge receipt of a Scholarship from the Government of Afghanistan

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## The effect of grazing different pasture herbage masses on rumen pH in lactating dairy cows

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**Introduction** The optimal use of grazed grass is identified as a key component of profitability in Irish dairy production systems (Shalloo *et al.*, 2004). Pre-grazing mass, also known as herbage mass, affects herbage quality and is one factor that can influence herbage intake (DMI) at grazing. O'Donovan and Delaby (2008) illustrated that swards with higher herbage mass reduced the feeding value of grass and thus reduced DMI. However, it is also suggested that grazing high quality pastures can lead to low rumen pH (Gibbs *et al.*, 2007). Rumen pH is cited as an important factor related to milk fat %, fibre degradation, nutrient absorption and overall cow health and welfare (Kleen *et al.*, 2003). Unfortunately, most of the information on rumen pH derives from work done with feeding high grain diets. Little information is available on the rumen pH of grazing dairy cows. Hence, the objective of the current study was to investigate the effects of three different herbage mass treatments on dairy cow rumen pH.

**Material and methods** A systems study with three separate farmlets was established at the Teagasc Moorepark research farm and 20 dairy cows were allocated to each treatment. The three treatments were i) low herbage mass [LM] (1200kg DM/ha), ii) medium herbage mass [MM] (1600kg DM/ha) and iii) high herbage mass [HM] (2200kg DM/ha). The treatments operated for the duration of the grazing season (Apr-Oct). Stocking rates (2.9cows/ha) and post-grazing sward heights (4cm) were the same for all three treatments. Grass was allocated on a daily basis and no supplementary feed was offered. All cows were milked twice daily. Six lactating rumen-cannulated dairy cows were arranged into two 3x3 latin squares and allocated to each treatment for one period each of two weeks. The study was carried out in the autumn part of the grazing season (Aug-Oct). Rumen pH was measured on days 10 and 11 of each period by means of an indwelling rumen pH probe. The Ionode IJ44 pH probe (Ionode Pty Ltd., Australia) was maintained immersed in one location at the bottom of the rumen by utilising a 1.5kg stainless steel weight (Flyco, Ireland). The data were logged at 60-second intervals over the 48-hour period using a Delta Ohm HD 2105.2 datalogger (Delta Ohm S.r.l., Italy) which was strapped to the cow's back using a MuPack backpack (Cassidy Covers, Ireland) and which was connected to the pH probe via a 2m cable passing through a modified cannula bung (Bar Diamond, Inc., USA). Average rumen pH across the day was calculated, as was the amount of time spent below certain pH thresholds (see table). The data were analysed as a 3x3 latin square using the mixed procedure (PROC MIXED) of SAS with herbage mass treatment, experimental period, square, cow and their interactions included in the model.

**Results** There was no difference in the average ruminal pH of dairy cows when grazing grass of three different herbage masses. In addition, no effect of treatment was found on the amount of time during which rumen pH was less than pH5.2, pH5.5 or pH5.8. In comparison to data derived from lactating dairy cows on total mixed ration-type diets, it appears that the rumen pH of grazing dairy cows could be lower and the time they spend below certain thresholds greater. For example, Nocek *et al.* (2002) found that lactating dairy cows fed a total mixed ration diet spent 120mins/d below pH5.5. However, the data presented here do agree with the low pH values suggested by Gibbs *et al.* (2007) for grazing dairy cows.

**Table 1** The effect of grazing different pasture herbage masses on rumen pH in lactating dairy cows

	LM	MM	HM	s.e.	Significance
Average rumen pH	5.90	6.09	5.98	0.057	NS
Time spent at rumen pH <5.2 (mins/d)	37	0	52	37	NS
Time spent at rumen pH <5.5 (mins/d)	187	43	216	46	NS
Time spent at rumen pH <5.8 (mins/d)	518	288	360	62	NS

**Conclusions** In the current study herbage mass did not have an effect on dairy cow rumen pH. Further work is needed to expand on the very limited data available for grazing dairy cows, and to explore the mechanisms by which the low pH values seen in grazing systems are attained without the concomitant and expected problems in terms of lameness and milk fat concentration. Such work could re-define the guidelines for rumen pH in the grazing dairy cow specifically.

**Acknowledgements** The authors gratefully acknowledge the Dairy Levy Research Fund for financial support, and the help and co-operation of John Paul Murphy and the Moorepark farm staff.

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## Effect of rumen acid load on *in vitro* ruminal total bacteria and *Fibrobacter succinogenes* populations determined by real-time PCR

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**Introduction** One consequence of feeding excessive amounts of rapidly fermentable carbohydrates in conjunction with inadequate fibre to ruminants is subacute ruminal acidosis, characterized by periods of low ruminal pH. Fibrolytic bacteria are unable to maintain the pH inside their cells when ruminal pH is low (Russell and Wilson, 1996). Rustomo *et al.* (2006) evaluated the acidogenicity value (AV) of feeds using an *in vitro* laboratory technique. Danesh Mesgaran *et al.* (2009) demonstrated that various kinds of dairy diets with different NFC contents had different acidogenicity values. The aim of the present study was to evaluate the effect of rumen acid load on ruminal total anaerobic bacteria and *Fibrobacter succinogenes* populations determined by real-time PCR.

**Material and methods** Various commercial dairy diets (forage:concentrate as 1:1) containing different levels of non-fibre carbohydrates (NFC) were provided as low (LNFC), medium (MNFC) and high (HNFC) to obtain different AV (Danesh Mesgaran *et al.*, 2009). The concentration of NFC in LNFC, MNFC and HNFC was 336, 366 and 371 g/kg DM. The acidogenicity values of the diets were determined using the procedure as described by Wadhwa *et al.* (2001). One-gram (DM) of each diet was weighed and incubated, in triplicate, with 30 ml of buffered rumen liquor comprising 60% buffer and 40% rumen liquor. The buffer was made up at 20% of the strength of the Tilley-Terry (1963) buffer. Cysteine hydrochloride monohydrate (0.025% wt/vol) was added just prior to incubations. The incubations were carried out in 100 ml bottles held in a water bath at 38.7 °C. After the incubation, samples were taken for DNA extraction (0.2 ml). Then, 2 ml of the each bottle content were located into micro tubes containing 50 mg (excess) of CaCO<sub>3</sub> powder. The mixture was shaken manually for 5 s, then centrifuged at 4000 rpm for 10 min before analysis of Ca content of the supernatant using Atomic Absorption. The AV was calculated as the product of Ca concentration (from the analysis) and fluid volume (30 ml) divided by the sample weight. DNA was extracted from the samples using the Bioneer Accuprep Genomic DNA Extraction Kit. The 16s rRNA gene-targeted primer sets used in the present study were forward: GTTCGGAATTACTGGGCGTAAA and reverse: CGCCTGCCCTGAACTATC. Cycling conditions were 95 °C for 5 min, forty cycles of 95 °C for 15 s, 61 °C for 1 min and 72 °C for 30 s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1 °C/s increment from 65 to 95 °C, with fluorescence collection at 0.1 °C at intervals. Total bacteria concentration was determined relative to bacterial standard. *Fibrobacter succinogenes* population was expressed relative to quantification of the total bacterial population. Data were analyzed as a complete randomized design using GLM procedure of SAS (2003). Model was: Y = Mean + Treatment + residual.

**Results** The AV of LNFC, MNFC and HNFC was 10.7, 11.2 and 11.9, respectively. Data on Table 2 showed that the *Fibrobacter succinogenes* population in LNFC were significantly higher than the other diets (P < 0.05).

**Table 1** *In vitro* DNA concentration of total bacteria and the population of the *Fibrobacter succinogenes* relative to total bacteria in commercial dairy diets containing different levels of NFC.

Bacteria	Treatments			s.e.d	P
	LNFC	MNFC	HNFC		
Total bacteria (ng/μl)	34.55	44.27	44.65	5.72	> 0.05
<i>Fibrobacter succinogenes</i>	0.1281	0.0310	0.0298	0.03	< 0.05

**Conclusions** The results of the present study demonstrated that increasing in NFC in commercial diets caused a decrease in ruminal AV and the population of *Fibrobacter succinogenes*. It was previously indicated that the rate at which rumen fluid pH changed followed a pattern similar to changes in the AV (Rustomo *et al.*, 2006), and the differences in AV and pH changes likely were associated with the fermentability of the feeds. Therefore, higher NFC content in HNFC led to higher available nutrients for bacterial growth, and consequently lower pH. This situation made a condition in which a rise in total bacteria might be assumed, with a decline in relative fibrolytic bacteria population, as demonstrated by the present results.

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## Effect of different sources of pectin feedstuffs on milk yield and composition of early lactating Holstein cows

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**Introduction** The inclusion of high levels of cereal-based concentrates decreases rumen pH and the activity of rumen cellulolytic bacteria thus depressing rumen cell wall fermentation, digestibility and roughage intake. Citrus pulp has a high potential rumen degradability and apparent digestibility and, as with other pectin-rich foods such as sugar-beet pulp, causes a lower production of propionic and lactic acids than starchy foods. As a result, supplementation of roughages with pectin-rich foods generally has a less adverse effect than cereal grains on cell wall fermentation and digestibility and on roughage intake, thus resulting in similar or even greater total digestible organic matter intake (DOMI). The aim of the present study was to evaluate effects of different source of pectin feed stuff on milk yield and composition dairy cows.

**Material and methods** Eight primiparous early lactating Holstein cows ( $60 \pm 23$  days postpartum, weighing  $530 \pm 60$  kg) were assigned into a replicated  $4 \times 4$  Latin square design with four 3-wk periods. Cows were allocated into four diets which were: 1) 10% barely grain, 2) 10% sugar beet pulp, 3) 10% wheat bran and 4) 10% dried citrus pulp. Each experimental period was 21 days including 14 days adaptation period and 7 days collecting samples. Milk yield recorded daily and milk sample were taken from each milking times during the last 3 days of each period. Milk samples were subjected to analysis for CP, lactose, fat and SNF. Blood samples were collected from the jugular vein 2h after the morning feeding. Blood serum was collected after centrifuged at  $1,500 \times g$  for 40 min, frozen at  $-40^\circ\text{C}$ , and later analyzed for Glucose and urea N. Rumen samples were taken from each cow on the last days of each experimental period at about 3 hr after the morning feeding. Ruminal fluid pH was determined immediately. Differences in means were detected using the PROC GLM procedure of the Statistical Analysis System release 9.1 (SAS, 2004).

**Results** The dry matter intake (DMI), milk yield and composition, ruminal pH and blood metabolites are presented in the Table 1. DMI (kg/d), milk yield, milk composition, ruminal pH and blood metabolites were not affected by treatments ( $P>0.05$ ).

**Table 1** Dry matter intake, milk production, and composition and ruminal pH of dairy cow

Treatment	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM
Item					
DMI (kg day <sup>-1</sup> )	19.34	19.63	19.74	18.69	0.122
Milk yield (kg day <sup>-1</sup> )	28.68	28.3	28.81	27.37	0.201
Milk composition (%)					
Protein	3.1	3.03	3.16	3.12	0.046
Lactose	4.92	5.01	5.15	4.98	0.057
Fat	3.14	3.44	3.36	3.34	0.070
SNF	8.27	8.3	8.56	8.36	0.099
Ruminal pH	6.25	6.36	6.3	6.26	0.025

The data of blood metabolites are presented in the Table 2. There were no significant differences in blood metabolites between treatment diets ( $P>0.05$ ).

**Table 2** Blood metabolites of dairy cow

Treatment	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	SEM
Item					
Glucose (mg/dl)	55.8	53.8	54.1	53.5	1.026
Blood urea nitrogen(mg/dl)	24.37	25.62	25.87	26.25	0.278

**Conclusion** The obtained results indicated that substitution of sources of pectin feed stuff by barely grain were not effect on DMI, milk yield and composition, ruminal pH and blood metabolites. This result in agreement with (Castel, 1972; Feregos *et al.*, 1995). However, the addition of sources of pectin feed stuff at 10% level (dry matter basis) of the dairy cow ration instead of cereal grain; can decrease the cost of milk production without any negative effect on animal performance.

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## Investigation of milking equipment cleaning procedures in three geographical areas where milk chemical residues were identified in bulk milk

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**Introduction** Milk consumers and regulatory agencies demand that milk be free of harmful adulterants. Excessive residues may be a cause for concern for human health, export regulations for dairy products or interference with the manufacturing process. Inappropriate equipment cleaning procedures can increase milk bacterial levels and can cause chemical residues in milk. The objective of this study was to identify inappropriate equipment cleaning practises which may account for the high chemical residues found in bulk tank milk and to establish if the farm management practises identified differed depending on the processor location.

**Material and methods** Individual milk suppliers were identified from three milk processors in different geographical locations (A (n=17), B (n=14) and C (n=14)) with bulk milk showing higher than acceptable milk residue levels. These farms were visited and the cleaning procedures investigated. A questionnaire survey (39 questions) was conducted to establish the cleaning practises for both the milking machine and the bulk tank on these farms. Management practises which were considered likely to result in milk residues and which did not conform to those as recommended by Teagasc (O' Brien, 2008) were considered unsatisfactory. Samples of the farm mains water supply was tested on site for the presence of available chlorine. Data was analysed using the Chi-square test to identify any relationships between processor location and management practises.

**Results** Unsatisfactory milking equipment cleaning practises were observed on each farm visited and the occurrence of some management practises differed depending on the geographical area (Table 1). Re-using of the post-detergent rinse water as a post-milking rinse (prior to detergent wash) at the subsequent milking was more likely to occur at location A ( $P < 0.05$ ). Detergent wash solvent is recommended to be reused only on one occasion; however this solvent was re-used more than once at all three locations. A higher proportion of farms at locations A and C did not use sufficient rinse water to remove the detergent solvent compared to farms at location B ( $P < 0.05$ ). Insufficient rinse water usage was influenced by trough size, price of water (group schemes), short wash cycles in bulk milk tanks and a lack of knowledge of the importance of using sufficient water. In-correct levels of detergent tended to be used at locations B and C compared to location A ( $P < 0.06$ ), due mainly to a lack of measuring equipment. Using the incorrect chemicals for cleaning either the bulk tank or milking machine occurred more often at locations A and B compared to location C ( $P < 0.01$ ). A further unsatisfactory cleaning practise observed at all locations was the practise of not rinsing the detergent steriliser product from the plant immediately after the main wash cycle. Proportionally (0.94) of farms at location A had public group water supplies while (0.92) of farms at locations B and (0.67) at location C used a private well water source. The public water supply contained acceptable levels of available chlorine (0.2 to 0.8 mg/l). Thus, the presence of chlorine in this water at the levels recorded may only be a factor if the detergent solvents were not used as recommended. Machine installation in some instances can hinder successful cleaning of equipment by not allowing complete draining of equipment between each wash cycle. Proportionally (0.71) and (0.29) of milking machines visited had milk recorder jars and pipeline milking systems fitted. The higher proportion of these problem farms had recorder jars which may indicate that using sufficient rinse water is more critical with this milking system. The bulk milk tank was identified as being the main equipment source of the residue problem at location B (0.67) whereas the milking machine was considered the main equipment source at locations B (0.46) and C (0.46). While other factors may influence high chemical residues in milk the faults outlined were considered the likely factors influencing the residue levels observed on these farms.

**Table 1** Proportion of farms with cleaning procedure faults at three processor locations

Main cleaning faults observed	Location			Significance
	A (n=17)	B (n=14)	C (n=14)	
Re-using the post-detergent rinse water	0.06	0.00	0.01	$P < 0.05$
Using chemicals in post milk rinse	0.00	0.01	0.01	n.s
Re-using detergent solvent > once	0.03	0.04	0.07	n.s
In-sufficient rinse water for the milking machine	0.13	0.06	0.13	$P < 0.05$
Chemical products used for wrong task	0.09	0.08	0.01	$P < 0.01$
Incorrect levels of chemical used	0.01	0.05	0.05	0.06
Incorrect operation of cleaning system	0.04	0.02	0.01	n.s
Machine installation fault	0.02	0.01	0.02	n.s

**Conclusion** Unsatisfactory management practises in relation to the cleaning of milking equipment were observed on all farms and the occurrence of some individual faults differed between locations. It may be speculated that the factors influencing chemical residues in milk may be due to a single or multiple incorrect equipment cleaning practises. The factors highlighted from this investigation may be used as a template when solving chemical residue issues on farms in general.

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## Comparative grazing behaviour of Holstein-Friesian and Jersey dairy cows and their F<sub>1</sub> cross in pasture based production systems

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**Introduction** Animals suited to grazing systems should attain sufficient quantities of herbage and efficiently convert feed to product (milk solids). Crossing the Holstein-Friesian (HF) with the Jersey (J) breed is considered to result in an animal that is well suited to grazing systems (Harris *et al.*, 1999). The objective of this study was to investigate the grazing attributes of HF, J and Jersey×Holstein-Friesian (F<sub>1</sub>) cows under Irish pasture based production systems.

**Material and methods** Data from 108 animals were available; 37 HF, 34 J and 37 F<sub>1</sub>. Mean calving date for these animals was February 18th ±23 d 2007. During the experimental period pasture was the sole feed offered. Milk yield was recorded daily with milk composition and bodyweight (BW) determined weekly. Grass dry matter intake (GDMI) was estimated for each cow on 4 occasions during lactation using the n-alkane technique. Grazing behaviour measurements were recorded twice during lactation using IGER grazing behaviour headset recorders. Measurements were recorded over a 24 h period. Data were analysed using the mixed procedure of SAS. The linear model included the fixed effects of breed group, parity and measurement period. Calving day of year was included as a continuous covariate. Cow was included as a random repeated effect. Orthogonal contrast statements were used to determine differences between the HF and J breeds while the F<sub>1</sub> was compared to the performance of the mid-parent mean.

**Results** Daily milk yield was greater ( $P<0.001$ ) for the HF (16.9 kg/day) compared to the J (12.8 kg/day). The F<sub>1</sub> (15.7 kg/day) had a higher yield of milk ( $P<0.05$ ) compared to mid-parent mean. Bodyweight was higher for the HF (503 kg) compared to the J (373 kg). Bodyweight of the F<sub>1</sub> was 452 kg indicating an estimate for hybrid vigour of 14 kg. The HF had a higher ( $P<0.001$ ) GDMI than the J (16.7 v 14.6 kg/d). The F<sub>1</sub> had an additional 0.25 kg/d GDMI ( $P<0.01$ ) compared to the parent breed mean. Differences in grazing and ruminating behaviour between the breed groups are presented in Table 1.

**Table 1** Effect of dairy cow breed group on grazing and ruminating behaviour.

	Breed group			HF v J		F <sub>1</sub> v. parent mean	
	HF	J	F <sub>1</sub>	S.E.M.	P-value	S.E.M.	P-value
Grazing time (min/d)	646	637	662	20.1	NS	17.5	NS
Grazing bouts (number/d)	10	8.9	10.2	0.81	NS	0.58	NS
Grazing bout duration (min/bout)	79.5	83.9	71.7	4.99	NS	4.35	<0.05
Total bites (number/d)	40672	39433	39859	1236.7	NS	1078.0	NS
Bite rate (number bites/min)	57	59	60	1.10	NS	0.96	<0.05
Grazing mastications (number/d)	3963	4785	4252	384.0	<0.05	335.1	NS
Grass DMI/bite (g)	0.42	0.38	0.42	0.017	<0.05	0.015	NS
Ruminating time (min/d)	426	371	383	22.1	<0.01	19.2	NS
Ruminating bouts (number/d)	17.4	14.5	12.8	4.92	NS	4.29	NS
Ruminating bout duration (min/bout)	36.8	26.1	32.0	2.16	<0.001	1.88	NS
Ruminating mastications (number/d)	25782	21758	23425	1458.2	<0.01	1271	NS
<u>per 100 kg BW</u>							
Grazing time (min)	129	171	149	64.4	<0.001	45.5	NS
Grazing mastications (number)	796	1292	954	92.9	<0.001	81.1	NS
Ruminating time (min)	83.8	97.5	82.8	3.59	<0.01	4.78	NS
Ruminating mastications (number)	5174	5847	5177	343	0.052	294.2	NS
<u>per kg GDMI</u>							
Grazing time (min)	39.3	44	41.9	1.51	<0.01	1.31	NS
Grazing mastications (number)	241	329	273	25.9	<0.01	22.6	NS
Ruminating time (min)	25.4	24.9	23.6	25.38	NS	1.05	NS
Ruminating mastications (number)	1571	1501	1455	92.6	NS	79.4	NS

**Conclusions** Ostensibly, little difference in grazing behaviour was apparent between the breed groups. However, when expressed per unit BW and per unit GDMI, differences between the breed groups for grazing measurements were apparent. Evidence presented also suggests that the crossbred may in fact display hybrid vigour for some components of grazing efficiency, thus highlighting the suitability of F<sub>1</sub> cows to intensive grazing systems.

**Acknowledgements** Financial support from the Research Stimulus Fund (RSF-06-353) is gratefully acknowledged.

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## Comparative somatic cell score and milking characteristics of Holstein-Friesian, Jersey and Jersey×Holstein-Friesian cows at pasture

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**Introduction** Inferior udder health can be a source of economic loss to both producer and processor (Walsh *et al.*, 2007). In Ireland, some processors, as an incentive to improve milk quality, have introduced bonus schemes for herds achieving bulk somatic cell count (SCC) of 200,000 cells/ml or less. Milking characteristics are important functional traits of dairy cows which influence labour cost (Boettcher *et al.*, 1998), rate of involuntary culling (Berry *et al.*, 2005) and udder health (Rupp and Biochard, 1999). The aim of this study, carried out at the Ballydague research farm, was to investigate the somatic cell score (SCS) and milking characteristics of Holstein-Friesian (HF), Jersey (J) and Jersey×Holstein-Friesian (F<sub>1</sub>) cows in seasonal pasture based systems.

**Material and methods** A total of 329 lactations across three years (2006-2008) were available, 112 HF, 106 J and 111 F<sub>1</sub> from 162 cows; 65 HF, 48 J and 49 F<sub>1</sub>. Cows grazed as a single herd for the first two years of the study and in 3 separate treatment groups in year 3. In mid-April of year 1, cows were randomised across two grass based feeding systems; low concentrate (658 kg DM/cow) and high concentrate (1072 kg DM/cow). In year 2, all cows were managed in a similar fashion to the low concentrate group in year 1. In year 2, concentrate supplementation averaged 240 kg DM/cow. In year 3, cows were randomly assigned within breed to a 3×3 factorial experiment comparing the HF, J and F<sub>1</sub> cows under three stocking rate systems with concentrate supplementation amounting to 352 kg DM/cow. Mean lactation number was 1.74 lactations across the breed groups. Somatic cell count (SCC) was determined biweekly, from morning samples using a Bentley Somacount 300 (Bentley Instruments Inc., Chaska, MN). The natural logarithm of SCC was used to define SCS. A total of 8,048 test-day records were available for SCS analysis. Mean SCS records for cow within lactation were used to determine average SCS over lactation. Milk yield and milking characteristics were recorded daily throughout lactation providing a total of 13,446 test-day records. Average milk flow (AMF), peak milk flow (PMF) and milking duration (MD) were recorded daily using electronic milk meters. Average milk flow, PMF and MD were determined weekly and subsequently averaged within lactation. Data were analysed using the mixed procedure of SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA, 2003). The linear model included the fixed effects of breed group (HF, J and F<sub>1</sub>), parity, year and feeding treatment. Calving day of year and lactation length were fitted as continuous covariates. Cow was included as a repeated effect. Using the Akaike's information criterion, a compound symmetry structure for the residuals was determined as the most appropriate residual covariance structure for repeated measures over time within cows. Breed groups (HF vs. J) and the F<sub>1</sub> vs. the mid-parent mean, were compared using orthogonal contrast statements.

**Results** Milk yield was greater for the HF compared to the J, while SCS was similar for the two breeds (Table 1). Average milk flow was greater with the HF compared to the J. Peak milk flow tended to be higher with the HF. The F<sub>1</sub> had a greater milk yield, AMF and PMF compared to the mean of the parent breeds. This corresponded to hybrid vigour estimates of +1.0 kg (+5.8%), +0.10 kg/min (+7.9%) and +0.32 kg/min (+10.3%) for milk yield, AMF and PMF, respectively. Milking duration was similar across the three breed groups.

**Table 1** Effect of breed on milk yield, SCS and milking characteristics.

	Breed group			HF v J		F <sub>1</sub> v. parent mean	
	HF	J	F <sub>1</sub>	S.E.M.	P-value	S.E.M.	P-value
Milk yield (kg/d)	18.0	14.2	17.1	0.29	<0.001	0.26	<0.001
Somatic cell score	10.6	10.8	10.7	0.15	NS	0.13	NS
Average milk flow (kg/min)	1.36	1.09	1.33	0.037	<0.001	0.033	<0.01
Peak milk flow (kg/min)	2.93	2.65	3.11	0.154	0.067	0.140	<0.05
Milking duration (log s/d)	6.64	6.62	6.62	0.024	NS	0.023	NS

**Conclusions** Results from this study suggest no difference in SCS between the breed groups evaluated. While differences in milk flow were apparent between the breed groups, the findings indicate that F<sub>1</sub> cows will, on average, exhibit similar MD, ensuring consistency during the milking process in mixed breed/crossbred herds.

**Acknowledgements** Financial support from the Research Stimulus Fund (RSF-06-353) is gratefully acknowledged.

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## Animal performance and economic implications of Holstein-Friesian, Jersey and Jersey × Holstein-Friesian cows under seasonal pasture based systems

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**Introduction** In seasonal production systems such as Ireland the aim is to optimise milk production from pasture with limited concentrate supplementation. Ideally cows in such systems should efficiently convert pasture to product and maintain a calving interval around 365 days. Crossbreeding, particularly with the Jersey (J), is common practice in New Zealand and Lopez-Villalobos *et al.* (2000) reported Jersey×Holstein-Friesian (F<sub>1</sub>) cows to be more profitable than the parent breeds. The J breed would appear to offer potential for crossbreeding under Irish conditions with its small size, the prospect of improved reproductive performance, and high milk components which with the introduction of multi component price systems of payment is of particular interest in adding value to milk (Shalloo, 2007). The aim of this study was to provide comparative cow performance data and the implications of same for overall farm profitability of Holstein-Friesian (HF), J and F<sub>1</sub> cows under Irish seasonal pasture-based management.

**Material and methods** A total of 329 lactations from 65 HF, 48 J and 49 F<sub>1</sub> cows were available from a three year study; 2006, 2007 and 2008 (years 1, 2 and 3, respectively), conducted at the 'Ballydague' research farm. Cows were managed in a seasonal production system (Dillon *et al.*, 1995). Milk yield was recorded daily while milk components and bodyweight were determined weekly. A more detailed description of fertility measures is provided by Prendiville *et al.* (2010). The Moorepark Dairy Systems Model (MDSM) (Shalloo *et al.*, 2004), a stochastic budgetary simulation model was used to simulate a model farm integrating biological data for each breed group. This model incorporates animal inventory and valuation, milk production, feed requirement, land, labour and economic analysis. Variable costs including fertilizer, contractor charges, medical and veterinarian, artificial insemination, silage and reseeded, fixed costs (machinery maintenance and running costs, farm maintenance, car, telephone, electricity and insurance) and sales values (milk, cull cow and calf) were included at current prices (Teagasc, 2008). The model was limited to 40 hectare of land and 13t DM of grass/ha was assumed to be grown. Fertilizer application was assumed to be 250 kg of N/ha. A milk price of 27c/l at 33.0g/kg protein and 36.0g/kg fat with a ratio of the value of protein to fat of 2.6 to 1 was used. Cull cow price was assumed to be €366, €149 and €268 for the HF, J and F<sub>1</sub>, respectively (for details see Prendiville *et al.*, 2010). All calves were assumed sold at 4 weeks of age. Male calf value was €80, €0 and €30 for HF, J and F<sub>1</sub>, respectively, while female calves were valued at €330. Replacement rate was calculated as the proportion of cows that failed to become pregnant by the end of the 13-week breeding season plus a voluntary culling rate of 10% of the remaining cows. Fertility values obtained for the HF and J were not statistically different. Consequently, empty rates were 18% for the HF and J and 9% for the F<sub>1</sub>. Due to the differences in replacement rates milk yield was adjusted for parity structure. Differences in calving date were also assumed based on differences in calving to conception interval.

**Results** According to the model 96 HF, 114 J and 97 F<sub>1</sub> cows would be facilitated on the 40 ha land base. Milk yield was highest with the HF (5,651 kg), intermediate with the F<sub>1</sub> (5,272 kg) and lowest with the J (4,220 kg). Milk fat and protein content were highest for the J (5.32% and 4.03%), intermediate with the F<sub>1</sub> (4.77% and 3.88%) and lowest with HF (4.12% and 3.49%). Consequently, milk solids production was highest for the F<sub>1</sub> (456 kg) intermediate for the HF (430kg) and lowest for the J (395 kg). Milk returns were highest for the J (€172,816), intermediate for the F<sub>1</sub> (€171,790) and lowest with the HF (€158,675). Due to additional animal numbers labour cost was higher with the J (€32,811) compared to the HF and F<sub>1</sub> (€27,760 and €28,463, respectively). Replacement costs were lowest with the F<sub>1</sub> (€26,935), intermediate with the HF (€38,904) and highest with the J (€45,982). Livestock sales were highest for the HF (€28,675) and similar for the J and F<sub>1</sub> (€22,696 and €21,674). Total costs were €149,852, €167,089 and €137,786 for the HF, J and F<sub>1</sub>, respectively. However, overall farm profit was highest with the F<sub>1</sub> (€55,678) followed by the HF (€37,499) and J (€28,423). Farm profitability per hectare was highest for the F<sub>1</sub> (€1,392), intermediate for the HF (€938) and lowest for the J (€711). The additional profit generated by the F<sub>1</sub> is largely attributed to increased milk value and lower replacement costs i.e. improved reproductive efficiency.

**Conclusions** In Ireland the impending removal of EU milk quotas will result in land becoming the most limiting resource. Although many farmers view crossbreeding as a means of improving reproductive performance and herd health, results from this study indicate that despite the lower livestock sales (cull cow and calf revenue), overall farm profitability with F<sub>1</sub> cows may well be higher compared to HF cows, due to greater milk receipts coupled with improved reproductive efficiency and survival.

**Acknowledgements** Financial support from the Research Stimulus Fund (RSF-06-353) is gratefully acknowledged.

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## Estimation of the effect of dilution due to milk yield on milk somatic cell count across cow parities

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**Introduction** Subclinical mastitis is one of the most costly diseases in dairy cattle. However previously reported costs may have been over-estimated as a consequence of the dilution effect of milk yield on somatic cell count (SCC) (Green *et al.*, 2006). Some adjustment of SCC for the effect of dilution has recently been made (Green *et al.*, 2006), but without considering whether differences in milk yield and SCC exist among parities. The objective of the current study was to investigate the association between SCC and milk yield as well as calculating the dilution estimates of SCC due to increased milk yield for individual parities on grass based dairy production systems

**Material and methods** A total of 235,163 test day records from 23,791 cows in 366 Irish milk recorded herds between the years 2003 and 2005 were included in analysis. Herds with less than 10 cows milk recorded in a year were removed and only test-days between 5 and 305 days post-calving were retained. The association between test-day milk yield and test-day SCC was calculated within parity after pre-adjusting test-day SCC by using one of four methods (1.1 to 1.4) as described by Green *et al.* (2006). *Method 1.1.* there was no pre-adjustment of SCC. *Method 1.2* pre-adjusted SCC = SCC divided by corresponding SCC dilution estimate. The mean SCC for milk yield categories 10 to 20, 21 to 30, 31 to 40 and >41 litres was divided by the mean SCC for milk yield category 10 to 20 litres. This was done for each SCC category (<51, 51 to 100, 101 to 200, 201 to 300, 301 to 400, 401 to 1000, ≥1001 cells/mL) separately to generate a total of 28 dilution estimates. *Method 1.3* pre-adjusted SCC = SCC\*milk yield/mean milk yield for each parity. *Method 1.4* pre-adjusted SCC = SCC + (-regression coefficient of SCC on milk yield\*milk yield). Within parity, a mixed model was developed where the data only included test days SCC of <50,000 cells/mL, as these animals were presumed uninfected. SCC was the dependent variable and cow was included as a random effect. Herd and calving month were included as fixed effects and the regression on milk yield was estimated. The association between milk yield and pre-adjusted SCC was estimated, within parity, using linear mixed models with milk yield as the dependent variable. Fixed effect were days in milk (DIM) and exponential (DIM<sup>-0.05</sup>) (Wilmink, 1987; Green *et al.*, 2006). Confounding effects forced into each of the models as cow level fixed effects were farm (n=366) and calving month (January to December).

**Results** Mean SCC was 175,084, 198,274, 242,300, 286,218 and 348,485 cells/mL, for parities 1 to 5, respectively. Mean milk yield was 20.4, 23.3, 24.8, 25.3 and 25.3 litres in parity 1 to 5, respectively. When there was no pre-adjustment of SCC for milk yield (*Method 1.1*), a test day milk loss of 1.43, 2.08, 2.59, 2.56 and 2.62 litres was associated with an increase of SCC category from <51 cells/mL to >400 cells/mL in parity 1 to 5 animals, respectively. When *Method 1.2* was used to pre-adjust SCC a smaller reduction in test day milk yield of 1.40, 2.00, 2.43, 2.39 and 2.47 litres (parity 1 to 5 animals, respectively) was observed with an increase of SCC category from <51 cells/mL to >400 cells/mL. When *Method 1.3* was used to pre-adjust SCC an increase in test day milk yield of 0.97, 0.60, 0.17, 0.24 and 0.30 litres for parity 1 to 5 animals, respectively was observed with an increase of SCC category from <51 cells/mL to >400 cells/mL. When *Method 1.4* was used to pre-adjust SCC a reduction in test day milk yield of 1.29, 1.77, 2.24, 2.20 and 2.31 litres for parity 1 to 5 animals, respectively was observed with an increase of SCC category from <51 cells/mL to >400 cells/mL. Regression coefficients (β) used in *Method 1.4* were -0.274, -0.442, -0.445, -0.520 and -0.441 litres for parities 1 to 5, respectively. For example if the adjustment methods were applied to two cows with a SCC of 400,000 cells/mL with a milk yield of 15 and 25 litres, the SCC would be 400,000 and 384,615 cells/mL for *Method 1.2*, 294,118 and 490,196 cell/mL for *Method 1.3* and 400,004 and 400,007 cells/mL for *Method 1.4*. Thus it is possible that SCC is over adjusted in *Method 1.3*. The dilution estimates in the study changed the association between SCC and milk yield; with a decrease in milk loss compared to milk loss associated with no SCC pre-adjustment (*Method 1.1*) of 3, 5, 6, 7 and 6% (parities 1 to 5, respectively) following the first SCC pre-adjustment (*Method 1.2*), and 10, 15, 13, 14 and 12% (parities 1 to 5, respectively) for the third pre-adjustment (*Method 1.4*). For *Method 1.3* there was a decrease in the test day milk loss associated with increasing SCC of 167, 129, 107, 109 and 111% (parities 1 to 5, respectively). Methods 1.1 to 1.4 were statically significant (P<0.05).

**Conclusions** Two of the SCC dilution estimates used in the present study (*Method 1.2* and *1.4*) showed an increased SCC was associated with reduced milk yield. The remaining dilution estimate (*Method 1.3*) showed an increased SCC was associated with an increased milk yield. When the dilution estimates were investigated *Method 1.2* had the best fit (lowest log likelihood value) to count for dilution in a grass based milk production system. The results from the study can be used to quantify the changes in milk production for different cow parities due to increased SCC in grass based seasonal production systems. The dilution estimates developed in the study can also be used to generate accurate estimates of milk yield loss due to SCC and thus economic costs of increased SCC.

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## Genetic aspects of lambing difficulty in Scottish Blackface sheep

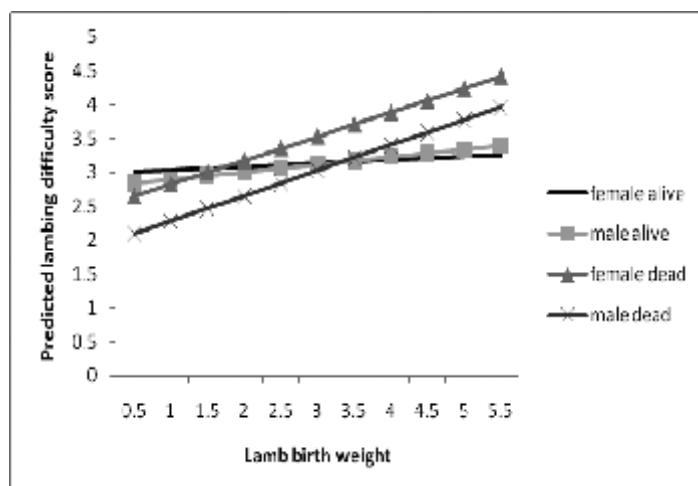
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**Introduction** Lambing difficulty is one of the main causes of lamb losses. It can affect both lamb survival and maternal ability, which are being actively selected for in modern sheep breeding. The available literature concentrates on environmental factors affecting the ease of birth, with special attention given to birthweight and sex of the lamb, litter size and presentation. Previous studies reported that there is an intermediate optimum birth weight at which lambs are less prone to experience dystocia (Sawalha *et al.*, 2007). Lamb birth weight is related to litter size, with singletons more likely to be too heavy, and lambs born in multiple litters more likely to be too light, to meet this optimum. The genetic basis to lambing ease was realised when breed differences in the occurrence of lambing difficulties were first reported (Grommers, 1985). The aim of this study was to evaluate the genetic component of lambing difficulty in Scottish Blackface sheep and to explore key environmental components affecting the occurrence of dystocia in this population.

**Material and methods** Data on lambing difficulty (LDIFF) from 8201 lambs, born between 2001 and 2007 from 2,344 Blackface ewes on two SAC hill farms, was used for this study. LDIFF scores and the proportion of lambings that fell into each category were reported from a similar data set by Lambe *et al.*, (2006), using a 0-7 scale, where 0 = no difficulty, 1 = normal presentation but assisted, 2 = 1 leg back, 3 = 2 legs back, 4 = head back, 5 = back legs first, 6 = breech, 7 = 2 lambs together. The data were re-coded as a binary trait (ASSIST), with 0 = no assistance and 1 = assistance given. Significant environmental factors were investigated using multiple linear models in the statistical package 'R' (Venables and Smith, 2009). Multiple regression was used to examine the effects and interactions of gender, birth weight and whether lambs were born dead or alive (D/A) on ASSIST and LDIFF. ASReml (Gilmour *et al.*, 2002) software was used for the genetic analyses (including heritability ( $h^2$ ) estimates), using a sire (of lamb) model for the analyses for ASSIST and LDIFF, with fixed effects of age of dam (5 levels), birth rank (4 levels), farm (2 levels), D/A (2 levels), gender (2 levels), year of birth (7 levels), lamb birth weight and ewe pre-lambing condition score (covariates) and interactions among birth weight, gender and D/A. The analyses assumed binomial distribution and used a logit link function. To analyse LDIFF as a trait of the ewe ( $\sigma_a$ ), the same fixed effects were fitted in an animal model, with random sire ( $\sigma_s$ ; of lamb), common environment (c; litter effect) and repeatability (r; of dam) effects fitted.

**Results** The total percentage of lambings that were assisted was shown in Figure 1. In lambs born alive, males with birth weight female lambs of the same weight. This trend becomes reversed for



**Figure 1** Interactions of gender, birth weight and lambing difficulty score

**Table 1** Genetic properties

	$h^2$	s.e.
ASSIST (sire)	0.380	0.088
LDIFF (sire)	0.084	0.018
LDIFF $\sigma_a$	0.005	0.011
$\sigma_s$	0.105	0.022
c	0.177	0.016
r	0.183	0.012

The estimate of heritability for the binomial analysis (AASIST) is moderate and higher than that for LDIFF. This is either due to the binomial methodology used or because the 0-7 category of LDIFF is not on a graded scale of severity. Most variation is attributed to non-genetic components of c and r for the animal model analyses.

**Discussion** The perception of male lambs experiencing more difficult births than female lambs reported in previous studies is justified only by the interaction described above. Binomial analyses for dystocia resulted in higher heritability estimates and would be the preferred method of analysis for the estimation of EBVs for dystocia in sheep.

**Acknowledgements** Defra, Scottish Government, EBLEX, HCC and QMS for funding, M. Steel, C. Brockie and A. McLaren for data collection.

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## Genetic associations between Johne's disease and susceptibility to *Mycobacterium bovis* and *Mycobacterium avium* subsp *avium* in Irish Holstein Friesian dairy cows

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**Introduction** Johne's disease in cattle is caused by *Mycobacterium avium* subsp *paratuberculosis*. The indications are that the prevalence of Johne's disease has increased in Ireland since the introduction of the single European market (Good *et al.* 2009). A recent study has demonstrated that significant genetic variation exists for susceptibility to *M. a. paratuberculosis* infection in Irish Holstein Friesian dairy cows, indicating that genetic improvement is possible (Berry *et al.* 2009). Nevertheless, data on Johne's disease occurrence is not collected routinely on Irish dairy farms. The shared evolutionary history of, and similar immunopathological response post-infection to, *Mycobacterium bovis* and *Mycobacterium avium* subsp *avium* may allow them to be used as indicators of susceptibility to *M. a. paratuberculosis* infection in cattle. National single intradermal comparative tuberculin test data collected routinely as part of the Irish bovine tuberculosis eradication programme may therefore provide a means of indirect selection for Johne's disease resistance in Irish dairy cattle. The objective of this study was to estimate the genetic associations between resistance to *M. a. paratuberculosis* infection and measures of susceptibility to *M. bovis* and *M. a. avium* infection.

**Material and methods** Serological response to *M. a. paratuberculosis* was used as a measure of cow susceptibility to Johne's disease. Data on animal serological response to *M. a. paratuberculosis* were obtained from 44 Irish herds from the Irish Department of Agriculture, Fisheries and Food between June 2004 and November 2005. Blood samples were collected from all lactating and non-lactating animals older than 12 months, and *M. a. paratuberculosis* specific enzyme linked immunosorbent assay (ELISA) was conducted. Serological response to *M. a. paratuberculosis* was dichotomised as seropositive (sample to positive control optical density ratio greater or equal to 70) or seronegative (sample to positive control optical density ratio less than 70). Only cows with a known sire, and herds with at least two ELISA positive cows (one of which was home bred) and 10 or more tested cows were retained. The final dataset included in the analysis of serological response to *M. a. paratuberculosis* consisted of 4,581 cows from 38 herds. The single intradermal comparative tuberculin test (SICTT) was used as a measure of susceptibility of cows to *M. bovis* and *M. a. avium* infection. The test involves injecting *M. bovis*-purified protein derivative (PPD) into the neck of each animal, and comparing the reaction induced to that produced by *M. avium*-PPD (a measure of sensitisation to environmental mycobacteria). Susceptibility to *M. bovis*-PPD responsiveness was dichotomised as standard reactor (a *M. bovis*-PPD reaction 4 mm or greater than the *M. avium*-PPD reaction) or nonreactor (a *M. bovis*-PPD reaction equal to the *M. avium*-PPD reaction). Susceptibility to *M. avium*-PPD responsiveness was dichotomised as standard reactor (a *M. avium*-PPD reaction 4 mm or greater than the *M. bovis*-PPD reaction) or nonreactor. National SICTT records between November 2000 and December 2007 were available for inclusion in the analysis. An episode was defined as a herd restriction initiated by two or more standard reactors (with at least one of the animals being home bred), and terminated by 2 consecutive clear herd tests. Cows that calved outside the normal age for a given parity, that had inconclusive SICTT results, or that moved into the herd within six weeks of the SICTT (it takes three to six weeks to develop a positive reaction to the test post infection) were discarded. Following edits, only episodes with at least one standard reactor and ten or more tested cows were retained; 19,663 *M. bovis*-PPD responsiveness records from 640 episodes and 15,824 *M. avium*-PPD responsiveness records from 479 episodes remained. Genetic and residual (co)variance components between serological response to *M. a. paratuberculosis* and susceptibility to *M. bovis*-PPD and *M. avium*-PPD responsiveness were estimated using bivariate linear animal models in ASREML (Gilmour *et al.* 2009). The likelihood ratio test of nested models was used to determine whether the genetic correlations differed significantly from zero.

**Results** Heritability estimates for serological response to *M. a. paratuberculosis*, susceptibility to *M. bovis*-PPD responsiveness and susceptibility to *M. avium*-PPD responsiveness were 0.07 (standard error [SE]=0.03), 0.03 (SE=0.01) and 0.03 (SE=0.01) respectively. A weak non significant genetic correlation of 0.03 (SE=0.32; P>0.05) was estimated between serological response to *M. a. paratuberculosis* and susceptibility to *M. bovis*-PPD responsiveness. However, a strong positive genetically correlation of 0.84 (SE=0.20; P<0.05) was estimated between serological response to *M. a. paratuberculosis* and susceptibility to *M. avium*-PPD responsiveness

**Conclusions** The results from this study suggest that selection for reduced *M. avium*-PPD responsiveness may indirectly increase resistance to *M. a. paratuberculosis* infection. *M. avium*-PPD responsiveness data is collected routinely within the national bovine tuberculosis eradication program; therefore, it should be possible to develop breeding programs to select for increased resistance to Johne's disease, via the SICTT. However, data in this study were few and results should therefore be interpreted with caution.

**Acknowledgements** Financial support from ERAD and Stimulus Research Fund (RSF-060328) is gratefully acknowledged

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## Genetic variation in serological response to *Mycobacterium avium* subspecies *paratuberculosis* and its association with performance in Irish Holstein-Friesian cows

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**Introduction** Paratuberculosis, also referred to as Johne's disease, is a contagious and chronic disease in ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Few estimates of the genetic variation in measures of susceptibility to MAP are available and even less have attempted to elucidate the genetic associations between measures of susceptibility to MAP and performance in dairy cattle. The objective was to quantify the genetic variation in susceptibility to MAP, as measured by serology, and to determine the genetic association between MAP serological response and performance in Irish Holstein-Friesian dairy cows.

**Material and methods** Data on animal serological response to MAP originated from two sources. In 2004 and 2005 blood samples were collected from all lactating and non lactating animals >12 months in 34 herds with suspected MAP infection. The second data base, originating from a paratuberculosis prevalence survey in 2007, was conducted on a random sample of Irish cattle herds. Serological response to MAP was measured using a MAP ELISA (Institut Pourquier, France). The sample to positive (S/P) ratio was used for interpreting test results. A positive animal had an S/P ratio of  $\geq 70$ . The last paratuberculosis test observation in time per animal was retained and animals with no known sire or maternal grandsire were discarded. Only herds with at least two ELISA positive animals (see definition below), of which at least one was home bred were retained. Three herds with less than 10 animals remaining following all previous edits were discarded. The final dataset consisted of 4,789 cows from 44 herds. (Co)variance components were estimated in ASREML (Gilmour *et al.*, 2008). Heritability estimates for serological response to MAP were estimated using both a univariate animal linear mixed model and a univariate animal threshold model. In the animal linear mixed model, the dependent variable was the normalised S/P ratio as well as the S/P ratio dichotomised into infected (S/P ratio  $\geq 70$ ) or not-infected (S/P ratio  $< 70$ ). For the threshold model analysis, a generalized linear mixed model with a logit link function was used. Fixed effects included in both models were herd-year of test, month of test, parity of animal (1, 2, 3, 4,  $\geq 5$ ), stage of lactation (6 stages), heterosis, recombination loss, and Holstein breed proportion. Genetic correlations between serological response to MAP and performance were estimated using a series of bivariate sire mixed linear models. Fixed effects included in the models for the performance traits included contemporary group, Holstein breed proportion, heterosis, and recombination loss.

**Results** Within the edited dataset, 211 (4.4%) of the 4,789 animals were MAP-positive (i.e., S/P ratio  $\geq 70$ ). Note that these estimates of herd-prevalence should not be extrapolated to the general Irish cattle population. The heritability for serological response to MAP measured on a continuous scale was 0.04 (SE=0.02) when estimated using an animal linear mixed model. Heritability of serological response to MAP when analysed as a binary trait was 0.07 (SE=0.028) and 0.14 (SE=0.069) when estimated using a linear and threshold animal model, respectively. Table 1 summarises the genetic correlations between serological response to MAP and performance. The genetic correlations with milk, fat and protein yield were negative or close to zero with the strength of the negative genetic correlations being greater for yields in parity 2 and 3 animals. Serological response to MAP was positively correlated with milk protein percent and negatively correlated with calving interval.

**Table 1** Genetic correlations between MAP (treated as a dichotomous variable) and milk production, fertility and survival.

Trait	Parity 1	Parity 2	Parity 3	Range of standard errors
Milk yield	-0.07	-0.15	-0.08	0.086 to 0.094
Fat yield	-0.04	-0.41	-0.41	0.080 to 0.095
Protein yield	0.05	-0.18	-0.12	0.090 to 0.105
Fat %	0.07	-0.18	0.03	0.082 to 0.097
Protein %	0.16	-0.03	0.06	0.082 to 0.091
Somatic cell score	0.12	0.15	0.05	0.115 to 0.134
Calving interval	-0.59	-0.34	-0.13	0.132 to 0.179
Survival	0.06	0.24	0.16	0.239 to 0.248

**Conclusions** These results clearly indicate significant genetic variation in serological response to MAP exist and are consistent with previous international estimates. Although the response variable used in the present study only measures the immune response and is not a measure of the clinical symptoms, it may, however, be an approximation of the ability to control the infection. No strong genetic correlations with performance were evident with the possible exception of calving interval.

**Acknowledgements** The financial support from Research Stimulus Fund (RSF-06-0328) is gratefully acknowledged

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## Transcriptional activities of bovine lactoferrin (LTF) gene promoter haplotypes

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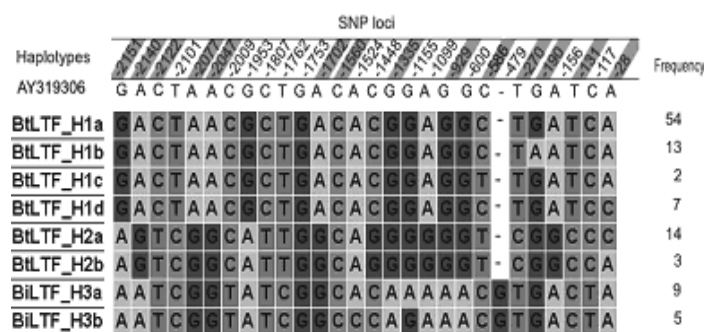
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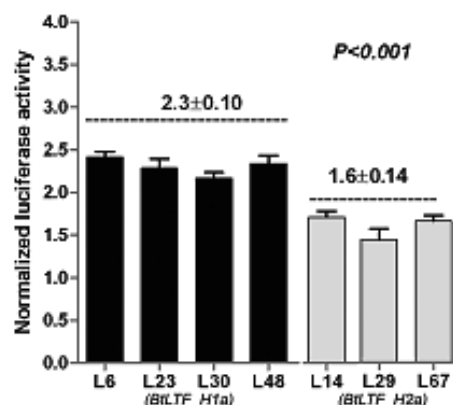
**Introduction** Bovine lactoferrin is an important innate immune protein with a wide range of host defense functions including anti-microbial and anti-viral properties. Genetic polymorphisms present in the promoter region of the lactoferrin (*LTF*) gene have the potential to affect gene expression (Teng 2002). Once characterized, such polymorphisms can be used for the identification of designer herds which produce increased levels of lactoferrin protein in milk. The objectives of this study were to identify major *LTF* promoter haplotypes and to investigate the *in vitro* transcriptional activities of the *LTF* promoter haplotypes.

**Material and methods** The experimental population comprised of Holstein-Friesians (n=47), Jersey (n=6), Jersey X Friesian cross (n=5), Norwegian Red (n=7), Montbéliard (n=4), Norwegian Red X Friesian cross (n=1) and *Bos indicus* (n=8). A total of 31 single nucleotide polymorphisms (SNPs), previously identified through sequencing of 2.2 kb bovine *LTF* promoter (n=80) (O'Halloran *et al.*, 2009), were used to deduce haplotypes. To identify promoter haplotypes, the locus wise genotype data of SNPs were analysed. Haplotype analysis was performed using EM algorithm in the Arlequin software 3.11. Haplotype frequencies were estimated considering the abundance of an individual haplotype over the total haplotypes in the population (n=156). Two bovine *LTF* haplotypes (BtLTF\_H1a and BtLTF\_H2a) were chosen for the *in vitro* promoter assay. Promoter region (stretching from -2200bp to +1bp relative to the transcription start site) of seven animals (four representing the haplotype BtLTF\_H1a and three representing BtLTF\_H2a) were cloned into a pGL4.17 luciferase expression vector system (Promega Corp.). The basal transcriptional activities of the *LTF* promoters were evaluated *in vitro* using a mouse mammary epithelial cell line (NMuMG). The data of the normalized luciferase activity (promoter assay) is compared by 't' test and presented as mean  $\pm$  standard error.

**Results** A total of 51 unique haplotypes were identified in the study population of which 8 haplotypes represented >70% of the population (Figure 1). *In silico* analysis of the 2.2 kb promoter revealed two major haplotypes (BtLTF\_H1a and BtLTF\_H2a) that differed at ten SNP loci that altered putative transcription factors binding sites for both constitutive (at -28, -1702) and inducible (at -131, -270, -586, -2047, -2077, -2122, -2140 and -2151) expression. Transfection studies demonstrated that these two haplotypes differed significantly ( $P<0.001$ ) in their basal promoter transcriptional activity (Figure 2). Cows with the BtLTF\_H1a haplotype were expected to have greater lactoferrin protein concentration in milk compared to herd mates with the BtLTF\_H2a haplotype.



**Figure 1** Major promoter haplotypes of the bovine *LTF* gene (2.2 kb)



**Figure 2** *In vitro* basal activities of the bovine *LTF* promoter haplotypes.

**Conclusions** Two predominant *LTF* promoter haplotypes were identified (BtLTF\_H1a and BtLTF\_H2a). Transfection studies in mammary epithelial cells showed that promoter constructs of the BtLTF\_H1a haplotype had increased transcriptional activity *in vitro* compared to constructs of the BtLTF\_H2a haplotype. A *LTF* haplotype based selection strategy in breeding may aid in increasing lactoferrin protein concentration in the bovine milk to promote and maintain health and well being in the dairy cow and the consumer. It may also facilitate identification of designer herds for production of milk with high content of lactoferrin.

**Acknowledgements** This research was supported by the Department of Agriculture, Food and Fisheries, Govt. of Ireland under the Food Institutional Research Measure (FIRM).

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## The impact of selection on milk production on the lactoferrin content of milk in Irish Holstein-Friesians

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**Introduction** Bovine lactoferrin is an important innate immune protein with a wide range of host defence functions including anti-microbial and anti-viral properties. This bioactive is found naturally in bovine milk and is considered to confer health benefits to both consumer and dairy cow. Currently, the Irish total merit index, the Economic Breeding Index (EBI) does not include the lactoferrin content of milk. The objective of this study was to determine what associations, if any exist between the lactoferrin content of milk and other milk constituents and to make inferences into the impact of selection on milk production on milk lactoferrin content.

**Material and methods** Milk samples (n=865) from 620 Holstein-Friesian cows stationed at five Teagasc research stations in the south of Ireland were obtained during routine weekly milk recording between May and October 2009. Experiments in operation during this time investigated the impact of a) level of herbage mass offered, b) grazing stocking rate, c) feeding system d) autumn calving and e) different concentrate feeding levels and grazing stocking rate on overall animal performance. All animals were on a basal grass based diet and included spring and autumn calving cows. Lactoferrin content (mg/L milk) for all milk samples was determined using the Bovine Lactoferrin ELISA quantification kit (Bethyl Laboratories) at Enfer Laboratories, Naas, Co. Kildare. Milk composition data corresponding to the milk samples analysed for lactoferrin content was also obtained. Only test-day records between 5 and 305 days in milk were retained. From the remaining records, only a single lactoferrin result per cow was retained resulting in 580 records. Milk lactoferrin content was positively skewed and was therefore transformed using the logarithm to the base ten. Phenotypic and genetic (co)variance between lactoferrin content and milk production were estimated using an animal linear mixed model in ASREML (Gilmour *et al.*, 2009). Fixed effects included in all models were cow parity, experimental treatment, stage of lactation and milking time (AM or PM).

**Results** The heritability of lactoferrin content was 0.30 (standard error = 0.10). The coefficient of genetic variation was 6.5%. The genetic correlations between lactoferrin and milk yield traits were positive or close to zero, while the phenotypic correlation between lactoferrin content and yield traits was negative. Lactoferrin was positively correlated with protein percent and casein content and negatively correlated with lactose percent, urea and SCS. However, the size of the standard errors of the genetic correlations were large and only the genetic correlations between lactoferrin content and protein percent, and lactoferrin content and casein (kg) differed ( $P < 0.05$ ) from zero.

**Table 1** Genetic and phenotypic correlations<sup>†</sup> between milk lactoferrin content and milk constituents

	Genetic	Phenotypic
Milk (kg)	0.07(0.246)	-0.16(0.046)***
Fat (kg)	0.24(0.277)	-0.11(0.045)*
Protein (kg)	0.30(0.247)	-0.06(0.046)
Lactose (kg)	0.00(0.260)	-0.20(0.044)***
Fat Percent	0.21(0.305)	0.01(0.045)
Protein Percent	0.43(0.191)*	0.26(0.043)***
Lactose Percent	-0.37(0.237)	-0.30(0.042)***
Urea (kg)	-0.31(0.308)	-0.09(0.044)
Casein (kg)	0.38(0.196)*	0.21(0.045)***
SCS	-0.36(0.326)	0.29(0.043)***

<sup>†</sup> \* $P < 0.05$ , \*\*\* $p < 0.001$

**Conclusion** The existence of a moderate heritability estimate coupled with significant genetic variation clearly indicate that genetic selection for lactoferrin content is possible. These results indicate that genetic selection for milk yield will not have a correlated response on the lactoferrin content of milk. However, more data and a larger number of animals are required to obtain more precise estimates of the genetic correlations.

**Acknowledgements** The RobustMilk project is financially supported by the European Commission under the Seventh Research Framework Programme, Grant Agreement KBBE-211708. The content of this paper is the sole responsibility of the authors, and it does not necessarily represent the views of the Commission or its services. The authors gratefully acknowledge the assistance of Dr. Fiona O'Halloran.

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## The influence of genetic selection on the milk fatty acid profile of spring calving dairy cows

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**Introduction** Bovine milk represents a unique source of nutrients and bioactive components that act in synergy as well as independently and that can positively affect human health. However, the fatty acid (FA) content of bovine milk is not ideal, containing on average 65 to 70% saturated fat (SAT), 25 to 32% monounsaturated fat (MONO), and 3 to 5% polyunsaturated fat (Soyeurt *et al.*, 2006; Grummer, 1991). A more favourable combination for human health would be 30% saturated, 60% monounsaturated and 10% polyunsaturated fat. It is well known that the relative proportions of FA in milk can be altered through nutrition, but less is known on the potential of genetic selection for different FA composition. Of particular interest is the impact of current national breeding strategies on milk FA content. The objective of this study was to investigate the influence of genetic selection, using the Irish total merit index, the Economic Breeding Index (EBI) on milk FA throughout lactation.

**Material and methods** Three genetic groups of Holstein-Friesians (HF) were established from within the Teagasc Moorepark herd: a high genetic potential group of North American HF with an average EBI of €77 (HighNA; n=46), a group representing the Irish national average genetic potential of North American HF with an average EBI of €49 (LowNA; n=46) and a group of high genetic potential New Zealand HF with an average EBI of €89 (HighNZ; n=48). Weekly milk samples collected from all cows for both AM and PM milk recordings between June 2008 and June 2009 were analysed using Mid-Infrared Spectrometry (MIR; Foss Milkoscan FT6000). The time period used in this study spanned over two experiments which investigated the impact of a) different concentrate feeding levels and grazing stocking rate, and b) different calving dates and grazing stock rates on overall performance on a basal grass based diet. Prediction equations previously derived and since validated on a selection of these animals (Soyeurt *et al.*, 2010) were used to predict the FA composition of all milk samples using the MIR spectrum. The weighted average FA constituent was calculated for each week of lactation. A total of 4,135 records between days 5 and 305 of lactation from 140 cows remained. The effect of genetic selection on the content of SAT, unsaturated fat (UNSAT), MONO, short chain FA (aliphatic tails <6 carbons; SCFA), medium chain FA (aliphatic tails 6 - 12 carbons; MCFA) and long chain FA (aliphatic tails >12 carbons; LCFA) in milk fat was investigated using mixed linear models; cow was included as a random effect with a compound symmetry covariance structure assumed among records within cow. Experimental treatment, parity and week of lactation were included as fixed effects in all analyses.

**Results** Mean (standard deviation) SAT, UNSAT, MONO, SCFA, MCFA, LCFA (g/100g fat) across all three genetic groups was 66.2 (3.68), 35.7 (3.70), 29.8 (3.37), 9.4 (1.00), 46.6 (4.54), 44.4 (5.07), respectively. Mean (standard deviation) fat percentage for each of the groups was 4.5 (1.20) for the HighNA, 4.4 (1.16) for the LowNA and 4.8 (1.16) for the HighNZ. Experimental treatment had a significant effect on all FA investigated ( $P < 0.01$ ). The HighNZ cows produced more ( $P < 0.05$ ) SAT and less ( $P < 0.05$ ) UNSAT and MONO than either the HighNA or the LowNA groups (Table 1). The HighNZ cows also had higher ( $P < 0.05$ ) content of SCFA and MCFA in fat (Table 1). There was no difference between the high genetic merit and low genetic merit North American HF for any fat constituent. Although statistically significant, all differences reported are biologically small.

**Table 1** Effect of genetic group on milk fat constituents (g/100 kg fat)

	SAT	UNSAT	MONO	SCFA	MCFA	LCFA
HighNA	66.1 (0.31) <sup>a</sup>	35.8 (0.31) <sup>a</sup>	29.9 (0.27) <sup>a</sup>	9.3 (0.08) <sup>a</sup>	46.5 (0.32) <sup>a</sup>	44.6 (0.36) <sup>a</sup>
LowNA	65.8 (0.30) <sup>a</sup>	36.1 (0.31) <sup>a</sup>	30.2 (0.27) <sup>a</sup>	9.3 (0.08) <sup>a</sup>	46.3 (0.32) <sup>a</sup>	45.0 (0.36) <sup>a</sup>
HighNZ	66.8 (0.29) <sup>b</sup>	35.1 (0.29) <sup>b</sup>	29.2 (0.26) <sup>b</sup>	9.6 (0.07) <sup>b</sup>	47.2 (0.30) <sup>b</sup>	43.7 (0.34) <sup>b</sup>
P-value	<0.01	<0.01	<0.01	<0.001	<0.05	<0.01

**Conclusion** These results indicate that genetic variation in milk FA composition exists and that HF cows of New Zealand ancestry have more SAT and less MONO and UNSAT per kg fat than HF cows of North American ancestry. In addition, within the North American animals, genetic selection for higher EBI does not appear to influence the FA profile of milk. Nonetheless, the routine availability of milk FA, predicted from MIR, facilitate the estimation of national breeding values for milk FA content thereby allowing the inclusion of milk quality in national breeding objectives.

**Acknowledgements** The RobustMilk project is financially supported by the European Commission under the Seventh Research Framework Programme, Grant Agreement KBBE-211708. The content of this paper is the sole responsibility of the authors, and it does not necessarily represent the views of the Commission or its services.

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## Development of a new crossbreed based evaluation for carcass quality of Piétrain boars in the Walloon Region of Belgium

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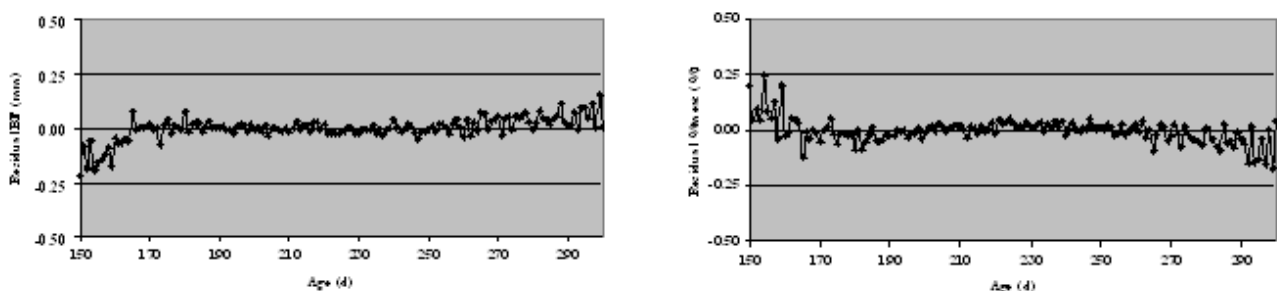
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**Introduction** Until recently, Piétrain boars in the Walloon Region were evaluated with performances recorded on their purebred progeny. However, these boars are mostly used in crossbreeding systems. Therefore, since 2007, a new genetic evaluation system has been developed in the Walloon Region. Piétrain boars are now evaluated on performances recorded on their crossbred progeny with Landrace sows. The aim of this study was to contribute to the new genetic evaluation system of these boars by the development of a genetic evaluation model for carcass quality. The objective was to develop a tool that allows selection of boars that produce carcass with a high lean meat percentage.

**Material and methods** Data provided by the on-farm performance recording system, also recorded at a central test station, were utilized in this study. Performances were recorded on live animals by ultrasound with the Piglog 105. This strategy provided data recorded on animals from the test station, measured the week before slaughtering, and on boars themselves and other related animals on their farms of origin. The data file contains 60,546 records from pigs between 150 and 300 days of age, originated from 56 822 different animals. Animals used needed to have a breed composition of at least 40 % Piétrain or Landrace. Recorded animals were entire males, castrated males or females. Traits analysed in this study were backfat thickness (BF) and meat percentage (%meat). The model developed was a multitrait animal model. Fixed effects were sex, contemporary groups and heterosis, modelled as regression on heterozygosity. A clustering algorithm created contemporary groups containing at least three animals measured at the same location in an interval of maximum 75 days. Random effects were additive genetic, permanent environment and residual. Additive genetic and permanent environment effects were modelled by random regressions using linear splines with three knots at 175, 200 and 250 days. Variance components were estimated by restricted maximum likelihood (REML) on random samples of the dataset and then confirmed by a Gibbs sampling algorithm on the total dataset. Fit of the models was tested by computing residuals from a BLUP (Best Linear Unbiased Prediction) evaluation. The model that could explain the greatest proportion of the variation in each trait and thus with the smallest residuals was selected. The t-test of Student was used to test whether the means of residual distributions were significantly different from zero.

**Results** Estimated heritabilities for BF and %meat were high and had a tendency to increase with age. Estimated heritability from 150 to 300 days increased from 0.56 to 0.75 for BF and from 0.55 to 0.69 for %meat. Genetic correlation was high between BF and %meat and varied from -0.90 to -0.93 between 150 and 300 days. Figure 1 shows the evolution of mean residuals of each trait with age between 150 and 300 days. According to this figure, mean residual is close to zero for both traits at any age. The means of residual distributions of the two traits are not significantly different from zero (P Value = 0.7977 for BF and P Value = 0.1476 for %meat).



**Figure 1** Evolution of mean residual with age between 150 and 300 days of age

**Conclusions** Given that BF and %meat had a high heritability and a high genetic variance (results not shown), genetic improvement of carcass quality is possible by genetic selection with these traits. Moreover genetic correlation was high between BF and %meat, so selection to increase %meat could be based on only one of these two traits. Selection on BF would be preferred because it is a trait that is measured while %meat is predicted from BF and loin muscle depth measurement by the Piglog 105. According to the study of residuals, it seems that the model developed fits well the data.

**Acknowledgements** The authors gratefully acknowledge the financial support of the Ministry of Agriculture of the Walloon Region of Belgium, the support of the Walloon Pig Breeders Association (AWEP) and the collaboration of the Walloon Agricultural Research Centre (CRA-W). Nicolas Gengler, a research associate of the National Fund for Scientific Research (Brussels, Belgium) acknowledges his support. Additional support was provided through grants 2.4507.02F (2) and F.4552.05 of the National Fund for Scientific Research.

## A comparison of ITS and IGS sequences of ovine nematodes

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**Introduction** Nematode parasitism of livestock is of economic importance worldwide. Accurate identification of nematode parasites is important in epidemiological studies as well and in selecting a control strategy. Traditional methods of identifying parasitic nematodes are based on morphological characteristics, which require expertise. DNA-based assays have the potential to rapidly and accurately identify the species infecting livestock. In recent years the intergenic spacer (IGS) and internally transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) have been exploited for the identification of parasitic nematode species. The aim of this study is to assess the feasibility of using the ITS and IGS regions as targets for a molecular assay based on species specific probes to identify parasitic nematode species relevant to sheep in Ireland.

**Material and methods** Eight species of parasitic sheep nematodes, belonging to the families Trichostrongylidae, Strongylidae, Trichonematidae, Molineidae, Chabertiidae and Trichuridae were used. DNA was extracted from a single adult worm from each species using a Roche High pure PCR template preparation kit according to the manufacturer's instructions. The entire ITS-1, 5.8s and ITS-2 region and IGS region of the rDNA were amplified using primer sets described either by Chilton *et al* (2004) or newly designed primers UniITS-F: 5'-GCGGGAAACAGTTAATCGC-3' and UniITS-R 5'-TCCCCGTTCACTCACTCGCCGTTA-3' for the ITS region and UniIGS-F 5'-ACCGTCGTGAGACAGGTTAG-3', UniIGS-R 5'-CTGCTCTAATGAGCCGTTTCG-3' for the IGS region. PCR reactions were carried out in 50 µl reaction volumes using 5 µl genomic DNA, 250 µM of each dNTP, 2.5 µM MgCl<sub>2</sub>, 0.6 µM of each primer and 1 U of *Taq* polymerase (Promega GoTaq®) with buffer supplied. Purified PCR products were then ligated into plasmids using a pGem® Teasy vector system (Promega). The ligated plasmids were transformed into high efficiency competent JM109 *E.coli* cells for multiplication. Purified plasmids were then sequenced (GATC Germany). The Align tool using the Smith-Waterman algorithm was used to compare the sequences (EBI, 2009).

**Results** The sizes of the ITS-1 fragments ranged from 770bp (*Trichuris ovis*) to 368bp (*Chabertia ovina*) (Table 1). The GC content of the ITS-1 ranged from 40% to 57%. The sizes of the ITS-2 fragments ranged from 409bp (*Trichuris ovis*) and 231bp (*Haemonchus contortus*) (Table 1). The GC content of the ITS-2 for all species ranged between 32% and 62%. The levels of homology between the sequences of the different nematode species are shown in Table 1. Amplification of the IGS region was unsuccessful.

**Table 1** Pairwise comparison of the percentage homology in the ITS-1 sequences (above diagonal) and ITS-2 sequences (below diagonal). ITS-1 sequence lengths are shown horizontally while ITS-2 sequence lengths are shown vertically.

	<i>T. colu</i> 387bp	<i>T. ovis</i> 770bp	<i>T. vitr</i> 390bp	<i>T. circ</i> 441bp	<i>O. venu</i> 376bp	<i>N. batt</i> 386bp	<i>H. cont</i> 405bp	<i>C. ovin</i> 368bp
<i>Trichostrongylus colubriformis</i> 238bp	-	40.2	97.7	84.7	66.5	70.8	80.9	66.7
<i>Trichuris ovis</i> 409bp	37.5	-	39.7	38.2	36.7	40.4	41.5	38.2
<i>Trichostrongylus vitrinus</i> 232bp	82.6	43.4	-	86.2	66.5	72.1	81.4	69.2
<i>Teladorsagia circumcincta</i> 246bp	81.1	44.6	78	-	62.3	68.8	79.9	68.5
<i>Oesophagostomum venulosum</i> 258bp	56.8	44.1	56.3	54.9	-	63.2	63.2	89.3
<i>Nematodirus battus</i> 231bp	65.1	38.9	62.6	63.8	52.6	-	64.6	68.5
<i>Haemonchus contortus</i> 231bp	75.1	43.1	88.4	74.4	54.2	58.9	-	65.7
<i>Chabertia ovina</i> 235bp	58.2	41.4	54.6	49.5	70.6	55.3	54.4	-

**Conclusions** Both ITS-1 and ITS-2 regions exhibit low levels of homology. The ITS-2 exhibited less levels in 22/28 (79%) of the pairwise comparisons. These results are promising and may well provide the target region on which to base a rapid species identification assay.

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## The effect of inbreeding upon some growth traits in Najdi calves

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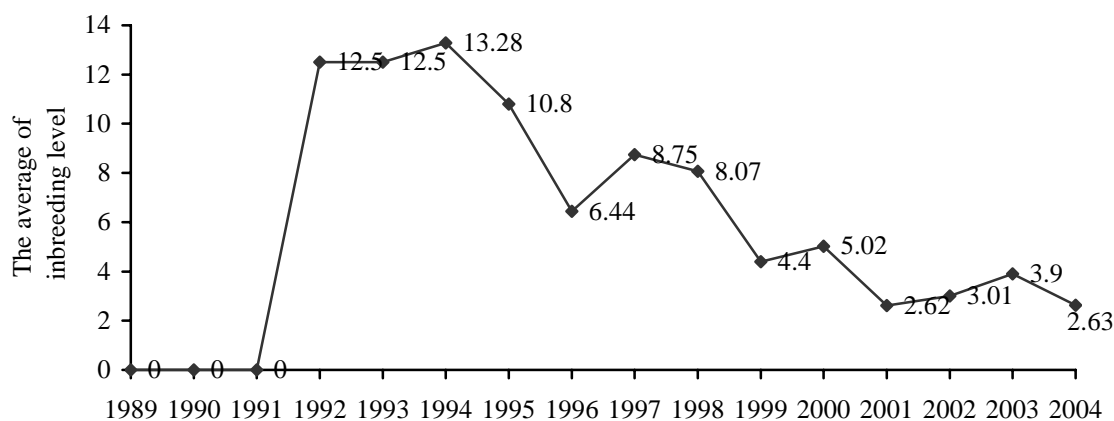
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**Introduction** In animals reared as small herds, accumulation of inbreeding is unavoidable. Inbreeding leads to change of genetic structure of population and to a decline in average phenotypic performance called inbreeding depression. Most evidence indicates that inbreeding adversely affects the growth and vitality of animals. Burgess *et al.* (1954) showed that inbreeding had a significant effect on weaning weight of Hereford calves. Therefore, estimates of inbreeding effects are needed to adjust records of individual animals in order to increase the accuracy of the selection of breeding animals and to facilitate the analysis of data. The present study was conducted to determine the levels of inbreeding and study the effect of inbreeding on birth weight and weaning weight of Najdi calves, born in Najdi cattle Research Station, located in Khuzestan province in Iran.

**Material and methods** In this study, 1281 records of Najdi calves were used. Data were obtained from the Najdi cattle Research Station of Khuzestan province in Iran. Two traits were considered: birth weight (BW) and weaning weight (WW). The calves were born from 1989-2004. Fixed effects, considered in analysis were birth year, birth season, sex of calf and dam parity. The coefficient of inbreeding of the individuals and average level of population inbreeding were calculated using Pedigree software (Sargolzaei *et al.*, 2006) from available pedigree records of animals. The impact of inbreeding on calves birth weight and weaning weight was studied by including inbreeding as a covariate in the model.

**Results** Total average of birth and weaning weight of the calves were  $18.08 \pm 3.17$  and  $42.6 \pm 13.10$  kg, respectively. In this particular population with 1435 animals, 1244 heads were with known parents, 36 heads were with only known dams and 155 animals were with unknown parents. The average inbreeding coefficient in this population was 0.78% with the maximum level of 25%. The change of the inbreeding level from 1989 to 2004 is presented in Fig. 1, shows a trend of reduction of inbreeding coefficient during studied years. In the total population, there were 186 inbred animals with the average inbreeding of 5.60%, which is much more than the average inbreeding of the entire population. In addition, the average inbreeding of male and female calves were estimated of 0.75 and 1.02, respectively. Regression coefficient of birth weight on percent of inbreeding was computed as  $-0.0627 \pm 0.031$  kg/percent of inbreeding, indicated that birth weight is depressed as 62.7 grams per percent increase in inbreeding. The same coefficient was found for weaning weight as  $-0.182 \pm 0.12$  kg/percent of inbreeding. Ferraz *et al.*, (2000) reported the average inbreeding level of Santa Gertrudis herd to be 0.0395, using 10 generations of records. Hays and Brinks (1980) reported a depressing effect of increased inbreeding on weight in beef cattle. However, in the study of Herford cattle by De Alexander and Bogart (1961), no evidence of an effect of inbreeding of the calf on birth weight.



**Figure 1** The change of the inbreeding level from 1989 to 2004

**Conclusions** The average inbreeding level of our studied population was low and the results of analysis showed that the inbreeding depression for birth weight and weaning weight was not significant. The lack of significance of the degree of inbreeding depression on studied weight traits indicate that either selection has been effective in offsetting inbreeding depression or that these traits are not subject to such inbreeding depression.

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## Determination of mitochondrial D-loop sequence of Iranian Moghani sheep breed

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**Introduction** The domestic sheep is an important livestock species in many developing countries. They have played a key role in food resources of Iranian plateau which is known as the origin of this species. Sheep are highly adaptable and versatile domestic animals, which has made them a critically important resource in human societies around the world. Molecular genetics have provided valuable information for investigating and conserving genetic resources. Mitochondrial DNA (mtDNA) sequencing has been used to elucidate the complexity and origins of many modern domestic livestock species, leading to a general theme of multiple maternal lineages. The sequence variation existing within mtDNA has proven a particularly useful tool for addressing such questions across a number of livestock species. Till now, four distinct mitochondrial maternal lineages (haplotype groups A, B, C and D) have been found in the domestic sheep.

**Material and methods** Blood samples from 10 Iranian Moghani sheep were collected from Jafarabad station in Ardabil province. DNA was isolated according to Miller *et al.* (1988) with minor modifications. The first half of the control region (CR1) region was selected for mtDNA analysis. The PCR products obtained from primers of F:5'-TAAGTGTGGGGTAACTATTT-3' and R:5'-AGTATTGAGGACGGGGTAA-3' were purified and then sequenced (MWG-BIOTECH AG, Germany). The sequences obtained were aligned and edited using Bioedit (Hall 1999). All similar sequences available were obtained from Genbank (NCBI) and phylogenetic relationships were drawn using MEGA4 (Tamura *et al* 2007).

**Results** After analyzing the sequences obtained, the length of the CR1 region in Moghani sheep was found to be around 420 base pairs. We obtained SNPs and 5 haplotypes (Table 1). All haplotypes were of the (Asian) A type. Neighbour-Joining tree of 29 mitochondrial sheep including the Mouflon, a wild sheep and European sheep was constructed. The tree contained four distinct branches: clades A, B, C and D. The Iranian Moghani sheep carrying A haplotype, was clustered in the A clade with another Iranian sheep breed, the Baluchi and one Turkish breed which was expected because of their geographical closeness. The branch style tree was drawn for analyzing samples in A haplotype group and results suggested that *Ovis urial* is the origin of this breed. Finally, the consensus sequence was registered in NCBI under accession number FJ531545.

**Table 1** Haplotype sequences in Moghani sheep breed

Sample	Haplotype	56*	75	83	90	303	314	318	322	327
Sample 1	1	C	T	T	T	T	C	T	A	A
Sample 2	2	A	T	C	C	C	T	C	G	G
Sample 3	3	A	C	T	T	T	C	C	A	A
Sample 4	4	A	T	T	T	T	C	T	A	A
Sample 5		A	T	T	T	T	C	T	A	A
Sample 6	5	A	T	T	T	C	C	C	A	A
Sample 7		A	T	T	T	C	C	C	A	A
Sample 8		A	T	T	T	C	C	C	A	A
Sample 9		A	T	T	T	C	C	C	A	A
Sample 10		A	T	T	T	C	C	C	A	A

\*Vertical numbers indicate the SNP position relative to the consensus sequence accession number: FJ531545

**Conclusions** This finding may arise from high levels of relatedness and a low effective population size (Ne) within the animals sequenced. Sequence analysis of the mtDNA from Moghani breed with another Iranian sheep showed high genetic diversity within some Iranian sheep breeds. However, more data from sheep breeds need to be analyzed to confirm these findings

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## Application of fixed regression test-day model for genetic evaluation of Iranian Holsteins

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**Introduction** Test-day (TD) models recently have received considerable attention for the genetic evaluation of dairy cattle. In fixed regression test-day models (FRM), TD records within lactation are taken as repeated measurements (Swalve, 2000). This model assumes constant additive genetic and permanent environmental variances throughout the lactation. Furthermore, it is assumed that the genetic and permanent environmental correlations among different TD records are 1. The objective of this study was to apply FRM to monthly test day milk yield trait in first lactation of Iranian Holsteins cattle.

**Material and methods** A total of 164,391 monthly test day milk records from 19,217 first lactations of Holstein cows calved between 1991 and 2008 and distributed in 172 herds in the Razavi-Khorasan region of Iran were analyzed. A single-trait animal model for fixed regression was applied to TD records of first lactation cows. In this model, fixed effect of contemporary groups of herd-year-month of production (HYM), linear and quadratic covariates of age of calving ( $A_{ijkt}$ ) and Holstein gene percentage ( $HF_{ijkt}$ ), third order Legendre polynomials for days in milk, random effects of additive genetic ( $a_j$ ), permanent environment ( $pe_j$ ) and residual ( $ME_{ij}$ ) were fitted. All genetic relationship among animals was also taken into account. The model equation was

$$y_{ijk} = \mu + HYM_i + \sum_{m=1}^2 \beta_m * (A_{ijkt} - \bar{A})^m + \sum_{m=1}^2 \delta_m * (HF_{ijkt} - \overline{HF})^m + \sum_{R=0}^{k-1} (\gamma_R * \phi_R(t)) + a_j + pe_j + ME_{ijk}$$

The covariance components and genetic parameters were estimated by REML method using DXMRR option of the DFREML software package.

**Results** Variance components, heritability and repeatability are in table 1. These results are contrary to result of most studies.

**Table 5** Estimates of variance components<sup>1</sup>, heritability ( $h^2$ ), and repeatability ( $r$ ) for monthly test-day milk yield

Trait	$\sigma_a^2$	$\sigma_{pe}^2$	$\sigma_e^2$	$\sigma_p^2$	Heritability	Repeatability
Milk yield (Kg)	4.686	11.97	13.92	30.57	0.153	0.544

<sup>1</sup>  $\sigma_a^2$  = additive variance,  $\sigma_{pe}^2$  = permanent environmental variance,  $\sigma_e^2$  = residual variance,  $\sigma_p^2$  = phenotypic variance

**Conclusion** In general, heritability estimate for monthly milk yield trait of first parity of Iranian Holsteins obtained in this study was low compared with estimates reported by other countries (Mostert *et al.*, 2004). Lower heritability observed here is due to the structure of the data set, lower pedigree information, method of estimation variance covariance components, and the model used for analysis of the data. However the low heritability estimates is attributed to a rather high environmental variance as a result of different system of keeping of dairy cows in Iranian herds that influencing performance of dairy cattle in Iran. Estimate of repeatability is within the range of other studies.

**Acknowledgments** The authors gratefully acknowledge the Ministry of Agricultural Jihad of Razavi Khorasan Province for supplying the data used in this study.

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## Estimation of genetic and environment trend for birth weight and weaning weight in Najdi calves

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**Introduction** The Najdi cow is native to Khuzestan province. Native cattle have some advantages over exotic breeds such as resistance to disease and use of native pastures with reduced food cost. In order to evaluate genetic improvement in a selected population, variation resulted from environment and genetic should be disentangled. This work required the estimation of genetic parameters for the traits of interest in the populations. Ebangi and *et. al.*, (2000) estimated direct genetic trends and maternal genetic trends for birth weight in Guadali cattle of 0.022 and -0.007 kg per year and for weaning weight of 0.228 and -0.103 kg per year, respectively. Thus, the objectives of the paper were investigating the effect of maternal additive genetic and maternal permanent environmental effect on birth and weaning weight of Najdi calves, born in Najdi cattle Research Station located in Khuzestan, Iran, using different models of Mayer and then, estimating genetic and environmental trend for birth weight and weaning weight.

**Material and methods** In this study, 1249 records of birth weight and weaning weight of Najdi calves were used for estimation of parameters and genetic, phenotypic and environmental trends. The calves were the progeny of 60 sires from 414 cows that calved over a 16-year period. This data collected from 1990 to 2005. To determine fix effects in model, data analysed with GLM procedure of Minitab program. DFREML software and animal model were used to estimate components of (co) variance and genetic parameters. The models included a direct effect, maternal effect, non-additive maternal permanent environment effect and environmental effect, associated with the animal, fitted as random effects. Sex, calf birth year, season of calving and parity were fitted as fixed effects. Best Linear Unbiased Prediction (BLUP) for direct additive and maternal breeding values were obtained for each animal from the best model, using single trait animal model.

**Results** Mean and standard deviation of birth weight and weaning weight for Najdi calves calculated  $18.08 \pm 3.17$  and  $49.56 \pm 13.10$  kg, respectively. Based on likelihood ratio test, model 4 was recognized as the best fit model for estimation of birth weight and weaning weight genetic parameters. Genetic and phenotype parameters for birth weight and weaning weight and trend of direct genetic, maternal genetic, environmental and phenotype are presented in table 1 and 2. In study of American Hereford cattle by Mattos *et al* (2000) estimates of direct and maternal heritability for birth weight were 0.24 and 0.16.

**Table 1** Estimates of direct and material heritability and components of variance for birth weight and weaning weight

trait	$m^2$	$h^2$	$\sigma_p^2$	$\sigma_e^2$	$\sigma_m^2$	$\sigma_a^2$	$r_{am}$
Birth weight	0.08	$0.37 \pm 0.11$	7.70	3.44	0.61	2.90	0.56
Weaning weight	0.1	$0.13 \pm 0.06$	103.79	81.88	10.51	13.28	-0.16

**Table 2** Trend of direct genetic, maternal genetic, environmental and phenotype

Estimated trend	Birth weight (kg)	Weaning weight (kg)
Direct genetic	0.06*	0.13*
Maternal genetic	0.03*	0.13*
Environmental	0.02 <sup>ns</sup>	0.04 <sup>ns</sup>
Phenotypic	0.07 <sup>ns</sup>	0.21 <sup>ns</sup>

**Conclusions** The results of this study show that ignoring maternal genetic and environmental effects in analysis model caused to overestimate direct heritability for studied traits. Because of negative genetic correlation between direct and maternal effects for weaning weight trait, methods of selection accounting for both direct and maternal genetic effects would result in greater economic selection response than selection based only on direct genetic effect. Genetic variation in studied years showed ascendant range for two traits, but no emphasizing on one selection index during different years caused to high fluctuation in the breeding value mean of traits in studied population.

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## Estimates of genetic parameters on economical traits in Khorasan Karakul sheep

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**Introduction** The main aim of sheep breeding is meat production in Iran. Karakul sheep are a dual purpose (meat and skin) breed in the eastern north of Iran. A high percentage of the sheep population is managed under a migratory system. Using the ranges as the major source of feed in harsh conditions, knowledge of genetic parameters for growth traits at various ages is needed (Bahreini *et al.* 2007).

**Material and methods** The data (4,167 records) used in the study, were collected at Karakul sheep Breeding station in Sarakhs on Razavi Khorasan province of Iran from 1995-2004. seven traits were considered: birth weight(BW), once month weight(W1), weaning weight(WW), 6 month weight(W6), 9 month weight(W9), yearling weight(YW), pre-weaning weight gain(G1) and post-weaning weight gain(G2) that the related mean and standard deviation were  $5.17 \pm 0.8$ ,  $12 \pm 2.39$ ,  $24.8 \pm 4.5$ ,  $33.5 \pm 5.5$ ,  $39.3 \pm 6.45$ ,  $42.7 \pm 4.7$ ,  $0.211 \pm 0.051$ ,  $0.085 \pm 0.025$  kilogram respectively. The JMP statistical package (JMP Ver. 7) and the method of unequal subclass analysis of variance were used to test the significance of the fixed effects. All of fixed effects were significant ( $p < 0.05$ ). Estimation of (co)variance components was carried out using the DF-REML (model 3) 1997 program. A simplex algorithm is used with 300 iterations. Effects of sex, age of dam, birth type and birth year were studied to design statistical model and they were significant ( $p < 0.05$ ) for growth traits. Variance component estimated in animal model by DF-REMEL software.

**Results** Direct heritability for body weights showed a tendency to increase with age measured (table 1). Because estimates of direct additive genetic variance component increased faster than the environmental variance components. Tendency for estimates of direct heritability to increase with age measured has also been reported in several studies (Yazdi *et al.* 1997). For all traits, estimates of maternal heritability were lower than the estimates of direct heritability. Estimates of maternal heritability tended to decline from birth to yearling weight. In general, the trend of increasing direct heritability and decreasing maternal heritability in Karakul sheep were the same in other breeds sheep reported in Iran. The present study estimates are within the range of literature.

**Table 1** The mean values, number of observation, direct and maternal heritability for the different traits

trait	Mean(kg)	Number of records	$h^2_a \pm se$	$h^2_m \pm se$
birth weight	$5.17 \pm 0.8$	4167	$0.16 \pm 0.03$	$0.03 \pm 0.01$
once month weight	$12 \pm 2.39$	2648	$0.15 \pm 0.01$	$0.01 \pm 0.00$
weaning weight	$24.8 \pm 4.5$	3157	$0.17 \pm 0.04$	$0.03 \pm 0.01$
6 month weight	$33.5 \pm 5.5$	2098	$0.13 \pm 0.03$	$0.00 \pm 0.00$
9 month weight	$39.3 \pm 6.45$	1614	$0.11 \pm 0.06$	$0.00 \pm 0.00$
yearling weight	$42.7 \pm 4.7$	1247	$0.13 \pm 0.03$	$0.00 \pm 0.00$
pre weaning weight gain	$0.211 \pm 0.051$	3157	$0.21 \pm 0.05$	$0.01 \pm 0.03$
post weaning weight gain	$0.085 \pm 0.025$	1247	$0.12 \pm 0.01$	-

Direct heritability( $h^2_a$ ), maternal heritability( $h^2_m$ )

**Conclusions** The low direct heritability estimates in growth traits of Karakul sheep is probably because of the low nutritional level in station and poor quality of pasture in area. The results showed that maternal effects do not need to be considered in selecting for growth traits of after WW in Sarakhs Karakul sheep.

**Acknowledgements** The authors gratefully acknowledge funding from Karakul Sheep Breeding Station in Sarakhs on Razavi Khorasan Province of Iran.

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## Single nucleotide polymorphisms in the growth hormone and insulin-like growth factor 1 genes are associated with production and fertility traits in dairy cows

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**Introduction** The *somatotropic axis* has been shown to be a key regulator of growth and development in animals and affects performance traits including milk production, growth rate, body composition and fertility. The action of growth hormone (GH) is mediated through controlling expression of many genes among which is the insulin-like growth factor-1 (*IGF-1*). A positive association between systemic concentrations of IGF-1 in the early postpartum period and subsequent cow fertility has been reported (Patton *et al.*, 2007). Additionally, we have also found associations between single nucleotide polymorphisms (SNPs) in *IGF-1* and *GH* and production traits in cattle (Mullen *et al.*, 2010). The objective of this study was to determine if an association exists between these SNPs and fertility and milk production traits in lactating dairy cows.

**Material and methods** DNA was extracted and subsequently genotyped for 10 SNPs in the *IGF-1* gene and 7 SNPs in the *GH* gene on 610 Holstein-Friesian dairy cows from 10 herds (6 commercial and 4 research herds) calving between 1997 to 2007. Pregnancy rate, assessed by ultrasound scanning initially 30 to 50 days and subsequently at 100 days post-insemination, and calving to 1st service interval (CFS) was available on 362 cows for the year 2006; 241 of these cows also had records of body condition score (BCS) at calving. Information was obtained from the Irish Cattle Breeding Federation on calving interval from 369, 242 and 148, first, second and third parity cows, respectively and on milk production for first, second and third lactation from 392, 237 and 152 cows, respectively. Experimental treatments within the four research herds were treated as separate herds and contemporary group was subsequently defined as herd-year-month of calving. The association between each of the SNPs and performance was determined using mixed animal linear models in ASREML (Gilmour *et al.*, 2009) accounting for the additive genetic relationship among animals. Analyses were undertaken within parity for milk production and calving interval. Fixed effects included in the model were Holstein breed fraction and contemporary group. Only one record on calving to first service interval, pregnancy rate to first service, and pregnancy rate overall was available for all animals and therefore the association between each SNP and these fertility traits was undertaken across all parities with parity, as well as contemporary group and Holstein breeding fraction, included in the model as a fixed effect.

**Results** No SNP was associated with calving to first service interval. The G allele of *IGF1i3* was associated with increased milk yield in lactation 1 (b=398 kg; SE=173.0 kg) and 2 (b=751.3 kg; SE=262.0), increased fat yield in lactation 1 (18.8 kg; SE=6.8 kg) and 2 (21.3 kg; SE=10.2 kg) and increased protein yield in lactation 2 (16.5 kg; SE=8.0). Both *IGF1i1* and *IGF1i2* were associated with BCS at calving (Table 1). None of the other seven *IGF-1* SNPs were associated with any of the performance traits evaluated. *GHi33* was the only *GH* SNP associated with milk production with the T allele associated with an increased in milk protein yield in lactation 2 of 9.8 kg (SE=5.0 kg). However, several of the *GH* SNPs were associated with fertility, as measured by calving interval, pregnancy rate to first service and pregnancy rate overall (Table 1).

**Table 1** Associations (standard errors in parenthesis) between SNPs in the *GH* and *IGF-1* genes and calving interval (days), body condition score ([Scale 1 to 5\*10]), pregnancy rate to first service (PRFS; %\*100) and pregnancy rate overall (Overall; %\*100)

SNP <sup>a</sup>	Allele substitution	Calving interval			BCS	PRFS	Overall
		Parity 1	Parity 2	Parity 3			
<i>IGF1i1</i>	A → T	-4.6 (6.2)	4.9 (7.9)	-9.6 (11.7)	-1.34 (0.52)*	1.75 (3.8)	-1.56 (2.22)
<i>IGF1i2</i>	C → T	-2.6 (6.8)	1.3 (8.8)	12.2 (12.2)	1.92 (0.57)***	0.17 (4.14)	1.07 (2.44)
<i>IGF1i3</i>	G → A	4.5 (13.1)	0.3 (16.5)	-3 (22.6)	0.29 (1.12)	1.71 (8.07)	3.73 (4.71)
<i>GHi32</i>	T → C	4.0 (6.6)	5.3 (8)	22.5 (11.4)*	0.08 (0.59)	10.03 (4.20)*	5.34 (2.48)*
<i>GHi33</i>	T → A	-4.4 (10.2)	-11.2 (14.2)	-15.4 (19.3)	0.66 (1.13)	5.5 (7.20)	1.49 (4.23)
<i>GHi35</i>	T → C	3.4 (6.2)	3.5 (7.7)	13 (10.5)	0.08 (0.59)	10.12 (4.14)*	5.01 (2.42)*
<i>GHi38</i>	T → C	4/0 (6.6)	5.3 (8)	22.4 (11.3)*	0.45 (0.59)	9.84 (4.22)*	5.29 (2.48)*

\* = P<0.05; \*\*\* = P<0.001 significance from zero. <sup>a</sup>: i = Intronic SNP

**Conclusions** SNPs in *IGF-1* were associated with milk production traits and BCS; however there was no evidence of an association with any fertility traits measured. SNPs in *GH* were associated with calving interval, pregnancy rate and milk protein yield. The associations found in this study warrant further investigation including additional sequencing to understand the causative mechanisms involved.

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## Sequence variations in the growth hormone and insulin-like growth factor 1 genes are associated with body size traits in Holstein-Friesian dairy cattle

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**Introduction** Feed costs make up a large proportion of the variable costs in dairy herds and a large proportion of the feed costs are attributable to maintenance which is influenced by animal size. Molecular markers associated with smaller body size but not antagonistic to performance would be useful to select for more efficient animals. The growth hormone (*GH*) and insulin-like growth factor 1 (*IGF-I*) genes were selected in this study as candidate genes for body size because of their known role in animal growth and development. The objective of this study was therefore to discover novel SNPs in these genes and to quantify their association with body size related production traits in dairy cattle.

**Material and methods** A panel of 22 cattle (four Belgian Blue crossbreds, four Charolais, four Simmental, four Aberdeen Angus crossbred and six Holstein-Friesians) was selected for SNP discovery. Regions of the *GH* and *IGF1* genes encompassing both promoter and regulatory flanking sequences were PCR amplified and sequenced. Sequence validation and *de novo* polymorphism detection was carried out using a combination of software packages including BLAST, ClustalW and Chromas. Identified SNPs were then genotyped across 848 HF sires with progeny in Ireland. The association between each SNP and performance was quantified using weighted mixed models in ASREML (Gilmour *et al.*, 2009) with genotyped individual included as a random effect. Year of birth (divided into five yearly intervals) and percent Holstein of the individual sire were included as fixed effects in the model. In all instances the dependent variable was de-regressed PTA for calving interval and functional survival, weighted by their respective reliability less the parental contribution. Genotype was included in the analysis as a continuous variable. A multiple regression model was developed within each gene separately by backward elimination of non-significant ( $P > 0.05$ ) SNPs.

**Results** Sequence analysis of ~ 13 kb across these two genes identified; 44 *de novo* SNPs in the *GH* gene and nine *de novo* SNPs in the *IGF-I* gene. Significant associations were found with both novel and previously identified SNPs across both genes with growth traits (Table 1). For example: in the *GH* gene, a C to T substitution in *GHi32*, was associated with increased carcass conformation of 0.05 (scale 1 to 15); and in the *IGF-I* gene a substitution of the A allele with a G allele in *IGF1i4* was associated with increased cull cow weight of 2.61 kg and an increase of carcass weight of 2.41 kg (SE= 1.01 kg). No SNP was associated with animal stature.

**Table 1** Allelic substitution effects (standard errors in parenthesis) between SNPs in the *GH* and *IGF1* genes and carcass conformation (Conform; scale 1 to 15), carcass fat (Cfat; scale 1 to 15), cull cow weight (Cull; kg) and the body size linear type traits, body depth (BD), chest width (CW), body condition score (BCS) and angularity (ANG) expressed in standard deviation units ( $\times 100$ )

SNP <sup>a</sup>	dbSNP	Allele	Conform	Cfat	BD	CW	BCS	Cull	ANG
<i>Igfl1i2</i>	<i>de novo</i>	C→T		5.14 (1.77)		-0.24 (0.11)			
<i>Igfl1i4</i>	<i>rs29012855</i>	A→G						2.61 (1.29)	
<i>Igfl1i6</i>	<i>de novo</i>	A→G					0.42 (0.21)	-3.40(1.50)	
<i>Igflr10</i>	<i>de novo</i>	C→T				-0.51 (0.23)			
<i>GHi63</i>	<i>rs41916256</i>	A→T		4.46 (1.84)					
<i>GHi36</i>	<i>rs41923523</i>	C→T			22.88 (8.65)				0.19 (0.09)
<i>GHi32</i>	<i>de novo</i>	C→T	5.04 (1.95)						
<i>GHi1</i>	<i>rs41923485</i>	C→T							0.35 (0.17)
<i>GHi17</i>	<i>rs41923483</i>	C→T			36.86 (12.13)				
<i>GHi19</i>	<i>rs41923481</i>	C→T					-0.22 (0.09)		
<i>GHi21</i>	<i>rs41923479</i>	A→G			-44.86 (13.31)				
<i>GHi24</i>	<i>de novo</i>	C→T		5.44 (1.99)					

<sup>a</sup> :*GHiX* = SNP X located downstream i.e. 3' of the *GH* gene, *Igfl1iY* = SNP Y located within an intron of the *IGF-I* gene.

**Conclusion** Analysis of the *GH* and *IGF-I* genes has identified SNPs associated with traits related to body size, reaffirming previous studies reporting their involvement in animal growth and development.

**Acknowledgements:** The authors acknowledge the Irish Cattle Breeding Federation, the Irish Dairy Research Trust, DAFF Food Institutional Research Measure and DAFF Research Stimulus Fund.

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Gilmour, A. R., Cullis, B. R., Welham, S. J. and Thompson, R. 2009. ASREML Reference Manual 2<sup>nd</sup> edition.

## Associations between the K232A polymorphism in the DGAT1 gene and performance of Holstein-Friesian dairy cattle in Irish and UK herds

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**Introduction** Marker assisted selection is a method to augment traditional methods of animal breeding with genomic information. Changes in the DNA sequence (i.e., polymorphism) of an animal have previously been shown to affect animal performance. One such example is the dinucleotide polymorphism K232A in the DGAT1 gene on BTA14. However, the effect of a polymorphism may differ by production system. The objective of this study was to quantify the association between the K232A polymorphism in DGAT1 in Irish and UK Holstein-Friesian cattle.

**Material and methods** Genotypes of the K232A polymorphism of 346 Holstein-Friesian sires with a reliability of at least 60% in both Ireland and the UK for milk production were available for inclusion in the analysis. For the purposes of this study the two alleles at the K232A polymorphism were called the K (lysine) and A (alanine) based on the amino acids encoded by the respective polymorphisms. Daughter yield deviations for milk yield, fat yield, protein yield, milk fat concentration, and milk protein concentration were obtained from the domestic genetic evaluations of the UK and Ireland. The association between the K232A polymorphism and performance was undertaken using weighted mixed models in ASREML (Gilmour *et al.*, 2009) with a polygenic effect. Fixed effects included in the model were country, the number of copies of the K allele (continuous variable) as well as an interaction between the number of copies of the K allele and country (i.e., Ireland or the UK). The dependent variable was the DYD of the trait of interest weighted by its respective reliability. Significance was based on the F-statistic.

**Results** The standard deviation of the DYD for milk yield, fat yield and protein yield in Ireland was 246.1 kg, 7.7 kg and 6.6 kg, respectively; the respective values in the UK were 211.6 kg, 6.2 kg, and 5.9 kg. The correlation between DYDs of the 346 sires in Ireland and the UK were 0.92, 0.85, and 0.90, for milk, fat yield and protein yield, respectively. The frequency of the AA, AK and KK genotypes in the 346 sires was 0.50, 0.41 and 0.09, respectively. Irrespective of country, an A (alanine) to K (lysine) allelic substitution was associated with a decrease in milk yield and protein yield but an increase in milk fat yield, milk fat concentration and protein concentration (Table 1). The association between the K232A polymorphism and both fat yield and concentration ( $P < 0.001$ ) as well as milk yield ( $P = 0.05$ ) differed by country, but no interaction was observed for either protein yield or protein concentration. However, in all instances only the size of the relationship with milk yield, fat yield and fat concentration differed between countries, not the direction of the relationship suggesting a scaling effect as opposed to a re-ranking effect. The size of the association between the K232A polymorphism and production was similar to previous reports in Ireland using 742 sires (Berry *et al.*, 2009) and the direction of the association with milk yield was similar to the direction of the association in a UK study that investigated the association between the K232A polymorphism and daily milk yield in 571 Holstein-Friesian cows (Banos *et al.*, 2008). The proportion of genetic variation explained by the K232A polymorphism for milk, fat and protein yield was 4.1, 14.0 and 0.01% in Ireland and 4.2, 7.2 and 0.6% in the UK respectively. Again these results are in agreement with the results obtained by Berry *et al.* (2009) from 742 Holstein-Friesian sires genetically evaluated in Ireland.

**Table 1** Allele substitution effect (A being substituted by K) of the K232A polymorphism on milk production in Ireland and the UK as well as the significance of the interaction between the effect of the polymorphism and country.

Trait	Substitution effect (standard error)		Significance of interaction	
	Ireland	UK	F-value	P-value
Milk yield (kg)	-77.61(15.21)	-66.93 (12.14)	16.6	0.05
Fat yield (kg)	4.47 (0.47)	2.58 (0.38)	76.6	$P < 0.001$
Protein yield (kg)	-0.90 (0.41)	-0.69 (0.34)	3.3	0.08
Fat concentration (%*100)	13.10 (0.89)	7.32 (0.49)	171.3	$P < 0.001$
Protein concentration (%*100)	2.90 (0.51)	2.12 (0.28)	22.8	0.27

**Conclusion** The K allele of the K232A polymorphism was associated with reduced milk yield and protein yield but increased fat yield and milk composition. The size of the associations was similar in both countries with the exception of fat yield and fat concentration which differed by country with a larger effect in Ireland which also had a larger variance. The K232A polymorphism can be used in Irish and UK breeding programs to increase the accuracy of selection for production traits.

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## Study on polymorphism of BMP-15 gene in Iranian native goats

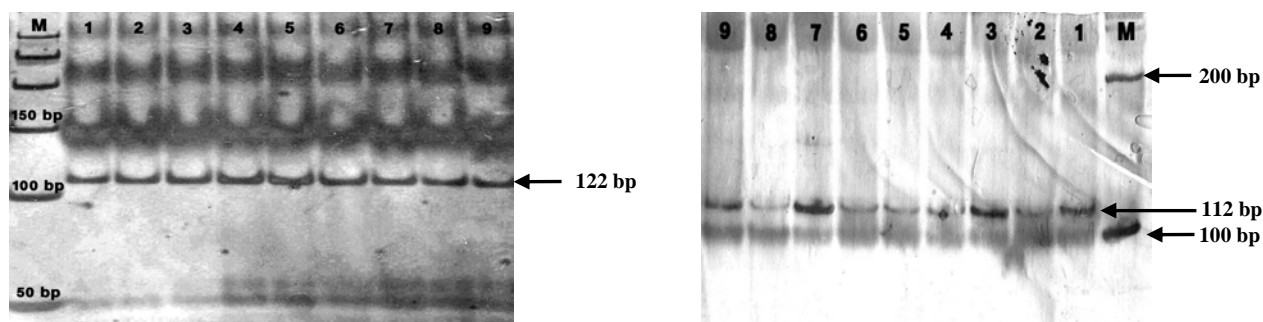
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**Introduction** Different mutations in the bone morphogenetic protein-15 (*BMP-15*) and the Growth Differentiation Factor-9 (*GDF-9*) genes have increased ovulation rate and infertility in a dosage-sensitive manner in sheep (McNatty *et al.*, 2005). Five naturally occurring mutations in exon 2 of the sheep *BMP-15* gene have been described. These mutations produce increased ovulation rate and twin and triplet births in heterozygotes, and complete primary ovarian failure in homozygotes resulting in total infertility in some prolific breeds of sheep (Galloway *et al.*, 2000). However, information on genes affecting the fertility of Iranian goats is scarce. The goal of the present study was to examine the polymorphism of the *FecX<sup>B</sup>* and *FecX<sup>G</sup>* loci in *BMP-15* gene in Iranian native goats.

**Material and methods** Jugular blood samples (7 ml) were randomly collected from 109 Iranian native goats by using of EDTA coated tubes. Caprine genomic DNA was isolated from whole blood samples by using a commercially available kit. The final DNA pellets were resuspended in 50 µl of sterile distilled water and stored in -20 °C for use. The *FecX<sup>B</sup>* allele in exon 2 *BMP-15* gene was amplified using the polymerase chain reaction (PCR) with primers forward: 5'-GCCTTCCTGTGTCCCTTATAAGTATGTTCCCTTA-3' and reverse: 5'-TTCTTGGGAAACCTGAGCTAGC-3' to amplify a 153 bp PCR product (Hanrahan *et al.*, 2004). Amplification was for 30 cycles in a 25 µl reaction mixture, with 100 ng of caprine genomic DNA, 1U of Taq DNA polymerase and 2.5 mM magnesium chloride at an annealing temperature of 57.5°C. The PCR products were digested with 10 U of *DdeI* enzyme (C/TNAG) overnight at 37 °C, and the resulting products were separated by 10% PAGE gel and visualized by silver staining. The resulting products of wild type animals will have a 122 bp and 31 bp fragments and the mutation type animals with *FecX<sup>B</sup>* variant will have a 153 bp fragment. A primer pair was also designed to detect SNP of the *FecX<sup>G</sup>* allele in *BMP-15* gene with *HinfI*. The primer sequences were as follows; Forward: 5'-ACTGTCTTCTTGTTACTGTATTTCAATGAGAC-3'; and Reverse: 5'-GATGCAATACTGCCTGCTTG-3' (Hanrahan *et al.*, 2004). Polymerase chain reactions were carried out. The amplification conditions for primers of the *FecX<sup>G</sup>* allele were as follows: denaturation at 94°C for 5 min; followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 63°C for 30 sec, and extension at 72°C for 20 sec; with a final extension at 72°C for 5 min. The PCR products of 8 µl were digested separately with 10 U of *HinfI* overnight at 37°C in a 20 µl reaction mixture, and the resulting products were separated by 10 % PAGE gel and visualized by silver staining. Primers amplified a 141 bp band. The wild type products could be cleaved by *HinfI* (G/ANTC) with a 112 bp and 29 bp fragments, the mutation type with *FecX<sup>G</sup>* remained uncleaved.

**Results.** In the present study the PCR products were separated by 6% PAGE and following digestion with restriction enzymes were separated by 10% PAGE (Figure 1). The basic finding of the current study was the absence of polymorphism at the *FecX<sup>B</sup>* and *FecX<sup>G</sup>* loci of *BMP-15* gene in Iranian native goats. All goats were monomorphic for exon 2 *BMP-15* gene.



**Figure 1** Polyacrylamide gel electrophoresis (10%) images for PCR product of the *FecX<sup>B</sup>* and *FecX<sup>G</sup>* digested with *DdeI* and *HinfI*, respectively.

**Conclusion** These results showed that there was no genetic polymorphism of *FecX<sup>B</sup>* and *FecX<sup>G</sup>* loci in *BMP15* gene in Iranian native goats. Further investigation should be directed at other loci of *BMP-15* gene or other genes, using larger sample sizes.

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## The effects of the nitrification inhibitor dicyandiamide on herbage production when applied at varying time points and rates in the autumn

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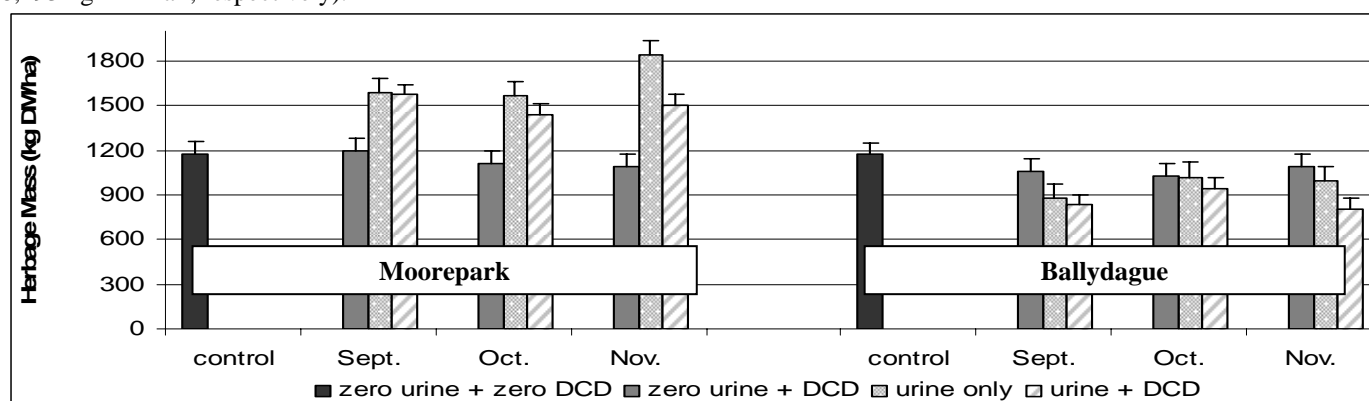
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**Introduction** Nitrification inhibitors such as dicyandiamide (DCD) have been shown to reduce nitrate leaching and nitrous oxide emissions (Moir *et al.*, 2007; Dennis *et al.*, 2008) by slowing the conversion of ammonium to nitrate in the soil. Nitrate is readily taken up by growing plants, but if surplus nitrate is available, such as when plant growth slows in winter, or under urine patches, it is likely to be lost through leaching. As well as reducing N losses, increased spring herbage production has been observed in New Zealand when DCD was applied in autumn and early spring. Moir *et al.* (2007) showed increases of up to 36% in annual herbage production from urine patches and up to 25% in non-urine areas when DCD was applied. The objective of this experiment was to investigate herbage production in spring and total production from February to September following the application of DCD on two soil types at three different times in autumn/early winter.

**Material and methods** This experiment was undertaken at Teagasc Moorepark Research Centre, Fermoy Co. Cork on two contrasting soil types. The soils were (1) free-draining acid brown earth of sandy loam to loam in texture at Moorepark (MPK) and (2) a moderate to heavy brown earth of sandy loam texture with evidence of an iron pan at Ballydague (BD), approximately 5 miles from Moorepark. The experiment was a randomised block design with three replicates of each treatment at each site. Two fertilizer N application rates were applied, 350 kg N/ha and 0 kg N/ha. There were 4 urine application timings, one application in September, October or November or not applied. Artificial urine was used (urea and water mix) and the quantity of N in the artificial urine was 1000 kg N/ha. DCD was applied at rates of 0, 5 and 10 kg/ha as a single application using a Kestrel spray-master sprayer, within 24 hours of each urine application. Plots were harvested every 4 weeks from February to November. All fresh samples were weighed and a sub-sample was dried at 40°C for 48 hours to determine dry matter (DM) yield. Data were analysed using PROC GLM in SAS. Data for each site were analysed separately.

**Results** Spring herbage mass (herbage production from October to March) was significantly ( $P < 0.001$ ) greater at MPK when urine was applied compared to when no urine was applied (Figure 1); and there was no significant effect at BD. There was no significant effect of DCD rate or application time on spring herbage production at either site. There was no significant effect of fertiliser on spring herbage mass. Annual herbage mass (herbage harvested from February to September, inclusive) increased significantly at both sites when fertiliser was applied ( $P < 0.001$ ). Herbage mass was 12,102 kg DM ha<sup>-1</sup> at MPK when fertiliser was applied compared to 6,519 kg DM ha<sup>-1</sup> when no fertiliser was applied. At BD herbage mass was 6,319 kg DM ha<sup>-1</sup> and 4,372 kg DM ha<sup>-1</sup> with and without fertilizer, respectively. There was a significant effect ( $P < 0.01$ ) of urine application on annual herbage mass production at MPK. Herbage mass was 1,808 kg DM ha<sup>-1</sup> greater on swards which received urine in autumn/early winter compared to those that did not receive urine (10,101 v's 8,293 kg DM ha<sup>-1</sup>, respectively).



**Figure 1** Average spring herbage mass production (kg DM/ha) between October and March on plots receiving zero urine, urine in September, October or November with and without DCD.

**Conclusion** There was no significant effect of DCD rate or application on spring or annual herbage mass, at either site. Urine application increased herbage mass irrespective of time off application at Moorepark; however it had no effect at Ballydague. Fertiliser N had no effect on herbage mass in spring but it significantly increased annual herbage mass at both sites. This experiment is currently being repeated for a second winter.

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## The use of nitrification inhibitors as a strategy for increasing grass yields: can this environmental tool also benefit agronomic efficiency?

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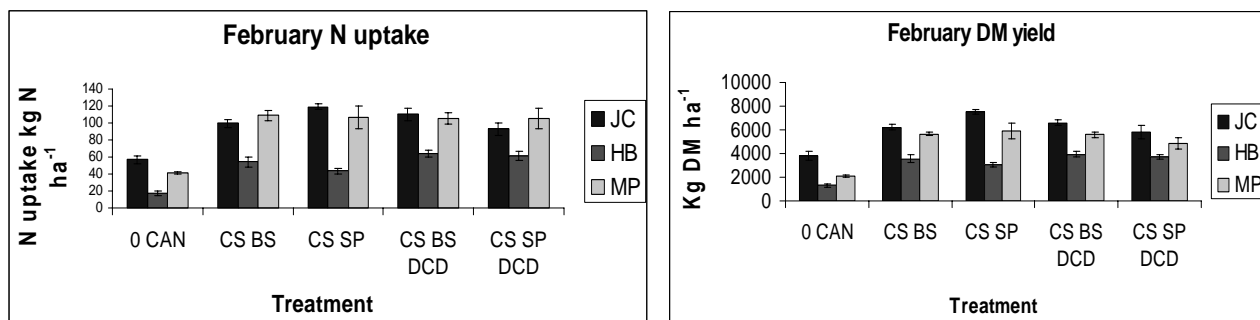
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**Introduction** Nitrogen is an essential element for plant growth, and is often the key limiting nutrient in grazed pasture systems. DCD (dicyandiamide) is a nitrification inhibitor that acts by specifically inhibiting ammonium oxidation, thus help keeping nitrogen in the ammonium form. Nitrate unlike ammonium is more prone to leaching and also denitrification which both result in a loss of nitrogen from the soil system through transport in soil solution and gaseous emissions respectively. DCD has been reported to reduce denitrification of excess nitrate within the soil by up to 73% (Di and Cameron, 2006) and increase herbage yield (Zaman *et al.*, 2009). With increased nitrogen retention in the soil there is in theory going to be more available to aid plant growth. Higher herbage yields as a result of improved nutrient efficiency will allow for lower fertiliser application rates and a reduction in input costs. The objective of this project is to evaluate the role of DCD in increasing herbage production.

**Material and methods** There were 5 treatments, with two spreading methods 1. Cattle slurry (CS) splashplate (SP) and 2. CS Bandsread (BS) both  $\pm$  DCD and a control. The experiment was conducted over three sites – (JC) Johnstown Castle, Co. Wexford, (HB) Hillsborough, Co. Down and (MP) Moorepark, Co. Cork. The three sites were arranged in a randomised block design with 6 replicates per treatment. Plot size measured 5 x 2 m. Cattle slurry was applied at a rate of 33 m<sup>3</sup> ha<sup>-1</sup> in March, June and October which are typically important dates for slurry application before/after winter and after first cut silage. DCD was mixed with the slurry at a rate of 15% of the slurry NH<sub>4</sub>-N content (10 kg DCD ha<sup>-1</sup> avg.). The herbage was harvested from the October application in February, harvesting of the February and June applications took place 8 – 10 weeks post amendment. Harvesting was carried out using a Haldrup plot harvester which had a cutting width of 1.5 m and cut a central strip through each plot. Sub-samples of the herbage were taken for DM% (dry matter) determination and from the dried sample chemical analyses was carried out for %N, P, K, S, Mg, Ca & Na determination. Statistics were carried out using SAS v. 9.1 and an ANOVA was carried out to test for significant differences ( $p < 0.05$ ) between treatments. The factors in the model were spreading method, DCD, site and their interaction.

**Results** The results below show total DM% yield and the total nitrogen uptake, from slurry applied on 9 March 2009 and the control. (Figure 1).



**Figure 1** Total DM% yield and total nitrogen uptake from slurry applied in March. Error bars indicate SEM.

For both DM yield and nitrogen uptake there was a lot of variation between the different sites. The HB site had lower DM yield and N uptake for each treatment compared to JC and MP. The slurry applied at all three sites did have a large positive effect on herbage yield, relative to the control. Bandsread and splashplate slurry application methods did not appear to differ in terms of herbage yield produced or in nitrogen removed in the herbage.

**Conclusions** The success of each of the slurry treatments regardless of spreading method or DCD content was dependent upon the site on which it was applied. More data will be needed in order to further investigate the effect of site, DCD and slurry application method on herbage production.

**Acknowledgements** This research is financially supported under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture and Food, Ireland.

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## The effects of autumn rotation length and cutting height on a perennial ryegrass-white clover sward

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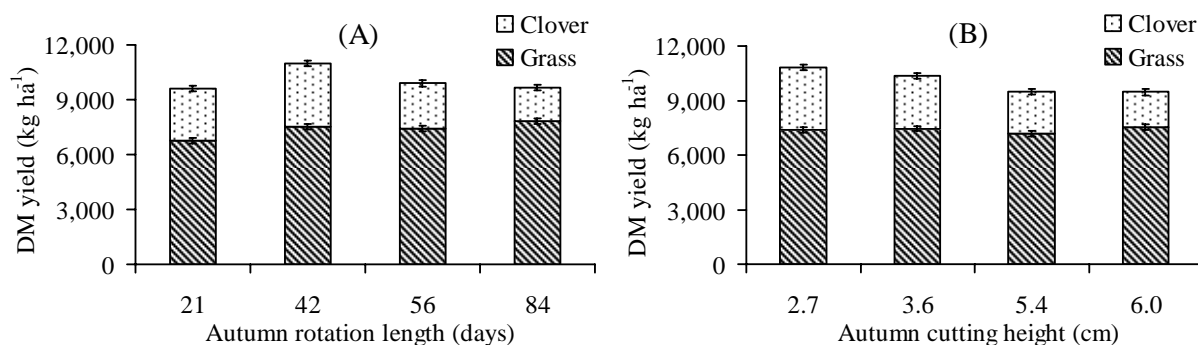
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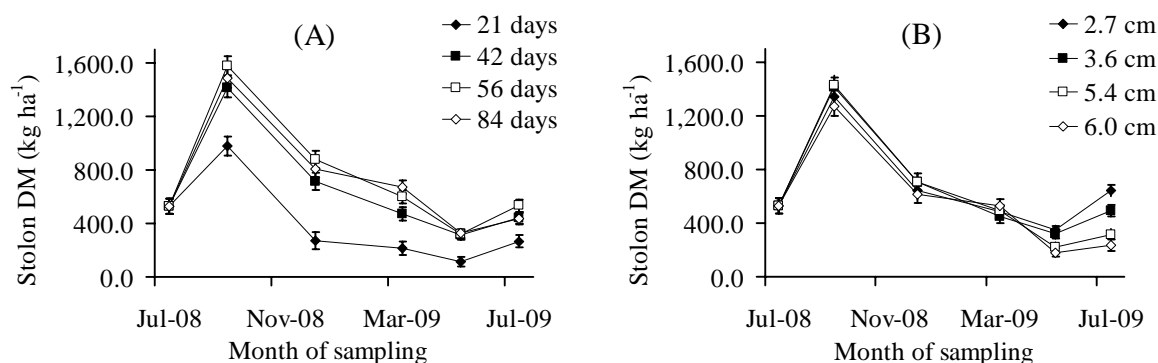
**Introduction** The proportion of white clover in grass swards usually declines in winter. The extent of this decline and subsequent recovery can affect white clover persistence. The objective of this experiment was to examine the effects of autumn rotation length and cutting height on herbage production and persistence of a white clover-perennial ryegrass sward.

**Material and methods** This experiment was conducted on a permanent grass-clover sward at Solohead Research Farm (52°51'N, 08°21'W) and involved 4 rotation lengths (21, 42, 56 or 84 days) and 4 cutting heights (2.7, 3.6, 5.4 or 5.9 cm above ground level). Treatments were imposed between July and December, 2008. Between March and June, 2009 all plots were harvested at 28-day intervals to a cutting height of 4.5 cm. Plots (8 m × 2 m) were laid out in a randomised complete block design with a factorial arrangement of treatments and 5 replications. Herbage dry matter (DM) yields, clover contents of herbage and clover stolon DM masses were measured. Results were analysed using PROC MIXED in SAS. Time (sampling date) was included as a repeat measure for stolon DM masses. All two-way and three-way interactions were examined.

**Results** For all results, there were no interactions between the two treatment factors; rotation length and cutting height ( $P > 0.05$ ). The highest mean herbage and mean clover herbage DM yield was associated with the 42-day rotation and with the two lower cutting heights ( $P < 0.001$ ) (Figure 1), while lower mean grass DM yield was only associated with the 21-day rotation ( $P < 0.001$ ). Mean stolon DM mass changed typically over time and was affected by interactions between time and rotation length (Figure 2 (A)) and between time and cutting height (Figure 2 (B)). The lowest mean stolon DM mass was associated with the 21-day rotations. Lower cutting heights increased stolon masses only on the final sampling date ( $P < 0.05$ ).



**Figure 1** Mean clover and grass dry matter (DM) yield (kilogram hectare<sup>-1</sup> (kg ha<sup>-1</sup>)) produced between July 2008 and July 2009 for (A) rotation length in autumn and (B) cutting height in autumn. Error bars indicate the standard error of the mean.



**Figure 2** Changes in mean clover stolon dry matter (DM) mass (kilogram hectare<sup>-1</sup> (kg ha<sup>-1</sup>)) over time for (A) rotation length in autumn and (B) cutting height in autumn. Error bars indicate the standard error of the mean.

**Conclusions** A 42-day rotation combined with a low cutting height (2.7 to 3.6 cm) in autumn gave the most desirable outcome in terms of annual herbage yield, clover yield and stolon mass during the following spring/summer. Short (21-day) rotations should be avoided during the autumn because of their negative impact on stolon mass.

**Acknowledgements** This project was funded by the Department of Agriculture and Food Research Stimulus Fund (RSF07-511).



## Using NIRS to predict composition characteristics of *Lolium perenne* L. cultivars

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**Introduction** Near infrared absorbance spectroscopy (NIRS) has become a preferred tool for the routine analysis of agricultural products. This is due to its rapid multi-constituent analysis capability and low sample cost, once a calibration is established. The most expensive step is the development of the prediction equation and this critical stage dictates its future performance as an analytical tool. One important potential application of the NIRS system is in quantifying quality attributes of large numbers of grasses in variety evaluation programmes. This paper reports the development of new predictive calibrations for four chemical attributes of grass composition, namely buffering capacity (BC), crude protein (CP), dry matter digestibility (DMD) and water soluble carbohydrate (WSC), using data from the DAFF national grass variety evaluation programme in Ireland.

**Material and methods** Ryegrass samples were collected from replicated field plots used in grass variety evaluation studies over a number of years in Ireland (McGilloway, 2003). A 300g sub-sample was collected from each harvested plot ( $n = 4170$ ) and dried at 80°C for 17 hours. Milled (1 mm sieve) samples were scanned on a NIRsystems 6500 (Foss UK Ltd., Warrington, UK). Absorbance ( $\log 1/\text{reflectance}$ ) was measured every 2 nm between 400–2500 nm and the spectra stored. WinISI (Infrasoft International, Port Matilda, PA, USA) selected 695 of these samples to provide unique information for the equation using the Neighbourhood H statistic. These 695 samples were analysed for the four attributes (BC, CP, DMD and WSC) as described by McEniry *et al.* (2006). Mathematical treatment of the spectra was carried out on WinISI. Standard normal variate (SNV) and Detrend scatter correction and a 1,4,4,1 derivation were carried out on the spectra to minimise the effects of background noise. A modified partial least squares regression (MPLS) technique was used to correlate the spectral data to the laboratory values. MPLS divides the data to a small number of independent factors and correlates these factors with the reference values. To validate the equation the sample set was divided into five equal groups of 139 samples. Each of these groups was removed in turn as a validation set and an equation formed using the remaining 556. This equation was then used to predict the validation set and the NIRS predictions were compared with laboratory methods.

**Results** The equation formed had strong correlations between reference and NIRS predicted values (Table 1, 0.92–0.99). The validation set had slightly lower correlations for each of the constituents but still remained strong (Table 2, 0.90–0.99). The standard error of each of the constituents was slightly higher for the validation set compared to the full data set. According to Sinnaeve *et al.* (1994) an SD/SECV ratio of greater than 3.0 is acceptable for quantitative analysis, and all four composition variables analysed were greater than this value (Table 2, 3.26–11.05). The BC and DMD ratios were closest to the threshold, but the cause was not evident in the data and not associated with the range of variation, as although DMD was smallest and CP greatest, BC and DMD were similar.

**Table 1** MPLS statistics of calibration for each of the four composition variables

Constituent	N	Mean	SD	SEC	R <sup>2</sup>
Buffering capacity (mEq/kgDM)	685	386.73	62.80	17.41	0.9231
Crude protein (g/kgDM)	688	149.50	41.17	3.50	0.9928
DMD (g/kg)	695	796.82	45.43	12.57	0.9235
WSC (g/kgDM)	684	197.22	61.60	9.79	0.9747

**Table 2** The cross validation statistics of each of the four composition variables

Constituent	SECV	1-VR	SD/SECV
Buffering capacity	19.41	0.9044	3.26
Crude protein	3.74	0.9917	11.05
DMD	13.10	0.9168	3.47
WSC	10.39	0.9715	6.13

**Conclusion** The equation described in this paper is both accurate ( $R^2 > 0.9$ ) and robust ( $\text{SD/SECV} > 3.0$ ) in the quantitative analysis of dried milled *L. perenne* samples. However, reasons for differences in SD/SECV ratio between parameters merits further study. As these samples were from different cultivars of different

maturity and ploidy, as well as from different years and time of season, the equation has the potential to be used on a wide range of ryegrass herbage, particularly those from the national grass variety evaluation programme in Ireland.

**Acknowledgements** The authors gratefully acknowledge funding from DAFF

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## Yield and chemical composition of contrasting maize cultivars at sequential stages of maturity

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**Introduction** Forage maize of high starch content is a high quality feed for ruminants that supports high dry matter intakes and animal performance. However in Ireland, unsuitable climatic conditions can result in poor yields compared to other forages such as grass silage. This study evaluated the effects of maturity at harvest on the yield and composition of the cob and stover components of maize cultivars selected for cold tolerance, high biomass and conventional forage maize silage use.

**Material and methods** Six cultivars of forage maize selected as conventional (Tassilo and Beethoven), cold tolerant (Andante and Nescio) and high biomass (Atletico and KXA 7211) were sown under plastic mulch on 7 May 2008. Plots from the three replicate blocks of a split-plot design were harvested on 16 September, 7 October and 28 October. Samples of whole crop, stover and cob were precision chopped and sampled for chemical analyses. Data were analysed using GLM procedures of SAS software (SAS, 2002).

**Results** Whole-crop yield and the proportion of cob present both increased with later harvest dates (Table 1). Delaying harvest also resulted in higher DM and starch content of cobs. The *in vitro* dry matter digestibility (DMD) of stover decreased with later harvest. High biomass cultivars had a lower cob DM content and cob proportion compared to the other cultivars.

**Table 1** Yield and physical composition of forage maize

<sup>1</sup>= harvest date; <sup>2</sup>=cultivar, <sup>3</sup>= water soluble carbohydrates; <sup>4</sup>=*in vitro* dry matter digestibility.

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , NS=  $P > 0.05$ .

H <sup>1</sup>	C <sup>2</sup>	Whole crop		Cob			Stover		
		DM yield (t/ha)	Proportion of crop (g/kg)	DM (g/kg)	Starch (g/kgDM)	WSC <sup>3</sup> (g/kgDM)	DM (g/kg)	DMD <sup>4</sup> (g/kg)	NDF (g/kgDM)
16 Sept	Tassilo	9.63	612	268	227	157	175	703	575
	Beethoven	13.66	651	242	276	175	179	667	605
	Andante	11.56	519	281	314	120	180	660	627
	Nescio	9.92	623	182	175	223	189	683	583
	Atletico	13.05	442	204	211	188	194	682	597
	KXA 7211	12.01	376	175	103	211	199	657	601
7 Oct	Tassilo	14.35	700	380	481	126	160	675	623
	Beethoven	15.33	678	367	545	117	172	618	684
	Andante	14.75	665	364	528	108	183	620	668
	Nescio	12.79	763	304	405	135	171	675	597
	Atletico	14.33	520	189	138	247	188	677	573
	KXA 7211	14.89	567	255	308	194	192	658	598
28 Oct	Tassilo	15.02	767	381	537	39	218	578	719
	Beethoven	15.52	738	446	612	31	244	545	755
	Andante	14.74	765	479	606	24	259	551	736
	Nescio	12.29	762	304	585	54	197	576	717
	Atletico	16.47	597	261	317	147	193	632	617
	KXA 7211	16.41	697	255	525	86	216	592	706
Sig	H	**	**	***	**	***	***	***	***
	C	***	***	***	***	***	**	**	***
	HxC	NS	NS	**	***	***	***	NS	*
SEM	HxC	0.829	51.5	17.0	36.6	12.6	7.9	16.8	17.6

**Conclusion** Later maturity at harvest increased whole-crop DM yield due to an increase in the yield of cob. This paralleled a rise in the starch content of the cob with later maturity. Cobs from high biomass crops had a lower DM and starch content than cold tolerant and conventional cultivars.

**Acknowledgements** Funding for this research was provided under the National Development Plan, through the Research Stimulus Fund, administered by the Department of Agriculture, Fisheries & Food, Ireland #RSF 5769.

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## Farm system N balances for contrasting intensive grassland dairy production systems

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**Introduction** Nitrogen (N) use efficiency is one of the key drivers of environmentally and economically sustainable agricultural production systems. However, the poor efficiency with which N is utilised within animal based systems is well documented (Jarvis, 1993). In recent years efficient N utilisation has become increasingly important because of economical and environmental concerns, combined with European Union policy such as the Water Framework Directive and Nitrates Directive. The objective of this work was to develop a N balance model to assess N use efficiency, N surpluses and N losses from spring calving grass based dairy production systems. This model was linked with the Moorepark Dairy Systems Model (MDSM; Shalloo *et al.*, 2004). Data from a five year study carried out at Moorepark and reported by McCarthy *et al.* (2007) was used to evaluate the N use efficiency of contrasting spring calving dairy systems. The model was validated by comparing data from Ryan *et al.*, (2006) against model outputs.

**Material and methods** The model simulates and determines the fate of N circulating within the farm system using a whole farm N balance model formulated in an Excel spreadsheet (Microsoft, 2003). The rationale for the development of the model presented in this paper is to assess the production efficiency of dairy farming systems in relation to N use efficiency by combining this model with the MDSM (Shalloo *et al.*, 2004). This allows the strategies and biological processes of agricultural systems to be evaluated. To integrate a set of alternative management strategies and accurately capture the consequences of contrasting systems from a variety of scenarios, a comprehensive range of feed and animal production alternatives for Irish grass based milk production systems were examined. The physical performance data were obtained from a five year study McCarthy *et al.* (2007) conducted at Curtins Research Farm, at Moorepark Dairy Production Research Centre. The study consisted of 3 divergent strains of Holstein-Friesian cows consisting of high-production North American (HP), high-durability North American (HD), and New Zealand (NZ), managed across a variety of Irish pasture-based production systems - the Moorepark Blueprint system (MP), a high concentrate input system (HC), and a high stocking rate system (HS). Farm system N balances on an individual cow basis were calculated for each dairy production system simulated from the MDSM, at monthly stages during the year. All N imported into the dairy system, circulated within and exported from the dairy system was accounted for. Nitrogen input into the individual cow consists of N in feed consumed (grazed grass, silage and concentrates) and N required to replace the individual cow. The N outputs from the farm system are N leaving the system in products (milk, meat), not including N retained in the soil and crops. The sum of the annual inputs less outputs in the form of agricultural products is the annual N balance. Imports and exports of N were expressed as kg N cow<sup>-1</sup>. Nitrogen use efficiency was calculated as the proportion of imported N recovered in agricultural products.

**Results** The N input for the whole system, including the rearing of replacement heifers increased as the replacement rate of the different systems increased from 0.18, to 0.25, and 0.37 for the NZ, HD and HP, respectively. As replacement rate increased, the total N input per cow increased from 167.8 kg N cow<sup>-1</sup> for NZ strain, 183.0 kg N cow<sup>-1</sup> for HD strain and 199.6 kg N cow<sup>-1</sup> for HP strain (Table 1). The N surplus per cow was greater for the HD and HP strains (140.0 and 155.5 kg N cow<sup>-1</sup>, respectively) than for the NZ strain (127.7 kg N cow<sup>-1</sup>) (Table 1).

**Table 1** The annual farm system Nitrogen balance per cow

Genetic strain	HP	HD	NZ
Total kg input (kg N cow <sup>-1</sup> )	199.7	183.0	167.8
Total N output (kg N cow <sup>-1</sup> )	44.1	42.9	40.1
N surplus (kg N cow <sup>-1</sup> )	155.6	140.1	127.8
N use efficiency cow <sup>-1</sup>	0.221	0.234	0.239

**Conclusion** The results demonstrated that within pasture-based systems the lowest N surpluses were observed with the Holstein-Friesian cows combining high genetic potential for both production and fertility traits (HD and NZ strains), rather than those selected solely for increased milk production potential (HP strain).

**Acknowledgements** This project was part funded by the Department of Agriculture, Fisheries and Food Research Stimulus Fund 2005 – RSF05-201.

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## Effect of post-grazing sward height on grass production and performance of four beef heifer genotypes

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**Introduction** Feed is the largest variable cost on beef farms and efficiently managed grazed grass is the cheapest feedstuff available (Finneran *et al.*, 2009). Consequently, maximising animal performance from grazed grass should be the basis of sustainable beef systems. There is evidence that grazing to a lower residual sward height (4 cm *vs.* 6 cm) improves the yield and subsequent quality of swards (Fulkerson and Donaghy, 2001). However, research has shown mixed results of this practice on performance of the dairy cow (Maher *et al.*, 2003; Humphreys, 2009). Most research examining post-grazing sward height in beef cattle is confounded with stocking rate. Consequently, there is a deficit in information on this grazing practice for beef cattle, especially for late-maturing continental crossbreds, the predominant breed types in Ireland. The objective of this experiment was to evaluate the effects of two contrasting grassland management systems on grass production and performance of four late-maturing crossbred breeding heifer genotypes.

**Material and methods** One hundred and thirty-six heifers (10-24 months old) comprising of 4 genotypes: Limousin × Holstein-Friesian (LF), Limousin × Simmental (LS), Charolais × Limousin (CL), and Charolais × Simmental (CS) were blocked by genotype, live weight, body condition score (BCS), age and suckler beef value (economic breeding index), and from within block, randomly assigned to one of two grassland management systems: grazing to a post-grazing sward height of either 4.0 or 6.0 cm. There were two replications of each grazing system resulting in four groups of 34 animals on 40 ha. The stocking rate was 2.5 LU/ha equivalent to 195 kg of organic nitrogen/ha for each grazing system. The experiment was undertaken from early March to early November during which, animals were rotationally grazed on predominantly perennial ryegrass swards. Fresh herbage was allocated to each system once the target post-grazing residual height was achieved. Forty five percent of the land area of each system was conserved as grass silage, harvested on 4<sup>th</sup> June. Herbage surplus to grazing requirements (i.e. when farm cover exceeded requirements) was removed from the rotation by harvesting relevant paddocks for silage. Heifers were bred (May-July) to Blonde d'Aquitaine sires. At the end of the grazing season, they were housed and offered grass silage *ad libitum*. Animal live weight, BCS and ultrasonic fat and muscle depths were measured. To permit adjustments in gut fill, final live weight was obtained 7 days post-housing. Grassland measurements included, pre and post-grazing compressed sward heights (rising plate meter), herbage mass (> 4 cm - lawnmower cuts), sward density, herbage production, farm cover and grazing days. Data were statistically analysed using PROC Mixed in SAS.

**Results** Pre-grazing sward height and herbage mass did not differ ( $P>0.05$ ) between grazing systems with mean values of 10.4 cm and 1920 kg DM/ha, respectively. By design, post-grazing sward height (4.4 *vs.* 5.6 cm) and herbage mass (484 *vs.* 920 kg DM/ha) was lower ( $P<0.001$ ) for the 4 cm than the 6 cm system. An additional 20 tonnes DM of surplus grazed grass (26 *v.* 6 t DM) was removed (as silage) from the 4 cm system compared to the 6 cm system. There was no difference ( $P>0.05$ ) in animal performance between the two grazing systems (Table 1). There were no genotype × grazing system interactions for all variables measured. Initial live weight was lower for LF than LS and CL, who in turn, were lower than CS ( $P<0.05$ ). Final live weight was greater ( $P<0.05$ ) for CS than CL and LF, with LS being intermediate ( $P>0.05$ ) to CL and CS, but live weight gain did not differ between the genotypes. Initial BCS was lower for LF than LS, CL and CS ( $P<0.05$ ), however final BCS did not differ ( $P>0.05$ ) between the genotypes. BCS gain was greater ( $P<0.001$ ) for LF than LS and CS with CL being intermediate.

**Table 1** Performance of four heifer genotypes on two grazing management systems

	Genotype						Grazing system			
	LF	LS	CL	CS	SEM	Sig.	4 cm	6 cm	SEM	Sig.
Initial live weight (kg)	419 <sup>a</sup>	453 <sup>b</sup>	451 <sup>b</sup>	470 <sup>c</sup>	10.7	*	449	447	6.8	NS
Final live weight (kg)	569 <sup>a</sup>	595 <sup>bc</sup>	585 <sup>ab</sup>	612 <sup>c</sup>	10.3	*	588	594	9.4	NS
Daily live weight gain (kg)	0.68	0.65	0.61	0.65	0.034	NS	0.63	0.67	0.037	NS
Initial body condition score (0-5)	3.04 <sup>a</sup>	3.18 <sup>b</sup>	3.16 <sup>b</sup>	3.23 <sup>b</sup>	0.043	*	3.15	3.16	0.027	NS
Final body condition score (0-5)	3.26	3.20	3.26	3.22	0.034	NS	3.22	3.25	0.022	NS
Body condition score change (0-5)	0.22 <sup>a</sup>	0.02 <sup>b</sup>	0.11 <sup>ab</sup>	-0.01 <sup>b</sup>	0.041	***	0.07	0.10	0.026	NS

**Conclusion** Potential exists to increase herbage production by grazing to a lower post-grazing residual height without sacrificing animal performance.

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## Effect of concentrate supplementation on milk production of spring calving dairy cows with restricted herbage allowance and subsequent carryover effects

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**Introduction** Irish dairy production systems are mainly pasture-based spring calving herds. Replacing grass silage with grazed grass in the diets of spring calving cows in early lactation will reduce production costs on Irish dairy farms. Dillon *et al.* (2005) has shown that 10% increase in the proportion of grazed grass in the diet will reduce the cost of milk produced 2.5c/litre. Also replacing grass silage with grazed grass in the diet of spring calving dairy cow in early lactation has milk production performance benefits, including increasing milk protein content (Kennedy *et al.*, 2005). However due to the seasonal pattern of grass growth in Ireland, grass supply in early spring is generally not sufficient to meet the cow's demand. As a result, it is sometimes necessary to restrict access to grass and supplement with concentrate in early lactation. The objective of this experiment was to establish the effect of concentrate supplementation on milk production of spring calving dairy cows in early lactation with restricted daily herbage allowance.

**Material and methods** Fifty-seven (24 primiparous and 33 multiparous) spring calving Holstein Friesian dairy cows (mean calving date 3 February; s.d. 13.1 days) were balanced on lactation number (2.3; s.d. 1.6), milk yield (22.2kg; s.d. 4.99), bodyweight (513kg; s.d. 62.99) and body condition score (3.19; s.d. 0.53) in a randomised block design. Animals were randomly assigned to a 3 treatment (n=19) grazing study - 3 concentrate (conc.) levels for a 44 day period (23 Feb. until 7 April). The treatments were: L1 - 1kg fresh weight (FW) conc./cow; L3.5 - 3.5 kg FW conc./cow and L6 - 6kg FW conc./cow. All cows grazed together for the duration of the experiment. Daily herbage allowance was governed using a post grazing height of 3-3.5 cm. Fresh herbage was allocated daily. Herbage mass and sward density was measured twice weekly by cutting four strips per grazing area. Pre and post-grazing sward heights were measured daily. Milk yield was recorded daily, milk composition and bodyweight (BW) were measured weekly. Body condition score (BCS) was also measured. Animal variables were analysed using covariate analysis in SAS. Parity, treatment, parity \* treatment interaction, days in milk and the pre experimental covariates were included in the model. Linear and quadratic effects on milk yield, milk fat, protein and lactose content, milk solids yield, solids corrected yield (SCM), BW change and BCS change were also tested.

**Results** Animal performance results are shown in Table 1. Mean pre-grazing sward height was 7.7cm (s.e. 0.15), while post grazing sward height was 3.2cm (s.e. 0.06). Mean pre-grazing herbage mass was 1120 kg DM/ha. Mean daily herbage allowance (DHA) was 12 kg DM/cow/day. Increasing conc. allowance from L1 to L3.5 significantly increased ( $P<0.005$ ) milk yield, solids corrected milk yield, milk protein yield and milk lactose yield. Increasing conc. allowance from L3.5 to L6 had no significant effect on milk production performance. Treatment had no effect on BW or BCS change with a large BW and BCS loss recorded across all treatments. Milk response to concentrate was 0.72kg milk/kg conc. increasing conc. from L1 to L3.5 with no response in milk production increasing conc. from L3.5 to L6. In terms of carryover effects; cows offered the highest level of concentrate, L6, continued to yield the highest four weeks after the trial ceased. However all groups equalised for milk yield eight and twelve weeks after trial completion.

**Table1** Effect of concentrate supplementation on dairy cow performance in early lactation

	kg conc FW*/cow/day			SED	Sig.	Linear
	1	3.5	6			
Milk yield (kg/day)	23.3	25.1	25.2	0.69	0.0156	0.0097
Milk fat content (g/kg)	4.17	4.24	4.09	1.12	0.4388	0.4750
Milk protein content (g/kg)	3.16	3.14	3.20	0.52	0.5821	0.4888
Milk lactose content (g/kg)	4.80	4.79	4.79	0.23	0.8654	0.6454
Milk solids yield (g/day)	1710	1832	1839	62.36	0.0804	0.0434
SCM yield (kg/day)	22.4	23.0	23.0	0.79	0.0737	0.0460
Bodyweight change (kg)	-49.3	-53.0	-44.3	12.11	0.7868	0.6875
BCS change	-0.66	-0.68	-0.63	0.06	0.7927	0.6835

\*FW = Fresh Weight

**Conclusion** These results suggest that where herbage allowance is restricted in early lactation, there is no response to feeding high levels of concentrate and that a moderate level of concentrate feeding will stimulate a milk production response. Cows across all treatments lost a substantial amount of body condition as indicated by the BCS change. This indicates that concentrate supplementation

will not prevent mobilisation of body reserves where herbage allowance is restricted in early lactation.

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## A comparison between cut and intensively grazed swards on DM yield of perennial ryegrass

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**Introduction** Grassland is of major importance to Irish agriculture due to our reliance on grazed grass and grass silage as the base feeds for livestock production. To achieve the optimum performance from a grass ley the most appropriate grass variety must be selected while considering the target production system and soil type with individual variety performance dependant on species, ploidy and production traits. Different cultivar evaluation protocols are employed throughout Ireland and Europe, and testing is generally conducted under cutting management practices. The protocols employed can generally be segregated into simulated grazing or conservation based cutting regimes, with some integrating both conservation and grazing. Wims *et al.*, (2009) reported that grass cultivars re-rank between simulated grazing and conservation based cutting regimes. However mechanical defoliation of cultivars may not be directly comparable to animal grazing in terms of dry matter (DM) production. The objective of this study was to compare the total DM and seasonal DM production of cultivars managed under either animal grazing or simulated grazing and to evaluate the effect of incorporating a silage harvest into these managements on total and seasonal DM production.

**Material and methods** Four managements were employed as follows:

- i) Animal Grazing (AG) (300 kg N/ha) incorporating 10 animal grazing rotations from February to November (n=60);
- ii) Simulated Grazing (SG) (350kg N/ha) incorporating 10 mechanical defoliations from February to November (n=60);
- iii) One-Cut Silage and AG (300 kg N/ha) incorporating 7 animal grazing rotations from February to November with silage harvested in late May (n=30);
- iv) Simulated One-Cut Silage (335kg N/ha) incorporating 8 mechanical defoliations from February to November with silage harvested in late May (n=30);

Managements were either classified as AG (Managements i and iii) or as SG (Managements ii and iv). Ten cultivars were assessed across the four managements with three replicates per cultivar for each management. The cultivars used were Abermagic, Aberstar, Astonenergy, Dunluce, Magician, Millenium, Navan, Spelga, Twystar and Tyrella. All plots were harvested with an Etisa mower and fertiliser was applied within two days of defoliation. A 1.5m wide strip was cut from the AG plots with the remainder of the plot grazed with dairy cows. All dung pads were removed after each grazing. Within each management the overall grazing season was divided into spring (harvests between February 1<sup>st</sup> and April 10<sup>th</sup>), summer (harvests between April 11<sup>th</sup> and August 6<sup>th</sup>) and autumn (harvests from August 7<sup>th</sup> onwards). All data were analysed using analysis of variance in SAS. Cultivar, management and the effect of silage harvest were included in the model. Interactions were tested for and none were found so interactions were excluded in the final analysis.

**Results** Cultivar and management had a significant effect ( $P < 0.05$ ) on both seasonal DM yield and total DM yield. The SG managements yielded 13.2 t DM/ha (s.d. 1393.3) which was significantly ( $P < 0.001$ ) higher than the AG managements yielding 10.2 t DM/ha (s.d. 1474.6). In the autumn period management had a significant effect ( $P < 0.001$ ) on DM yield, the difference between managements was 167 kg DM/ha and this is not considered to be biologically significant. Silage harvest also had a significant effect ( $P < 0.001$ ) on total DM yield, summer DM yield and autumn DM yield but not on spring DM yield, as there was no difference in harvest strategy during the spring period. Silage harvest had a significant effect ( $P < 0.001$ ) on autumn DM yield, however this difference of 175 kg DM/ha is not considered to be biologically different. Managements that incorporated a silage harvest yielded 12.8 t DM/ha (s.d. 1721.8) which was 18.0% higher than managements without a silage harvest.

**Table 1** Effect of grass cultivar evaluation management and integration of silage harvest on seasonal and total DM production

	AG	SG	Silage Harvest	No Silage Harvest	Significance		SED
					Management	Silage	
Spring DM Yield (kg DM/ha)	1515	1998	1772	1741	$P < 0.001$	N.S.	59.69
Summer DM Yield (kg DM/ha)	5787	8446	8140	6093	$P < 0.001$	$P < 0.001$	150.71
Autumn DM Yield (kg DM/ha)	2884	2717	2888	2713	$P < 0.001$	$P < 0.001$	72.27
Total DM Yield (kg DM/ha)	10185	13161	12800	10546	$P < 0.001$	$P < 0.001$	222.61

**Conclusion** Cultivars evaluated under simulated grazing had higher DM yields than cultivars evaluated under animal grazing. However as no interaction was found between cultivar and management, it indicates that simulated grazing is representative of the relative performance of a cultivar under animal grazing.

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## An evaluation of grass growth models for use in perennial ryegrass swards in Ireland

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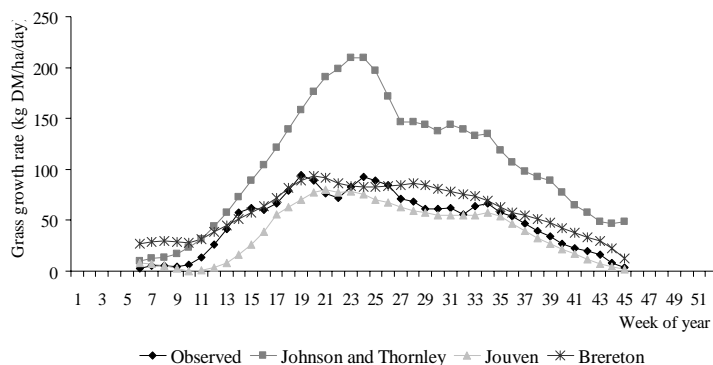
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**Introduction** There are several grass growth models available; varying from simple empirical to multi-component mechanistic. While empirical models have advantages in that they are usually conceptually and operationally simple, their application is restricted to prediction within the range of conditions to which the original data set relates. On the contrary, a mechanistic model is more likely to be effective across a range of conditions, given the correct inputs. Due to climatic factors the growth pattern for grass is highly variable thus making forecasting of dry matter (DM) yield at farm level difficult. The objective of this study is to compare the accuracy of prediction of three grass growth models using climatic and grass growth data from Teagasc Moorepark Dairy Production Research Centre over three years.

**Material and methods** The three models selected for evaluation were developed for perennial ryegrass swards in temperate climates. The three models were (1) an English model developed and described by Johnson and Thornley (1983), this is a mechanistic model based on individual plant processes, incorporating leaf area expansion and senescence where the above-ground dry matter is divided into four compartments described by structural weight and leaf area index; (2) an Irish model described by Brereton *et al* (1996) and modified by R. Schulte (unpublished), this static model provides a means to understanding the dynamics of a grazing management system subject to a variable herbage supply taking into account the vegetative and reproductive phase; and (3) a French model described by Jouven *et al* (2005), this is a mechanistic model designed to respond to various defoliation regimes, perform multiple-year simulations and produce simple outputs that are easy to use as inputs for other models, in which the biomass is subdivided into four compartments taking into account their biomass, age and digestibility. The inputs to all three models are meteorological data collected at Teagasc Moorepark. The modelled data were compared to grass growth data measured at Moorepark over the three years (2005, 2006 and 2007) using the methods described by Corral and Fenlon, 1977. The predicted and measured data were analysed using the mean percentage error (MPE) and the root mean square error (RMSE) annually and seasonally - annual data were split into approximately three equal periods to create seasons.

**Results** Model predictions of grass growth rate for 2005 are shown in Figure 1. Over the three years, the model that best fits the data is the Jouven model (Table 1), although it under predicts for the winter period. The Johnson and Thornley model repeatedly over predicts grass growth from about mid April onwards, particularly in the late spring and summer. The Irish model is over predicting grass growth during the winter period but it closely follows the observed trend during the remainder of the year.



**Figure 1** Observed and predicted Moorepark Grass Growth Rates (kg DM/ha/day) for 2005

**Table 1** Performance of the models

		Johnson	Jouven	Brereton
MPE	Feb-Apr	-113.60	28.22	-200.99
	May-Aug	-177.17	25.08	-17.31
	Sep - Nov	-193.46	38.37	-36.99
	annual	-161.80	30.42	-83.40
RMSE	Feb-Apr	25.06	33.32	19.34
	May-Aug	108.34	17.85	14.52
	Sep - Nov	55.04	15.62	15.22
	annual	72.78	23.49	16.45

**Conclusions** The Jouven model and the Brereton model closely predict grass growth when compared with the observed data. Comparison of a greater number of year's data will give more information on the accuracy of the model and highlight times of the year when some modification of one or all of the models is required to improve their predictive ability for perennial ryegrass swards in Ireland.

**Acknowledgements** This project is funded by a Teagasc Walsh Fellowship.

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## Evaluating the economic performance of grass varieties

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**Introduction** In Ireland 80% of the agricultural area is devoted to pasture, hay and grass silage. Dairy systems target calving to coincide with the onset of grass growth in spring. In the main grazing season animal production is achieved through an almost entirely grass based diet, with excess forage harvested and conserved as silage and used as the main feed source during the winter. Due to the high dependency on grass, varieties are selected on the basis of seasonal DM yields, adequate silage yields and mid-season quality. The economic value (EV) of an individual grass variety across a full production year has not been classified due to difficulties in quantifying the economic change in seasonal yield and quality parameters. Such a development to define the economic difference between grass varieties would guide the industry in selecting grass varieties that would significantly improve farm profitability.

**Material and methods** To derive the economic value, the Moorepark Dairy Systems Model (Shalloo *et al.*, 2004) which provides a comprehensive simulation framework integrating biological, physical and economic processes in a model of a 'typical' dairy farm, was used to simulate herd parameters, nutritional requirements, land use, inputs and outputs. Economic values were derived by simulating a physical change for each trait of interest independently and calculating the effect changing that trait has on the model output (Table 1). Each EV, expressed through the unit of measurement appropriate to each trait, was calculated as:

(net margin per ha) ÷ (unit change in trait of interest). A base milk price of 27 c/l was assumed.

**Table 1** Economic value per unit change in each trait of interest: DM yield, quality, persistency and silage yield

€/ kg change in DM yield			€/ unit change in DMD per kg							€/ %change/ha/yr	€/kg DM change in silage yield	
Spring	Summer	Autumn	Apr	May	Jun	Jul	Aug	Sept.	Persistency		1 <sup>st</sup> cut	2 <sup>nd</sup> Cut
0.27	0.03	0.16	0.01	0.02	0.02	0.02	0.02	0.02	4.96		0.09	0.06

**Results** The EV for spring DM yield is based on the financial benefit of a 1kg increase of grass DM yield in the spring. An increase in grass growth and hence increase in grass available to the cow reduces the requirement for silage or concentrate during this period with no effect on milk production. The EV for autumn DM yield is based on the same principal as that for spring yield. The lower EV for summer DM yield occurs as a result of grass not being limiting during this period, therefore each kg increase in DM yield is less valuable to the system. The EV for quality expressed per kg DM, is based on a 1% change in DMD and is calculated on a monthly basis. The EV for silage is based on a kg increase in silage DM yield above the average of all varieties for both 1<sup>st</sup> and 2<sup>nd</sup> cut. Based on a 10-year reseeding plan any variety which has a shorter lifespan and is therefore less persistent will result in a decrease of €4.96 per % change in persistency per ha per year. The EV of 10 varieties are shown in Table 2, excluding persistency. Persistency is currently reported as ground score which does not provide a realistic assessment on the persistency of a variety.

**Table 2** Economic values applied to a grazing system based on data measured at Moorepark

Variety	€ DM yield			€ % DMD	€ DM yield silage		€/ha per year
	Spring	Summer	Autumn	Quality	1st cut	2nd cut	Total economic value
Bealey	121.3	9.0	16.0	101.3	-28.6	-1.9	217
Dunluce	50.4	5.4	-17.6	111.9	24.2	35.5	210
Tyrella	70.5	8.0	21.9	29.4	10.2	-1.5	139
Greengold	31.3	-5.9	-34.3	77.6	22.6	29.1	120
Navan	-4.0	-3.9	4.8	107.9	-2.5	17.9	120
Malone	38.2	-7.3	-33.2	31.3	31.7	-1.9	59
Aberdart	-10.3	5.6	42.6	-19.5	-1.7	-32.1	-15
Foxtrot	-87.3	4.8	30.0	-59.8	20.8	0.3	-91
Twystar	-87.6	-3.4	11.6	-115.5	-11.7	-17.6	-224
Corbet	-128.7	-10.8	-27.5	-128.9	-34.1	-11.9	-342

\*No persistency data is available and therefore persistency is excluded from the calculations.

**Conclusion** The index will provide clear guidelines to farmers on the total merit of each variety to their system. The objective is to introduce an EV for every variety published on the National Recommended List for Grass Varieties in conjunction with the Dept. of Agriculture, Fisheries and Food, thus providing a tool to ensure the farmer have more confidence in choosing varieties that are suitable to their system as it allows a direct comparison between varieties using a common currency (€). Work to develop an accurate estimation of persistency is ongoing at Moorepark.

**Acknowledgements** The authors wish to acknowledge funding received by the Department of Agriculture, Fisheries and Food

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## Irish grassland: feedstock for a green biorefinery application

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**Introduction** ‘Green Biorefinery’ (GRB) is a novel alternative use of grass biomass. It is an integrated refinery concept using green biomass (pasture) as raw material, splitting it into **press cake** (solid fibre fraction) and **press juice** (liquid fraction). For GRB, there needs to be a thorough understanding of the relationship between the quality (desired fraction content) of the end product and grass biomass and composition (Grass, 2004). The aim of this paper is to assess the effects of botanical composition, nitrogen application and phenological growth stage on the presscake fraction yields (NDF, ADF), for a GRB application.

**Material and methods** Field trials were established on six commercial farms around the country, Wexford (52° N, 6° W), Cork (52° N, 8° W), Offally (53° N, 7° W), Roscommon (53° N, 8° W), Monaghan (53° N, 6° W), and Fermanagh (52° N, 8° W). Sites were cleared off each year in March. At each of the six sites, three annual nitrogen treatments (225, 90, 45 kg ha<sup>-1</sup> yr<sup>-1</sup>) as CAN were applied to plots (2.5 × 2.0 m) in four replications; a blanket dressing of phosphorus (30 kg ha<sup>-1</sup> yr<sup>-1</sup>) and potassium (120 kg ha<sup>-1</sup> yr<sup>-1</sup>) was applied as 0:7:30. A two cut silage system was implemented (May /June, July/Aug.). A strip cut from each plot using an Agria (Haag, Germany) was used to determine plot yield. Grass cores were taken from each plot and were sub sampled for determination of DM concentration (dried at 100 °C overnight). Chemical analysis was conducted on the sub samples dried at 40 °C for 48 h and ground through a 1-mm screen using a hammer mill. Samples were analysed for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest, 1963). The yields of the presscake fractions (NDF, ADF) were determined as the product of the mass fractions and the dry matter yields, summed over the two harvests to give annual fraction yields. Another sub sample of the harvested grass was taken from each plot, sorted into individual grass species, and analysed for relative abundance within the harvested sward. The growth stage was also assessed for the individual species (Moore *et al.*, 1991). Species found included *Lolium perenne* (Lp), *Poa spp.* (Poa), *Agrostis stolonifera* (As), *Holcus lanatus* (Hl), *Trifolium repens* (Tr), *Rumex obtusifolius* (Rumex) and *Ranunculus repens* (Rr).

**Statistical analysis** The diversity-interaction modelling approach (Kirwan *et al.*, 2009) was used to estimate the effects of species interactions on the herbage chemical composition. The fixed effects included botanical composition (the proportions  $P_i$  of the  $i^{\text{th}}$  species), species interactions (specified as  $P_i P_j$  among the  $i^{\text{th}}$  and  $j^{\text{th}}$  species), nitrogen application ( $N$ ), soil and climatic variables ( $C$ ) and growth stage at cutting ( $GS$ ). The form of the fixed effects model for each fraction yield ( $FY$ ) was:

$$FY = \sum_{i=1}^s \beta_i P_i + \sum_{\substack{i,j=1 \\ i < j}}^s \delta_{ij} P_i P_j + \alpha N + \lambda_k C_k + \phi GS$$

Where species within a functional group [grass (Lp, Poa spp., As, Hl) and forbs (Tr, Rumex, Rr)] were found to perform in a similar manner, the species coefficients were combined to give a composite functional group coefficient. A linear mixed model (LMM) was fitted to account for repeated measures. Model fitting was conducted for both the fixed and random effects, and the model of best fit was determined for each chemical component using Akaike’s Information Criterion (AIC). The compound symmetric random structure was the best fit for the random model of the fibre fractions. The final models presented are those that gave the lowest AIC value. All models were fitted using MIXED procedure in SAS (v. 9.1).

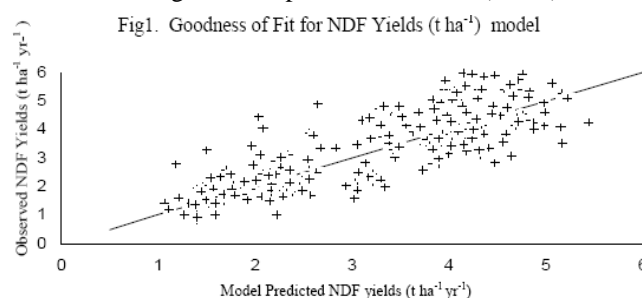
**Results** Figure 1 shows the goodness of fit for the fiber yield (NDF) model. The individual species contributed significantly to presscake yields (NDF/ADF). The grass functional group interaction with N (grass × N) rate was highly significant ( $P < 0.0001$ ) for increasing the fibre yields, indicating that fibre yield from grasses responded more rapidly to N fertilisation than fibre yield from forbs. The grass functional groups interaction with average daily rainfall reduced the fibre yields (NDF:  $P = 0.0121$ ; ADF:  $P = 0.0312$ ). The effect of the As interactions within the sward ( $P = 0.0233$ ) was significant for increased fibre (NDF) yields. The growth stage of Lp at time of harvest also increased the ADF yields ( $P = 0.0325$ ).

**Conclusions** The results of the field trials suggest the interaction effect of *A. stolonifera* with the other species in the sward, the phenological growth stage of *L. perenne* and nitrogen are favourable for producing higher fibre yields. These are important sward management factors for GRBs’ interested in increasing the harvestable fibre in the biomass for applications such as insulation material.

**Acknowledgements** The authors greatly acknowledge the Teagasc Walsh fellowship, Teagasc Johnstown Castle and Teagasc Grange Dunsany.

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## Utilisation of clear-fell forestry residues as a biomass energy source: Brash bale production, storage and comminution costs and analysis of brash bale contents

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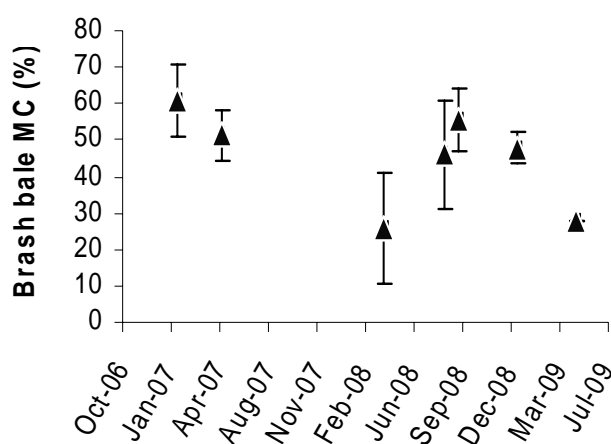
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**Introduction** Clearfell forestry residues (brash) consisting of branches, crowns, reject logs, off-cuts, small diameter stems and trees are estimated in Britain at 0.82 million oven dried tonnes (odt) per year (McKay, 2005). Brash has a large biomass energy potential but in the raw state the high-volume: low-density ratio renders it financially unviable. Machines that gather, compress, bind and produce brash bales may offer a means of recovery at acceptable cost. Utilisation of the energy content requires comminution of brash bales to woodchips of specific size of known moisture content (MC). The aim of this study was to determine: outputs from commercial scale brash bale production and conversion to wood fuel; and inherent properties of brash compared to parent logwood.

**Material and methods** Mature stands of Lodgepole Pine (*Pinus contorta*), and Sitka Spruce (*Picea sitenichis*) in a 10.2 ha forestry site at AFBI, Hillsborough, Northern Ireland were clear-felled during winter 2007. In a randomised block experiment with 4 replicates brash was formed into windrows within the Pine and Spruce areas. A John Deere 1490 D brash baler collected and processed brash into bound brash bales 0.6m diameter x 3.0m long, dropped in-situ then transported to roadside by a Valmet 860 forestry forwarder and row stacked up to 4m high. Randomly selected brash bales from each species were labelled, weighed fresh and stacked apart for long term mass and MC monitoring, carried out each year in 2007, 2008 and 2009. On three separate occasions during this period a Starchl 450HP mobile woodchipper with a



**Figure 1** Changes in brash bale moisture content from February 2007 to May 2009 (mean of species). Vertical bars +/- sem.

MC fluctuated widely during 2008/9 (Fig 1) indicating drying and re-moistening it was below 30% on two of the sampling dates. The brash bales passed through the chipper at an average of 69.2 sec/bale (sd 25.0) giving an output of 6.4 odt/hr. Logwood chipping gave a significantly higher ( $P<0.05$ ) rate at 8.05 odt/h.

Pine and Spruce brash bales, when compared to logwood, had significantly ( $P<0.005$ ) higher levels of P (848mg/kg vs 157 mg/kg DM), K (3928mg/kg vs 1076 mg/kg DM), Ge (20.24 MJ/kg vs 19.95 MJ/kg DM). The chipped brash had a significantly ( $P<0.05$ ) higher proportion by weight of fine particles <3.15mm, 9.7% vs 4.7% than the logwood. The mean cost for brash bale production was £7.98/odt. Comminution of brash bales and logwood to fuel ready chip had mean costs of £15.06 and £13.12/odt respectively.

**Conclusions** Brash baling was found to be an effective system for gathering and storing brash prior to chipping for utilisation as fuel. The bales were capable of drying naturally down to below 30% moisture under suitable weather conditions. However the rate of chipping was slower for brash bales than for logwood. Chipping costs were higher for brash than logwood. Brash had significantly higher nutrient (P and K) and gross energy levels and a higher proportion (by weight) of fine particles (<3.15mm) than logwood.

**Acknowledgement** This work was funded by the Department of Agriculture and Rural Development

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## The relationship between bulk density and energy input, in biomass pellet production

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**Introduction** The search for alternatives to fossil fuels has stimulated interest in lignocellulosic pellets for home heating. Currently all pellets used in Ireland are produced from wood sawdust. Sawdust has many advantages for pellet production, but its supply is limited by the scale of wood processing. If Irish pellet production is to keep pace with demand, other feedstocks will have to be found. The most likely options are wood residues, willow, miscanthus, rape straw and cereal straws. Significant quantities of these materials are readily available with rape and some cereal straws being thought of currently as a waste product. The objective of this research is to determine the pellet production rate for the various raw materials by analysis of material flows through the pellet mill; and to determine the relationships between bulk density, material flows and the energy input in the production process.

**Material and methods** Biomass pellets were produced using a Farm Feed Systems biomass pellet mill (Cinderford, Gloucestershire, England) located at Oak Park Research Centre. The raw materials were firstly tested for moisture content and then chopped using a Teagle Tomahawk straw chopper (Teagle Machinery Ltd., Truro, Cornwall, England)) with a 24 mm sieve. The pelleting process involves movement through a 5 kW hammer mill with 4 mm screen, addition of oil and water and then forcing of the material through a 6 mm die to form cylindrical pellets. The pellet mill has a rated capacity of 150 kg/h for wood pellet production from sawdust.

Each biomass was processed in this way until settings of material throughput and oil and water addition, which produced stable pellets, were found. The mill was run for 30 minutes to allow the system to reach steady state and then run for one hour at these steady state conditions. The raw material throughput was determined by weighing the amount of pellets produced in a measured time to give a kg/hr throughput rate. An Elster A1100 kWhr meter (Elster Metering Ltd., USA) was used to determine the amount of energy required by the pelleting system to create this quantity of pellets and then converted to a kW per kg of pellet produced figure. This was repeated five times for each biomass. Wood pellets were produced for comparison purposes. Raw material and pellets were tested for bulk density using CEN Standard TS 15 103. The GenStat Analysis of Variance Multiple Comparisons tool (Bonferroni Test) was used to test for statistical differences between biomass pellets.

**Results and Discussion** While stable pellets could be consistently produced for miscanthus, willow, wood and rape straw, pellet production from wheat and barley straw proved more difficult and less consistent. It was also noticed that the rated throughput of 150kg/h could not be achieved, as a maximum of 80 kg/h was seen for wood. A summary of the test results is shown in Table 1. As can be seen, the throughput rate for wheat and barley straw was significantly different to that of all the other products. This was due mainly to the very low bulk density of the raw material ( $<20\text{kg/m}^3$ ) after milling as compared to  $180\text{ kg/m}^3$  for miscanthus and  $210\text{ kg/m}^3$  for willow. The low bulk density led to flow difficulties and blockages throughout the pelleting system.

The throughput rate is proportional to the bulk density ( $R^2 = 0.895$ ). The energy input is inversely proportional to the throughput rate ( $R^2 = 0.85$ ). i.e. the more quickly the material can flow through the system, due to a low bulk density, the less energy is used in pellet production. This shows that the bulk density of the biomass has a greater influence on the energy used in pelleting, than the actual type of biomass to be pelleted.

**Table 1** Summary of data on different biomass materials

	Throughput rate (kg/hr)	Energy input (kW/kg)	Bulk Density ( $\text{kg/m}^3$ )
Wood	80	0.2	225
Willow	60	0.26	210
Miscanthus	55	0.28	180
Rape Straw	40	0.31	50
Barley Straw	10	0.4	18
Wheat Straw	15	0.35	20

**Conclusion** These results demonstrate that while it is possible to make pellets from almost any biomass, the energy efficiency in so doing varies greatly from one raw material to the next. The pelleting energy input: output ratio for barley straw is almost twice that of willow or miscanthus. However a full life cycle analysis taking into account crop production, transport, drying etc. is required to make any conclusions on the overall energy efficiency of each biomass.

**Acknowledgements** The authors would like to acknowledge funding received for this research from the Department of Agriculture's Research Stimulus fund

## Seaweed extracts as possible agents in improving the emergence of barley, oats and maize in Northern Ireland

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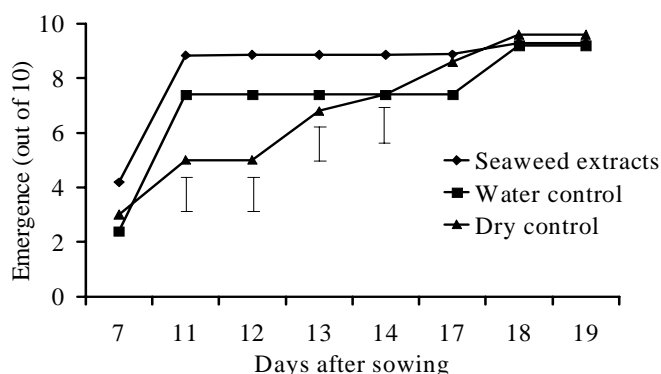
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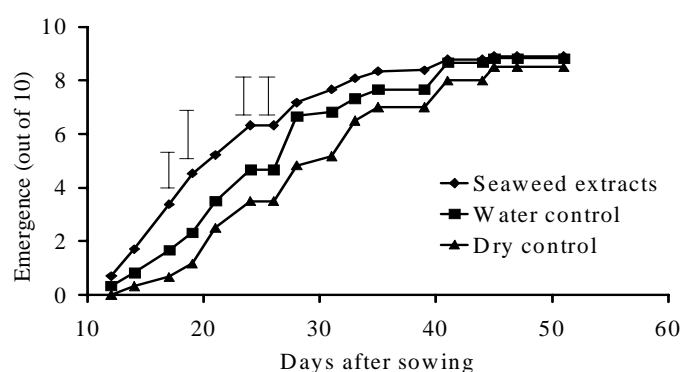
**Introduction** A combination of factors such as rising fertiliser prices, precarious world food supplies, climate change and restrictions on pesticide usage (due to resistance problems and EU regulation) has led to pressures on agricultural scientists to examine more sustainable options. One potential area is that of biostimulants, products which stimulate the plant's own defence systems to allow it to cope better with stress induced by such factors as salinity, drought, pests, diseases and temperature extremes. One of these, temperature, has particular relevance to N. Ireland where low temperatures in the spring affect the growth and emergence of forage maize. The area of the crop has expanded in recent years, but further expansion is constrained by the high cost of either growing under polythene or waiting until the frost risk has passed. Faster crop emergence could also help to suppress weed growth of temperate cereals, such as oats and barley, when grown under organic conditions. This paper reports on initial attempts to use extracts (biostimulants), derived from seaweed, to improve emergence in barley, oats and maize.

**Material and methods** In the first experiment, seeds of barley (cv. Westminster) and oats (cv. Firth) were either soaked for 18 h in water or a range of seaweed extracts (obtained from *Palmaria palmata*, *Delesseria sanguinea*, *Porphyra* sp., *Laminaria* sp. or *Ulva lactuca* by maceration in cold water) or in distilled water or left unsoaked. Ten seeds were then sown out in each of 15 cm diam. pots filled with peat-based compost, placed in a growth cabinet with 12 h light; 12h dark at 15°C and observed for emergence (out of 10). There were five replicates. In the second experiment, maize seeds (cv. Goldcob) were similarly treated, but with a range of commercially available seaweed products (Algifol (Neomed Pharma GmbH), Algaegreen (Oilean Glas Teo, Co. Donegal), Ecolicitor and Nematec (Bioatlantis Ltd., Co. Kerry)). Pots were placed in a growth cabinet at 10°C and again observed for emergence. There were six replicates. All data were analysed by Analysis of Variance using Genstat version 12.1.

**Results** In the first experiment, barley seedlings, grown at 15°C, emerged significantly more quickly following treatment with a range of seaweed products than when treated with either distilled water (water control) or left untreated (dry control) (Fig. 1). Although results for oats were in a similar direction they were not significant. Maize, grown at 10°C, germinated significantly faster when it had been pre-treated with a range of commercial seaweed extracts compared with water and dry controls (Fig. 2).



**Figure 1** Effect of seaweed extracts (meaned over products) on emergence of barley seedlings at 15°C. L.s.d. at 5% for comparison between extracts and controls



**Figure 2** Effect of seaweed extracts (meaned over products) on emergence of maize seedlings at 10°C. L.s.d. at 5% for comparison between extracts and controls

**Conclusions** These results indicate some potential for enhancement of emergence of a range of cereals with extracts obtained from seaweeds, and are similar to those obtained by Farooq *et al.* (2008), who found that priming maize seeds with salicylic acid improved emergence and uniformity both at its optimal temperature for growth (27°C) and at 15°C. However, as Khan *et al.* (2009) have indicated, the biostimulatory potential of many seaweed products has not been exploited due to lack of scientific data on growth factors and their mode of action. Further basic research is therefore needed before this potentially valuable bioresource could be exploited commercially as an emergence promoter.

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## Impact of the economic recession on emissions of greenhouse gases from agriculture in the Republic of Ireland

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**Introduction** Reductions in production, consumption, and transport also result in reduced emissions of greenhouse gases (GHG). However, recessions do not affect all sectors in the same way, and each sector's share of emissions will vary from country to country. Given the agricultural sector's unusually high contribution of 25.6 percent of total national GHG emissions (EPA, 2009), the aim of this analysis was to assess the impact of the economic recession on the projected path of emissions from the agricultural sector.

**Material and methods** The FAPRI-Ireland GHG model is a set of econometric, dynamic, multi-product, partial-equilibrium commodity models as described in Hanrahan (2001). In its current version, the model covers markets for grains, other field crops, livestock and milk and dairy products. The FAPRI-Ireland GHG model projects the output levels for the various enterprises of the agricultural sector. When given these output figures and the relevant emission factors it is possible to apply the GHG accounting methodology set forth in the National Inventory Report (EPA, 2009) to arrive at projected GHG emission levels. We used a baseline – to – scenario comparison approach to tease out the effects of the recession on emissions through shocks to several key prices. These shocks are in fact the first revisions to pre-recession projections from the FAPRI EU Gold model, which the FAPRI-Ireland GHG model accepts as exogenous inputs. The key prices are international commodity prices which largely determine the price Irish producers will receive for their products on international markets, and thus will partially determine Irish output levels. The updates to these prices will imply new emission projections, and with all other variables held at their previous values the resultant distance between the reference and recession scenarios' projections is the impact of the recession on Irish agricultural emissions through 2020.

## Results

Total CO<sub>2</sub> equivalent emissions from the agriculture in Ireland in mega tonnes per annum

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Reference	18.22	18.45	18.48	18.45	18.40	18.34	18.27	18.20	18.12	18.04	17.94	17.84
Recession	18.21	18.36	18.34	18.29	18.21	18.11	18.01	17.91	17.81	17.70	17.59	17.47
Percent difference	0.0%	0.5%	0.8%	0.9%	1.0%	1.2%	1.4%	1.6%	1.7%	1.9%	2.0%	2.0%

Percent difference between Reference and Recession scenarios for selected livestock populations

	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Cattle	0.0%	-0.1%	-0.2%	-0.1%	-0.1%	0.0%	0.1%	0.1%	0.1%	0.1%	0.2%	0.2%
Sheep	0.0%	-1.8%	-3.5%	-4.2%	-5.0%	-5.6%	-6.0%	-6.4%	-6.8%	-7.3%	-7.7%	-8.1%
Pigs	-8.1%	-12.8%	-14.5%	-17.8%	-23.2%	-28.7%	-33.2%	-37.1%	-40.4%	-43.3%	-45.9%	-48.4%

**Conclusions** The analysis shows that impacts on total emissions from the sector will persist but will be small in magnitude. The impact of the recession in 2009 is a 0.01 percent reduction in projected total GHG emissions relative to the reference scenario projections. By 2020, the impact of this recession will be to have reduced the projected level of emissions from the sector by only 2 percent. Whilst large percentage changes occur in the population figures for sheep and pigs throughout the projection period, no such effect is present in the cattle populations. With cattle being the most important livestock category in terms of emissions, the impact of the recession on the total emissions figure for the sector is severely dampened.

**Acknowledgements** The authors gratefully acknowledge funding from the ESRI.

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## The returns to farm afforestation in Ireland: a discounted cash flow analysis

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**Introduction** Irish afforestation rates have been in decline over the past 10 years and currently are more than 60 percent below government targets, despite increases in the value of the forest establishment grants and premium. This decline in afforestation rates has also occurred despite the decoupling of direct payments in Irish agriculture in 2005 and the opportunity for Irish farmers to stack their Single Farm Payment (SFP) entitlements and afforest up to 50 percent of their farm. The aim of this analysis is to calculate the returns to forestry under alternative opportunity costs from the conventional agricultural activities being superseded.

**Material and methods** The Discounted Cash Flow (DCF) approach allows for the comparison of investments with different cash flow profiles such as annual versus multi-period systems and is used to evaluate the afforestation investment decision. The Net Present Value (NPV) values an investment as the sum of the project's net cash flows discounted at the businesses' opportunity cost of capital (Boardman *et al.* 2001). This paper compares the NPV of three alternative afforestation options. The opportunity cost of land, is accounted for through the inclusion of foregone returns from five superseded activities; grazing land rental, lowland sheep, store to finish beef, spring barley and winter wheat. The cost and revenue assumptions for the five superseded enterprises are in Table 1.

**Table 1** Gross margin and working capital released per hectare for the superseded enterprises

	Land Rental	Spring Barley	Winter Wheat	Lowland Sheep	Store to Finished Beef
Gross Margin	€236	€260	€435	€365	€210
Working capital released	-	€291	€487	€50	€928

The returns to forestry are calculated using the Forestry Investment Valuation Estimator (FIVE), a Teagasc research and advisory support tool. In this analysis the FIVE is used to calculate the costs and returns to three typical Irish forest scenarios Sitka spruce (SS), Ash and Mixed (ash, SS and Japanese Larch (JI)). Details of the scenarios are outlined in Table 2. All three afforestation scenarios assume a plantation of 10 hectares, a rotation length of 40 years, productive area of 85%, 'normal' forest thinning and a discount rate of 5%. All agricultural prices and costs were inflated to 2009 levels, while forestry prices and costs are based on a ten year average. The resulting normalised margins for forestry and the superseded enterprises are held constant over the economic life of the project.

**Table 2** Assumptions for alternative forest scenarios

	SS	Ash	Mixed
Tree Mix	SS 80% / JI 20%	Ash 100%	SS 48% / JI 12% / Ash 40%
Yield Class (Productivity)	SS – 22 / JI – 12	Ash – 10	SS – 22 / JI – 12 / Ash – 10
Grant Premium Category (GPC)	GPC 3	GPC 5	GPC 3 (60%)/GPC 5 (40%)

**Results** The results indicate that the NPV in all cases are positive, except for Ash with a superseded enterprise of winter wheat. Despite receiving a higher annual premium, the Ash scenario has a lower NPV than SS regardless of the superseded enterprise. This is likely as a result of the significantly lower volume of timber produced. However over the last few years Ireland has seen an increase in the planting of broadleaves, which may have reflected the higher annual premium payable on broadleaves, as well as changes in the preferences of farm-foresters and increased afforestation on better quality soils. Table 3 below presents the NPV for each of the three afforestation scenarios with the five alternative superseded enterprises.

**Table 3** Investment performance in per hectare terms with alternative assumptions about the superseded activity

	Land Rental NPV (€)	Spring Barley NPV (€)	Winter Wheat NPV (€)	Lowland Sheep NPV (€)	Store to Finished Beef NPV (€)
SS	4,035	3,908	1,194	1,933	5,343
Ash	2,524	2,397	-317	422	3,832
Mixed	3,432	3,304	591	1,330	4,740

**Conclusions** Despite the decline in afforestation rates over the past 10 years, this analysis indicates that the returns to forestry compare favourably with the superseded enterprises examined. The existence of a re-planting requirement after clear-felling means that forestry is in effect a permanent decision and it is unclear as to whether or not farmers will perceive the higher returns from forestry as being sufficient to offset the permanent nature of the afforestation decision.

**Acknowledgements** The authors acknowledge funding from COFORD.

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## Development of a dairy processing sector model for the Irish dairy industry

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**Introduction** Reductions in the Common Agricultural Policy market support, as a result of its continued reform, will expose the Irish and EU dairy industry to increased downward price pressure and increased price fluctuation in the coming years. As a result there will be an increased requirement to reduce costs while at the same time focusing on product portfolio changes to increase revenues in Ireland, which exports 85% of its dairy products and so will be particularly exposed to the fluctuations of the world market in the future. The objective of this paper is to describe a milk processing sector model that simulates the production of dairy products in Ireland. The model was demonstrated by analysing the effect of cow breed and product portfolio change on overall return or net milk value and the individual milk component values.

**Material and methods** A processing sector model was developed which simulates the manufacture of dairy products. This is a mass balance model accounting for all inputs, outputs and losses. Model inputs including volume of milk intake, its composition, product portfolio and its composition are recorded and utilised in the dairy product simulations. The products produced in the model include cheese, butter, whole milk powder (WMP), skim milk powder (SMP), fluid milk and casein. Within each of the simulations milk is separated to cream and skim milk based on the final product requirements. The skim milk and cream are mixed together in order to develop the required final product specifications, excess cream or skim milk from the process are used in other product manufacture. The quantities of products and by-products that can be produced from the milk pool are calculated and multiplied by the product market values to estimate the market value of the portfolio. In this analysis the assumed values per tonne of product sold are: cheese €4,124, WMP €2,939, SMP €2,406, cream €2,150 and whey €570 (FAPRI). Milk collection, assembly, processing, distribution and marketing costs are applied to estimate all production related costs. The component values of protein (P) and fat (F) are calculated and the net value or return from raw milk is estimated. The net value and component values of milk vary as the composition of milk intake varies. To demonstrate this effect the component values of milk from three cow breeds are compared, each with varying F and P compositions: average Holstein Friesian (HF) (38.3 g/kg F, 33.4 g/kg P; CSO), Jersey (53.3 g/kg F, 40.6 g/kg P; Prendiville *et al.*) and New Zealand (NZ) (43.9 g/kg F, 36.5 g/kg P; McCarthy *et al.* 2006). Two scenarios were analysed to determine the effect portfolio has on overall returns. Scenario 1: 60% cheese, 30% WMP, 10% SMP; Scenario 2: 10% cheese, 30% WMP, 60% SMP. The marginal rate of technical substitution (Coggins and Hammond 1994) was used to calculate the value per kg of F and P whereby for each additional kg of F or P the overall milk revenue will increase depending on the product portfolio, product values and processing costs.

**Results** In scenario 1 1,000 L of HF, Jersey and NZ milk results in 63.3 kg, 76.0 kg and 68.9 kg cheese, 34.8 kg, 38.3 kg and 35.8 kg WMP and 8.9 kg, 8.9 kg and 8.8 kg SMP being produced respectively. In scenario 1, Jersey milk returns the highest net milk value at €426 while HF milk has the lowest value at €338, reflecting the relative low fat and protein content of HF. In scenario 2 HF, Jersey and NZ milk results in 10.5 kg, 12.7 kg and 11.5 kg cheese, 34.8 kg, 38.3 kg and 35.8 kg WMP and 53.1 kg, 53.3 kg and 52.5 kg SMP being produced respectively. In scenario 2, Jersey milk again returns the highest net milk value at €352 while HF milk has the lowest value at €286. Protein is valued higher than fat in both scenarios with the relative value remaining the same across breed and scenario.

**Table 1** Estimated net value of milk and component milk values for Scenarios 1 and 2

	Scenario 1			Scenario 2		
Cow breed	Net Value (€)	F (€/kg)	P (€/kg)	Net Value (€)	F (€/kg)	P (€/kg)
HF	338.46	4.76	7.23	285.99	3.99	6.07
Jersey	426.39	4.44	6.75	352.27	3.67	5.56
NZ	373.08	4.61	7.00	309.07	3.80	5.70

**Conclusions** The sustainability of the Irish dairy industry has been challenged with the low milk price seen in 2009 coupled with high feed, fertilizer and overhead costs. Change within the industry is essential as has been highlighted by the Prospectus reports <sup>6</sup>. The development and use of a processing sector model, would be a powerful decision support tool for the decision making process within the dairy industry.

**Acknowledgments** This research is funded by a Department of Agriculture, Fisheries and Food Research Stimulus grant.

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## A preliminary analysis of completion of a statutory farm safety code of practice document by farmers in Ireland

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**Introduction** The Safety, Health and Welfare at Work Act 2005 in Ireland permits farmers with three or less employees to complete a risk assessment (RA) document as an alternative to preparing a written safety statement, required under the previous 1989 Act. A statutory code of practice (COP) incorporating a RA document, an explanatory document and a farm health and safety DVD was prepared by a statutory advisory committee to the Health and Safety Authority and was then sent to all farms, nationally. The research aims of this paper are to (1) provide national estimates of the stated level of completion of the RA document and satisfaction levels with the COP documents along with levels of viewing of the DVD, and (2) to identify explanatory variables associated with statistically significant probabilities for completion of the RA document, satisfaction with the COP documents and DVD viewing.

**Material and methods** An additional survey was conducted among participants in the National Farm Survey (NFS) during the second half of year 2007. The questions posed were whether or not the RA document had been completed, the opinion of the farmer on the usefulness of the COP documents and whether the accompanying DVD was viewed by the farmer, or another household member(s). The population surveyed was 1,040 and this sample was weighted to represent a national population of 111,913 farms of at least 2 Economic Size Units (ESU) in accordance with NFS protocols (Connolly *et al.*, 2008). Logistic regression analysis using SAS (2004) was used to model the NFS data available for a reduced sample of 991 related to use of the COP components, allowing for up to three-way interactions, with the following potential explanatory variables: farm size; farm system; region; economic size; man days used; age of farmer; use of hired labour; number in household; educational attainment of the farmer; rural environmental protection scheme (REPS) participation, Teagasc client membership and whether the farmer and/or spouse had off-farm employment.

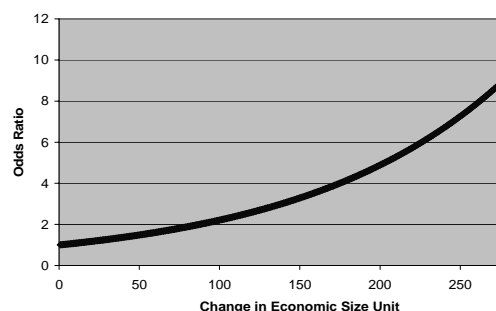
**Results** The nationally weighted results indicated that 41.5% of farmers reported completing the RA document and of the 54.2% who gave an opinion on utility of the COP documents, 74% considered these 'excellent' or 'good'. The DVD was viewed by 24.8% of the weighted farmer population and by one or more household members for 18.6% of farms. The model fitting process selected region ( $p=0.017$ ), Teagasc client ( $p=0.001$ ) and economic size unit ( $p=0.009$ ) as the main variables associated with completion of the RA document. Region was considered as a blocking factor and interpretation focussed on the other factors and the odds ratios for these are shown in Table 1. The relationship between odds ratio and change in ESU for RA document completion is shown in Figure 1.

**Table 1** Odds Ratios for RA Document Completion

Effect	Odds Ratio	Confidence Limits (95%)	
Teagasc Client (yes vs. no)	1.592	1.205	2.109
Economic Size	1.008	1.003	1.013

**Table 2** Odds Ratios for Viewing of DVD

Effect	Odds Ratio	Confidence Limits (95%)	
Age of Farmer	0.98	0.967	0.993
REPS (yes vs. no)	1.453	1.074	1.961
Household Number	1.111	1.018	1.212



**Figure 1** Odds Ratios and change in ESU for RA document completion.

Usefulness of the COP documents was modelled using a multinomial logit model which found that region, man days and their interaction ( $p=0.031$ ) were significant. For viewing the DVD, decreasing farmer age ( $p=0.003$ ), participation in REPS ( $p=0.015$ ) and household number ( $p=0.018$ ), were significantly associated and Table 2 shows the odds ratios for these responses. A quadratic effect occurred for household number ( $p=0.002$ ) where the odds ratio increased up to 5 household members and then decreased.

**Conclusions** The weighted survey results give national estimates of farmer use of the farm safety RA document, farmer perception of COP documents and viewing levels of the DVD. The exploratory statistical analysis of the sample population indicates that completion of the RA document was predominantly influenced by whether farmers were Teagasc clients and economic size and viewing the DVD by farmer age, REPS participation and household number. Overall the data obtained suggests that engagement with the COP materials is associated with farmer contact with outside influences (e.g. Teagasc, REPS).

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## Effects of dietary chromium-methionine supplementation on blood metabolites and insulin resistance index in fructose-fed diabetic rats model

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**Introduction** Insulin resistance (IR) is a problem in periparturient dairy cows (Hayirli, 2000). The transition period is a critical phase for dairy cows and can cause negative energy balance and physiological stresses that develops in to IR. Chromium has the potential for lowering plasma free fatty acids and cholesterol concentrations that potentiates predominantly the IR by causing secretion of inflammatory cytokines from insulin dependent adipose tissues. The role of chromium is probably associated with increasing the insulin internalization and amplifying insulin signalling through activation of cellular insulin receptors. The exact evaluation of chromium in ruminants, necessitates the use of modelling for simulation IR in laboratory animals (Jalal, 2007). The aim of this experiment was to evaluate the effects of organic chromium on blood metabolites and IR index in fructose-fed insulin resistant (diabetic) rats.

**Material and methods** Twenty six Wistar male rats (mean BW of  $225 \pm 25$ g), were provided by the Iranian Pastor Institute and housed individually in standard cages, in an air conditioned ( $22 \pm 2^\circ\text{C}$ ) room with a 12h light and dark cycle. All rats were nourished with 15g standard rat chow. After 1 week adaptation, 10 rats were used as healthy control group and 16 rats received fructose (10% weight/volume) in drinking water for 5 weeks. The insulin resistant (fructose-fed) rats were divided into two groups. Eight rats were fed 50 ppm chromium-methionine (Cr-met) supplement in the diet and the others remained in the previous feeding state for 6 weeks. Animals were blood sampled prior to chromium administration in order to test for IR inducing as well as after the end of the experiment for determination of blood serum parameters including fasting glucose, triglyceride, cholesterol and insulin contents. Centrifuged and extracted serum samples were stored at  $-20^\circ\text{C}$  and transported to Mashhad Medical University labs for analysis. IR index was calculated by HomA-IR (Homeostatic model assessment of IR) software (Oxford University). Data were analysed using general linear model of SAS (2000) as completely randomized design with analysis of covariance.

**Results** The effect of fructose on blood parameters are shown in Table 1. In the first period of the study, IR was induced significantly ( $P < 0.05$ ) in rats receiving fructose. Serum fasting glucose, insulin, IR index (HomA-IR) and triglyceride contents, were significantly increased ( $P < 0.05$ ) in the fructose-fed group in comparison with the control rats. These changes could be the result of the fructose metabolite effects on insulin signaling in adipose tissues. Gene expression of insulin signaling mediators under the indirect effect of increased triglyceride of blood serum is another possibility. According to the results obtained in the second period of this study, IR index significantly ( $P < 0.05$ ) decreased after Cr-met supplementation (Table2). The fasting insulin, glucose and triglycerides concentration in Cr-met treated group was lower ( $P < 0.05$ ) than the fructose-fed insulin resistant group.

**Table 1** Blood serum content of the rats in first period of experiment

Variables	unit	Treatment	
		Control (n=10)	Fructose-fed (n=16)
Glucose	mmol/l	$10.02 \pm 0.12$	$10.48 \pm 0.90^*$
Insulin	mU/l	$3.89 \pm 0.05$	$5.09 \pm 0.04^*$
HomA-IR	-	$1.81 \pm 0.03$	$2.27 \pm 0.03^{**}$
Triglyceride	mmol/l	$1.459 \pm 0.011$	$1.711 \pm 0.003^*$

\*  $p < 0.05$  \*\*  $p < 0.01$

**Table 2** Comparison of blood serum parameters after Cr-met administration

Variables	unit	Treatment		
		Control (n=10)	Fructose-fed (n=8)	Cr-met + fructose-fed (n=8)
Glucose	mmol/l	$10.46 \pm 0.16^a$	$11.56 \pm 0.16^b$	$10.91 \pm 0.16^a$
Insulin	mU/l	$4.22 \pm 0.35^a$	$6.10 \pm 0.27^b$	$5.32 \pm 0.21^a$
HomA-IR	-	$1.68 \pm 0.04^a$	$3.38 \pm 0.11^b$	$2.78 \pm 0.09^c$
Triglyceride	mmol/l	$1.74 \pm 0.11^a$	$2.42 \pm 0.09^b$	$2.17 \pm 0.07^c$

The significant differences between treatments are shown with small alphabetic characters ( $p < 0.05$ ).

triglyceride in insulin resistant rats. It is possible that the mode of action of chromium supplementation follows the similar model in ruminant animals, although more studies are required.

**Acknowledgements** The authors are grateful to the WHO Agency in Iran and the excellent center of animal science of Ferdowsi University of Mashhad for their supports.

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## Microbiological identification in *Kundi*, an Intermediate Moisture Meat (IMM) product

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**Introduction** Meat and meat products are high in nutritive value and because of this they can only remain fresh for a short time before spoilage sets in, but simple preservative techniques can reduce spoilage. One such simple technique is Intermediate Moisture Meat processing (IMM). Obanu (1981), observed that IMM are shelf stable under the tropical climate without refrigeration and may be eaten directly with or without rehydration. Ogunsola and Omojola (2008) stated that IMM is used to describe meat products that have less than 30% of moisture. *Kundi* is one of such IMM products that is easy to prepare. It is therefore the objective of this study to identify the microbial loads in *Kundi* products.

**Material and methods** Beef used for this study was obtained from the slaughter slab of the department of Animal Science, University of Ibadan, Oyo state, Nigeria. Semimembranous muscle from the hindquarter, weighing 2kg from 2-3 years old White Fulani animals was used. Meat was trimmed of all external fats, nerves, blood vessels, excess epimysial connective tissues and deboned and wash with water. The chunks were held overnight for 24 hours at 4 °C, cut into sizeable small portion of 70 – 90grams of 6cm - 8cm wide. *Kundi* process involves two methods of preservations; boiling and drying. Meat samples were boiled in a pressure cooker for 30 minutes at 100°C and then oven dried at 170 °C for 3 hours. Microbial status was determined by isolating and identifying and characterizing the organisms according to the method described by Norris and Ribbon (1971). The organisms were identified using their colour and the shape of their colonies. The identification was carried out monthly during six month of storage. Samples were stored at room temperature on the shelf according to Sonaiya (1997) in the departmental laboratory, they were not packaged and samples gave 32.09 % as the final moisture content.

**Results** Fungi and mold were identified; they include *Aspergillus flavus*, *Aspergillus niger*, *Penicillium spp*, *Rhizopus spp*, *Mucor spp* and *Fusarium spp*. Most of the Fungi isolated were xerophilic, which are organisms that are capable of growth at low water activity ( $a_w$ ) of less than 0.83  $a_w$  are well adapted to dry arid partially dry food (Pitt, 1975). Alonge (1984) screened some Fungi isolated from *Kundi* products for aflatoxin. No toxin was detected because all the organisms identified grow at a very low water activity e.g *Aspergillus flavus* grows at  $a_w$  of 0.75. Leister *et al* (1981) reported that aflatoxin cannot be found in meat products with water activity  $a_w$  below 0.83. The dried meat in this study and that study by Alonge (1984) had  $a_w$  values below 0.83.

**Table 1** Xerophilic Fungi isolated from *Kundi* incubated at 73°C

Microbes	Descriptions
<i>Aspergillus flavus</i>	Large bright – green colonies with yellowish centres, Sterigmata, Uniserate
<i>Aspergillus niger</i>	Black radiating colonies with large conidia heads was seen
<i>Penicillium spp</i>	Had distinct blue – green white at first then coloured after conidial matures
<i>Rhizopus spp</i>	The fungus quickly filling the culture plate with a dense colony aerial mycelium at first white and later becomes grey
<i>Mucor spp</i>	Fast growing fungus filling a Petri plate with colony aerial mycelium at first white and later becoming dark grey brown or yellow
<i>Fusarium spp</i>	This fungus was at first white in colony or woody then it frequently becomes pale in the hyphae or in the substrate

**Conclusion** Results shows that at 6 months of storage, microbiological organisms identified were fungi and molds, and since *Kundi* is a ready to cook meat product, and that the organisms identified are not toxigenic but xerophilic, thus *Kundi* is a good Intermediate Moisture Meat.

**Acknowledgements** Author gratefully acknowledges funding from P.G. School University of Ibadan. Nigeria

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## Comparison of the nutritional and digestion characteristics of two forms of preserved lucerne forage fed to mature horses

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**Introduction** Lucerne *Medicago sativa* (also known as alfalfa) is fed to horses in many forms, ranging from ground meal, pelleted, hay, or dry chaff to fermented or ensiled product. Currently there is little data concerning the preservation processing of lucerne impacts its energy availability and digestibility characteristics in horses (NRC, 2007). Lucerne chaff contains high levels of nutrients such as protein and calcium compared to grass hay (Cuddeford, 1994), can improve electrolyte imbalances and hoof problems, and be a good feed for older horses. Horses have also been shown to retain lucerne for longer than oat straw in their gut (Cuddeford *et al.* 1995), and lucerne hay has higher dry matter and protein digestibility, and enhanced mineral absorption compared to grasses (Crozier *et al.*, 1997). Lucerne may also be useful in the prevention of gastric ulceration in horses (Andrews *et al.*, 2005). Legume forages generally contain more soluble carbohydrates than grass forages (Fonnesbeck, 1968), making lucerne hay more digestible than grass hay, however the impact of preservation method has not been investigated. The current trial was conducted to determine whether controlled fermented Lucerne had better energy availability for horses compared to a conventional dried lucerne chaff. Ascertaining nutritional differences caused by preservation methods will allow correct inclusion of lucerne in equine rations.

**Material and methods** Twelve non-racing thoroughbred horses were kept in 3.6 x 4 m pens, bedded on wood peelings. Following a seven day adaptation period, using a dry lucerne chaff and a complete feed (in reducing amounts per day), each horse received either controlled fermented (CF) lucerne (Fiber Pro, Fiber Fresh Feeds Ltd, Reporoa, New Zealand) or a lucerne chaff (LC) control diet. The diet was fed in two daily feeds for two seven day periods in a cross-over design, giving 12 replicates per diet. The amounts fed were based on an iso-energetic daily intake according to standard DE and DM levels (NRC, 1989). Horses were monitored throughout the trial for body weight (by weigh tape) and condition score. Any feed refused was weighed and recorded on a daily basis. Faecal score (1-5 scale, where 1=diarrhoea and 5=hard pellets), body condition score (1-5, where 1=emaciated and 5=obese) and faecal samples were taken at the end of each seven-day period. Samples of both feed and faeces were analysed for dry matter, gross energy (by bomb calorimetry), total ash and acid insoluble ash. Total digestibility was calculated as per the methodology of Bergero *et al.* (2005). Data was analysed by the GLM procedure of Unistat 5.5 (Unistat UK Ltd.), with the cross-over designated as a time replicate.

**Results** Horses fed CF lucerne had significantly higher dry matter intakes ( $p<0.001$ ), due to less feed refusal. There were no significant differences between faecal output on a dry matter basis, and faecal scores for both forage diets were consistently good (4 or higher). Faecal ash was 37% higher ( $p<0.001$ ) for the CF lucerne, even though the ash levels in the original forages, on a DM basis, were similar (10.5% for the chaff and 10.8% for the CF lucerne). The gross energy of the diets was 18.2 MJ/kg for the chaff and 18.9 MJ/kg for the CF lucerne. However, when horses were fed the CF lucerne they consumed over 20% more energy ( $P<0.001$ ) than when fed lucerne chaff. The total amount of energy excreted did not vary significantly between the diets, even though there was 17% more gross energy per kg faecal material ( $P=0.003$ ) for the horses fed the dry lucerne chaff. The CF lucerne product resulted in 32% more retained energy per day ( $P=0.0007$ ) compared to the dry lucerne chaff. Digestible energy (DE) for the CF lucerne was 22% higher ( $P=0.021$ ) compared to the dry chaff form.

**Table 1** Consumption and excretion characteristics of CF lucerne or dry lucerne chaff fed to non-racing thoroughbred horses

Parameter	CF lucerne	Lucerne Chaff	SEM
Dry matter intake (kg/d)	7.88 <sup>a</sup>	6.53 <sup>b</sup>	0.300
Faecal ash (%)	15.2 <sup>a</sup>	9.6 <sup>b</sup>	0.394
Gross energy faeces (MJ/kg)	19.88 <sup>b</sup>	20.21 <sup>a</sup>	0.096
GE consumed (MJ/d)	148.90 <sup>a</sup>	118.64 <sup>b</sup>	5.511
Retained energy (MJ/d)	87.64 <sup>a</sup>	60.00 <sup>b</sup>	6.968
Digestible energy (MJ/kg feed)	11.08 <sup>a</sup>	9.03 <sup>b</sup>	0.457

Figures in rows containing different letters are significantly different ( $P<0.05$ ).

**Conclusions** The trial demonstrated that CF lucerne is higher in DE compared to standard values given for conventional dry lucerne chaff. When total feed digestibility was calculated, there were no significant differences between the forms of lucerne. This indicated a potential high level of variance in the digestibility of the other nutrients present in the forages, which requires further elucidation.

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## Foot health in sheep – prevalence of hoof lesions in UK and Irish sheep

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**Introduction** Lameness in sheep is usually attributed to the infectious condition of footrot, the etiology, prevalence and genetic background of which has previously been documented. Far less is known about other (non-footrot) hoof lesions that cause lameness in sheep, in particular the extent to which they are prevalent in our sheep populations and the relative degree to which they cause pain and lameness. There is a dearth of surveillance data in this area and so the purpose of this paper is to present prevalence data of such hoof lesions recorded in different breeds and crosses in the UK and Ireland, in relation to prevalence of footrot, and to report recent data on lameness prevalence in the Irish Republic.

**Material and methods** As part of a wider study investigating the genetic basis to footrot (Nieuwhof *et al.*, 2008), between 2005 and 2008, records of hoof lesions were collected on 4,360 Blackface, 5,940 Texel, and 962 Welsh Mountain sheep from 27 farms across the UK. None of the farms had more than one breed running together as one flock. Apart from footrot lesion scores (reported by Conington *et al.*, 2008) on a 5 point scale, each hoof was scored for the presence or absence of white line degeneration (shelly hoof), interdigital fibroma, white line abscess, Contagious Ovine Digital Dermatitis (CODD), Granuloma and Pedal joint sepsis. Whether or not the hoof was abnormally-shaped (Mis-shapen) and overgrown was also noted. In N. Ireland, with the exception of interdigital fibromas, the same data were collected on two occasions in 2009, approximately 6 weeks before and after lambing, respectively, of on 6 hill and 6 lowland farms across N. Ireland (approximately 150 ewes per farm). The genotypes used for the hill farms were purebred Blackface, Swaledale X, Cheviot X, Lley X and Texel X Blackface and for the lowland farms they were Texel X, Belclare X, Charolais X, Cheviot X, Lley X, Romney X, Suffolk X. Prevalence (presence in any hoof) of each condition was expressed as a % of animals affected at each scoring occasion. In the Irish Republic, a total of 1353 records on lameness (0/1) representing 694 Belclare, 148 Cambridge, 249 Suffolk and 262 Texel ewes managed on the same farm were used for this study. All cases of lameness were examined and cases of footrot recorded. Ewes were classified on an annual basis as having had footrot or not.

**Results** The percent prevalence of each recorded lesion is shown in Table 1 according to genotype and source of data. It also shows the percentage of records with mis-shapen and overgrown hooves. There were significant breed differences in the percent prevalence (and confidence interval, CI) of lameness in the Irish Republic sheep, shown in Table 2 ( $p=0.02$ ). With the exception of the data on Texel sheep, the highest prevalence of all lesions in the UK and NI data sets was shelly hoof. This was mostly consistent across breeds and higher than footrot lesion prevalence. Very low prevalence levels were recorded for White line abscess, CODD, Granuloma and Pedal Joint Sepsis for all sheep. Large between-farm differences was recorded.

**Table 1** Prevalence of hoof lesions after inspection if sheep had  $\geq 1$  hoof affected (%)

	Texel	B/face	Welsh Mt.	Hill breeds (NI)	Lowland breeds (NI)
Number	5,940	4,360	962	1592	1800
Footrot <sup>1</sup>	23.3	17.3	15.5	16.6	13.1
Shelly hoof	19.5	47.4	53.0	56.7	40.0
Interdigital fibroma	10.2	7.1	12.0	-	-
White line abscess	0.4	0.4	0	0	0.2
CODD	0.1	0.1	0	0	0.4
Granuloma	0.25	0.9	0	1.3	1.7
Pedal joint sepsis	0	0.7	0	0	0
Mis-shapen	27	16.2	7.1	11.7	10.4
Overgrown	24.5	19.3	22.6	-	-

<sup>1</sup>Hoof lesions on 0-4 point scale defined as Conington *et al.*, (2008)

**Table 2** Lameness in Irish sheep

	Mean	CI
Suffolk	10.2	7.0-14.7
Texel	13.1	9.4-18.5
Belclare	7.1	5.2-9.5

**Discussion** The prevalence data give some indication of the extent to which sheep are subject to abnormal hoof lesions. Shelly hoof is the most significant problem for the majority of the flocks recorded, although the extent to which it causes lameness is not known.

After footrot, interdigital fibromas, was reported to have the next highest prevalence although again, the extent to which they cause lameness is unknown and hence further studies on this are required.

**Acknowledgements** Financial support for the studies reported here was received from The Scottish Government, Defra, HCC, Eblex, Genesis Faraday, DARD, AgriSearch. The authors are grateful to staff at SAC, and AFBI for data collection and to participating sheep farmers.

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## Breed difference and heterosis estimates for milk production and udder health among Holstein, Friesian and Norwegian Red dairy cattle

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**Introduction** Crossbreeding provides a means to increase the health and efficiency of animals, by introducing favourable genes from other breeds, by removing inbreeding depression, and by maintaining the gene interactions that cause hybrid vigour (VanRaden and Sanders, 2003). The Norwegian Red (NR) is a potentially useful breed for crossbreeding under Irish conditions (Walsh *et al.*, 2008). A large farm participatory study was established to determine the benefit of crossbreeding with the NR and to generate data to enhance breeding value estimation for the NR breed and crossbreds in Ireland.

**Material and methods** Data were available from the farm participatory study described by Begley *et al.* (2009). The design was a contemporary comparison; both parent breeds and crosses between them were present on each farm. During the spring of 2004, NR semen from 10 proven AI sires was distributed to generate crossbred females. In June 2004, 393 purebred NR heifer calves were imported to Ireland. All heifers calved for the first time during the spring of 2006. To augment this dataset, herds containing both HF and NR genetics were also identified from the national database. A number of data edits and restrictions were carried out and were comparable to those used in the national genetic evaluations. All herds were milk recorded and thus 305 d milk yield and Somatic cell count data were available from the ICBF. Somatic cell count data were transformed to SCS for normalization of the residuals. A herd-year-season (HYS) variable was created as the combination of herd and date of calving. Each HYS contained records from at least one cow containing a minimum of 50% NR genetics. In Ireland, the Holstein (HO) and Friesian (FR) are considered to be distinct breeds for the purpose of genetic evaluations. The final dataset contained a total of 5,874 cows (8,614 lactations), including 2207 Holstein, 449 NR, 33 FR, 2479 HO×FR and 660 NR×HO, 46 NR×FR. A total of 3,747, 1009 and 790, HO, FR and NR breed equivalents were represented in the dataset. The figures in brackets relate to the breed fraction equivalents. Pedigree data were collated and breed and heterosis effects were estimated by regressing the breed fractions (NR, HO and FR) and proportion of heterozygosity, respectively, on the phenotypic data using the statistical package DMU (Madsen and Jensen, 2008).

$Y = \text{HYS} + \text{age}(\text{parity}) + \beta_1\text{NR} + \beta_2\text{FR} + \beta_3\text{HO}\times\text{FRh} + \beta_4\text{NR}\times\text{HOH} + \beta_5\text{NR}\times\text{FRh} + \text{permanent environmental effect} + \text{animal effect} + e$

Parity and HYS were considered as fixed effects, while age at calving was nested within parity. The other effects in the model were included as random effects.

**Results** The HO the NR had numerically lower 305 d yields of milk (-151 kg), fat (-8.61 kg) and protein (-3.84 kg). However, these were not significantly different. The FR was observed to have lower milk yield (-284 kg;  $P < 0.05$ ), and lower protein yield (-8.13 kg;  $P < 0.05$ ) compared to the HO. Statistically significant heterosis estimates, were observed for 305 d milk yield for the NR×HO, (+120 kg), fat yield (+5.88 kg) and protein yield (+5.09 kg). A genetic superiority for udder health, as indicated by SCS (-0.15), was observed for the Norwegian Red compared to the Holstein. Heterosis for SCS (-0.003), was not significant.

**Table 1** Breed and heterosis estimates for milk, fat, protein yield and SCS based for FR, NR, HO×FR, NR×FR and NR×HO compared to the HO

	Milk	SE <sup>1</sup>	P-value <sup>2</sup>	Fat (kg)	SE	P-value	Protein (kg)	SE	P-value <sup>1</sup>	SCS	SE	P-value
Average	5795			232			202			1.98		
FR	-284	131.45	<0.05	-1.25	4.965	0.80	-8.13	4.077	<0.05	-0.07	0.048	0.14
NR	-151	92.98	0.10	-8.61	3.388	<0.01	-3.84	2.795	0.17	-0.10	0.029	<0.001
HO×FR	+124	79.61	0.12	+1.02	3.059	0.74	+3.52	2.507	0.16	+0.02	0.311	0.95
NR×FR	+176	170.06	0.30	+3.59	6.495	0.58	+6.13	5.338	0.25	+0.06	0.064	0.35
NR×HO	+120	53.13	<0.05	+5.88	2.001	<0.01	+5.09	1.646	<0.01	-0.003	0.0191	0.87

**Conclusions** This study provided an insight into the relative production potential and udder health characteristics of HO, FR, NR, HO×FR, NR×FR and NR×HO dairy cows under Irish production circumstances. Heterosis estimates observed in the current study demonstrate that the NR×HO cows are capable of producing milk yields similar to that of HO cows under grazing conditions. HO×FR cows are expected on average to milk a little less. The variation in heterosis levels observed between the breeds highlight the potential requirement for specific heterosis coefficients to be included in routine genetic evaluations in Ireland.

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## Effect of prestarter concentrate feeding system on calf performance

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**Introduction** The decision on when to wean calves is often based on the level of solid food intake (Morrison *et al.*, 2009). If pre-starter concentrates encourage early dry food intake, as demonstrated with pigs, earlier weaning may be possible. Therefore the objective of this study was to examine the impact of a pre-starter feeding system, designed to encourage dry feed intake and reduce the age at weaning, on calf health and performance.

**Material and methods** Thirty-nine calves (Holstein-Friesian and Holstein-Friesian crosses) were assigned to one of three rearing systems based on gender, genotype and birth weight. The three treatments were: (1) milk offered twice daily until 6 weeks of age with calves weaned over a 5-day period (TAD); (2) milk offered twice daily until day 14 then once daily with abrupt weaning at 5 weeks (OAD) and (3) as for OAD treatment with addition of prestarter (PRE). Calves commenced the study at 5 days of age and were fed individually using teated buckets. Starter concentrate was available at all times for calves on the TAD and OAD treatments and from day 15 onward for calves on the PRE treatment. Prestarter concentrate was available at all times with PRE calves with all calves having access to drinking water. The prestarter contained whey, toasted soya seed, palm oil, wheat flour, oat flakes, yeast and vegetable extracts. A skim-based milk replacer containing 220 g crude protein and 180 g fat/kg DM was mixed at a rate of 140 g/l and offered at 4 l/day until weaning commenced for calves on the TAD treatment. Calves on the OAD and PRE treatments were offered 4, 4.5, and 3 l/d of milk replacer from days 5-7, 8-14 and 15-35 respectively and post-weaning all calves were offered *ad libitum* grass silage and concentrate. Calf performance and feed intakes were recorded. Intakes were analysed by ANOVA with fixed effects for sex, genotype, birth weight and feeding system. Weekly live weights and body size data were analyzed by repeated measures analysis using the Genstat REML procedure. This fitted a model with week as the time factor and fixed effects for sex, birth weight and feeding system and a week by fixed term interaction.

**Results** Milk replacer intake for each treatment is presented in Table 1. Rearing system had no effect on starter intake or total intake until day 42, although calves offered the TAD tended ( $P < 0.10$ ) to have an increased total intake compared with OAD calves. Rearing system had no effect on calf live weight (Figure 1), however calves offered the PRE and TAD systems had a greater body size and condition score throughout the study compared with OAD calves.

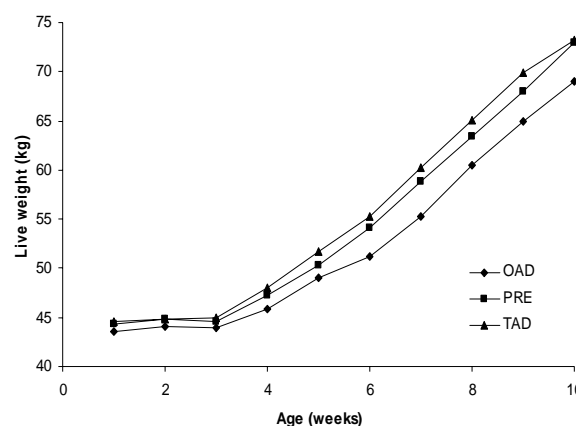
**Table 1** Effect of rearing system on total intake of dietary components and calf growth and development

	Feeding system			SED	Sig.
	TAD	OAD	PRE		
Intake until day 42 (kg DM)					
Milk replacer	18.63 <sup>b</sup>	14.35 <sup>a</sup>	14.38 <sup>a</sup>	0.09	***
Starter concentrate	12.77	13.71	14.84	1.59	NS
Prestarter concentrate	-	-	0.77	-	
Silage intake <sup>1</sup>	0.047 <sup>a</sup>	0.187 <sup>b</sup>	0.103 <sup>a</sup>	0.038	**
Total DMI	31.48	28.21	30.09	1.634	NS
Growth and development					
Live weight (kg)	55.8	52.7	54.9	1.97	NS
Withers height (cm)	79.6 <sup>b</sup>	78.0 <sup>a</sup>	79.2 <sup>b</sup>	0.60	*
Heart girth (cm) <sup>2</sup>	84.1 <sup>b</sup>	82.0 <sup>a</sup>	83.4 <sup>ab</sup>	0.78	*
Body condition score	2.35 <sup>b</sup>	2.17 <sup>a</sup>	2.29 <sup>b</sup>	0.05	*

<sup>1</sup>Total intake from day 35 to 42 for OAD/PRE calves and only day 42 for TAD calves

<sup>2</sup>Heart girth is the circumference of the calf's body behind the front legs

<sup>abc</sup> values with different superscript are significant different ( $P < 0.05$ )



**Figure 1.** Effect of rearing system on calf live weight

**Conclusions** Feeding system had no effect on total dry matter intake however the quantity of milk replacer within the diet was reduced with once daily feeding systems. Offering prestarter to calves fed once daily improved body size development resulting in calves that were similar in size and stature to those offered milk replacer twice daily throughout the 42 day period.

## References

Morrison, S. J., Carson, A. F., Matthews D. and Mulholland, M. 2009. Proceedings of British Society of Animal Science Annual meeting, 84

## The effects of mannan oligosaccharide and *Streptococcus faecium* addition to milk replacer on calf performance

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**Introduction** Calf diarrhoea is a significant health issue in dairy rearing enterprises, with 38% of producers reporting it to be a problem (Morrison *et al.*, 2009). Non-antibiotic administration techniques commonly used to help prevent the invasion and growth of these pathogenic bacteria include that of offering competitive bacteria (probiotics) or substances in the milk replacer that prevent the pathogenic bacteria from adhering to the gut wall and therefore prevent scours from occurring (e.g. mannan oligosaccharides (MOS)). Previous research reported positive effects of probiotics on calf health, but there are no published data in the literature on the effect of probiotics on calf health and performance in a group feeding situation using computer controlled feeders. Group housing and feeding of calves increases the risk of disease transmission but is becoming a more common rearing practice. This necessitates investigation of management and nutritional factors that affect calf health and performance in group housed environments. The addition of probiotics or MOS has been shown to have a positive impact on starter intake and with starter intake being a key indicator to signal when to commence weaning (Morrison *et al.*, 2009), inclusion in the calf's diet, through promotion of starter intake, could potentially reduce the age at weaning. The objectives of the current study were to examine the effect of offering calves milk replacer containing either MOS, probiotic (*Streptococcus faecium*) or a combination of both (MOS+PRO) on the growth and health of dairy calves in a group situation fed using computer controlled milk and concentrate feeders.

**Material and methods** Thirty-one Holstein-Friesian, 3 Norwegian Red and 46 Holstein-Friesian crossbred calves were assigned to 1 of 4 dietary treatments based on zinc sulphide turbidity score (ZST), birth weight, gender and genotype at 4 days of age. Calves were born between 31 January and 23 April. Treatments included: 600 g/calf/day of a 23% crude protein milk replacer with no supplement added (C); C with the addition of 10 g/MOS/ calf/day; C with probiotic (*Streptococcus faecium* EEC Reg. no. EI705 -  $5 \times 10^9$  CFU/g) included in the milk replacer (PRO); and C with both MOS and PRO included (MOS+PRO). Calves were fed individually, within a group pen, using computerised milk and concentrate dispensers. Milk replacer was offered at a rate of 600 g/d until concentrate intake averaged 500 g/d for 4-days at which stage the computer-controlled programme automatically reduced milk replacer intake. When calves consumed an average of 1.5 kg/d of concentrate over a 4-d period, milk replacer feeding was finished. Calf health, feed intakes and performance was recorded until 10 weeks. Due to unequal replication for the various treatments, continuous data were analysed using the Genstat REML procedure for the analysis of variance of unbalanced data. This fitted a fixed model with effects for gender, genotype, birth weight, ZST score and treatment. Health data were analysed by binomial and Poisson regression with birth weight, ZST score, gender and treatment included in the model. Pairwise t-tests were used to test for treatment differences.

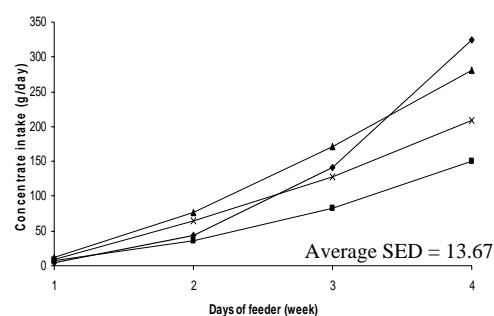
**Results** Milk replacer intake for each treatment is presented in Table 1. Daily concentrate intake during week 4 was greater ( $P < 0.05$ ) with calves offered MOS or PRO compared with calves offered C (Figure 1). However, weaning age was not significantly affected by supplementation with MOS or PRO. Faecal score was significantly lower with calves offered supplements, although no difference in the number of scour episodes was detected. The inclusion of supplements had no significant effect on calf growth compared with calves offered the C treatment (Table. 1).

**Table 1** Treatment effects on intake and performance of calves

	C	MOS	PRO	MOS+PRO	SED	P-value
Number of calves	19	19	21	21		
Milk replacer (kg DM)	25.6	23.3	25.4	26.5	1.12	0.07
Concentrate (kg DM)	17.7	17.4	18.6	17.5	1.56	0.83
Weaning age (day)	52	49	50	52	2.06	0.47
Mean faecal score <sup>#</sup>	1.16 <sup>b</sup>	1.11 <sup>a</sup>	1.11 <sup>a</sup>	1.09 <sup>a</sup>	0.02	0.03
No. of scour episodes	0.31	0.28	0.37	0.17	0.15	0.94
Live weight (kg)	56.0	55.3	57.2	54.9	1.1	0.71

<sup>#</sup>1=normal consistency; 2=slightly liquid; 3=moderately liquid and 4=primarily liquid

<sup>a</sup>, <sup>b</sup> values with different superscript are significant different ( $P < 0.05$ )



**Figure 1.** Weekly concentrate intake until week 4 of calves offered no supplement (■), MOS (●), PRO (▲) or MOS+PRO (×)

**Conclusions** Calves offered MOS or PRO, despite having increased concentrate intake at an earlier age, had a similar live weight and body size compared with unsupplemented calves throughout the study. MOS, PRO and MOS + PRO addition to dairy calf diets reduced the faecal score of calves compared with unsupplemented calves, although no reduction in the incidence in scour was observed.

## References

Morrison, S. J., Carson, A. F., Matthews D. and Mulholland, M. 2009. Proceedings of British Society of Animal Science Annual meeting, 84

## Effect of a range of concentrate intake thresholds to conclude weaning on the performance of calves fed using computerised milk and concentrate dispensers

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**Introduction** One key criterion used to signal the time to wean calves is solid food intake but if milk is fed at high levels solid feed intakes can be relatively low. Low solid feed intakes prior to weaning can result in post-weaning growth slumps (e.g. Hill *et al.*, 2006). In an effort to smooth the transition from liquid to solid food based diets, Morrison *et al.* (2010) examined the use of concentrate dependent weaning programs with computerised calf feeding machines. By initiating and concluding the weaning process when calves consumed 0.5 and 1.5 kg of concentrate respectively, the authors found weaning age and rearing costs were significantly reduced while live weight at 20 weeks of age onward was similar compared with calves weaned at fixed age. Therefore the objective of the present study was to investigate the effect of a reduced concentrate intake threshold to conclude the weaning process on the performance of calves reared using computer-controlled feeders.

**Material and methods** Group-housed Holstein-Friesian calves ( $n = 70$ ), were randomly assigned to milk feeding systems with either weaning at 8 weeks (C) or weaning based on a range of concentrate intakes. All calves were fed individually through the feeding system with milk replacer mixed at 125 g/l throughout the study. For control calves, milk replacer was offered at a rate of 4 l/d from 4 to 51 days of age and then reduced in steps from day 51 to weaning at day 56. For calves weaned based on concentrate intake, milk replacer was offered at a rate of 4 l/d until concentrate intake averaged 500 g/d for 4 consecutive days at which stage the computer-controlled programme automatically reduced milk replacer intake. When calves on the concentrate dependent treatments consumed an average of 0.8 (L), 1.0 (M) or 1.5 kg/d (H) of concentrate over a 4-d period, milk replacer feeding ended. If calves did not achieve a concentrate intake of 500 g/d and initiate the auto weaning facility before day 51, they were weaned according to the standard treatment. Four calves were removed from the study for health reasons unrelated to treatment resulting in 16-17 calves per treatment. All data were analyzed by analysis of variance using the Genstat REML procedure. This fitted a model with individual calves as the subjects, and fixed effects for sex, birth weight and weaning system plus the sex by weaning system interactions. Female calf live weight from week 15 onward were analyzed by ANOVA with week 1 value used as a covariate.

**Results** Total milk replacer intakes until weaning were 23.7, 17.3, 17.7 and 19.1 kg DM/calf respectively, for calves on the C, L, M and H treatments respectively (Table 1). Weaning age was reduced by up to 13 days on the concentrate dependent treatments, resulting in calves that were up to 16 kg lighter at weaning ( $P < 0.001$ ). However at 40 weeks of age, weaning treatment had no effect on calf live weight.

**Table 1** Performance of calves weaned at a fixed age or based on concentrate intake

Performance parameters	Weaning system				SED	Sig.
	C	L	M	H		
Milk replacer intake until weaned (kg DM)	23.7	17.3	17.7	19.1	1.14	***
Concentrate intake until weaned (kg DM)	35.9	9.7	9.4	14.9	2.90	***
Weaning age (day)	56	43	43	47	2.5	***
Live weight at weaning (kg)	71.7	55.6	57.6	59.5	2.17	***
Feed cost until weaning (£)	44.31	27.57	27.99	31.58	1.983	***
Gain : kg DM until weaning	0.49	0.50	0.55	0.50	0.033	NS
Gain : £ feed until weaning	0.66	0.48	0.53	0.53	0.043	***
Live weight at 40 weeks of age (kg)	250	242	237	241	9.9	NS

**Conclusions** Weaning calves based on concentrate intake reduced the age and live weight of calves at weaning and reduced feed costs however no difference in live weight or body size was found at 40 weeks of age. Reducing the concentrate intake threshold to signal weaning below 1.5 kg tended to further reduce feed intake, feed costs and weaning age without effecting live weight at 40 weeks of age. Therefore the results from the current study indicate calves can be weaned when consuming 0.8-1.0 kg concentrate without reducing future growth and development.

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## Starch processing wastage as a feed for Holstein bulls

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**Introduction** Industrial wastes comprise a vast range of organic matters which is usually considered useless and an environmental pollution, while most of them have high nutritional value and could be used in livestock nutrition. The waste remained after separating starch and gluten from wheat flour in starch processing factories is one of these valuable and nutritious feeds. In this experiment, a mixture made from starch processing waste, wheat bran and salt by a commercial name of "Powerfeed" was used in Holstein bulls fattening rations to investigate their performances.

**Material and methods** Twenty-one Holstein bulls were randomly assigned to three groups so that the mean live weights of each of the 7 bulls in each group were almost the same. Bulls in group 1 (control) were fed with a total mixed ration (TMR) comprising of 24% alfalfa hay, 34% barley grain, 11% barley straw, 7% rapeseed meal, 5% wheat bran, 11% corn silage, 7% beet pulp, 0.6% mineral-vitamin supplement and 0.4% sodium bicarbonate to met their energy and protein requirements according to the NRC 2001. For the TMR of bulls in groups 2 (5% Powerfeed) and 3 (10% Powerfeed), barley grain was replaced with Powerfeed at amounts of 5% and 10% respectively. Bulls in each group were housed in their individual boxes with free access to the fresh water. The experiment lasted 105 days comprising 15 days adaptation and 90 days of fattening. The TMR was fed to the bulls twice a day at 08:00 and 20:00 hours so that they could obtain their feed requirements at *ad-lib* levels. The body weight of bulls was measured at 30-day intervals and their relative feed conversion ratios were then measured. Data were analyzed using the GLM procedure of SAS 2001.

**Results** The dry matter content and the crude protein (CP), calcium, phosphorus, sodium, crude fibre and the fat composition of Powerfeed used in this study were: 890, 157, 11.2, 11.2, 30.3, 56.1 and 33.7 g/kg, respectively. As Table 1 shows, there were no significant differences in daily feed intake, average daily gain and the feed conversion ratio of bulls in control group compared to those in bulls of 5% and 10% Powerfeed groups.

**Table 1** The effect of Powerfeed contained total mixed rations on the performances of Holstein bulls

Items	Treatments			
	Control	5% Powerfeed	10% Powerfeed	SEM
Daily feed intake (Kg)	12.3	12.4	12.2	0.26
Average daily gain (g)	766.1	808.0	748.9	39.3
Feed conversion ratio	21.6	17.4	17.7	2.15
Average start weight (Kg)	246.0	253.1	248.7	11.4
Average finish weight (Kg)	321.9	331.1	322.9	12.1

**Conclusion** Since the daily feed intake, average daily gain and the feed conversion ratio of Holstein bull calves were not affected by the use of this supplement as part of their total mixed rations in this study and due to the relatively lower feed expenses paid for Powerfeed compared to that for barley grain in Iranian feed markets, the use of Powerfeed (especially at 5% level) in bull calves fattening rations is recommended.

**Acknowledgements** The financial support from the Veterinary Research Unit of University of Tehran is gratefully acknowledged.

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## Challenges and opportunities for European agriculture

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Agriculture is at the nexus of science, economics and politics. If one of these disciplines is lacking or misdirected in the framing of agricultural policy then the sector plunges into instability and potential crisis.

The challenges at the present time are becoming clearer.

The income accruing to the Agricultural sector has been dropping since records began – apart from the occasional “price spike”. This decline is fudged by the conversion of income figures into the return per labour unit employed – which it is assumed falls by 2% a year. But this is almost incidental as the key policymakers for the sector tend to be neither scientist nor economists.

The basic absence of a clear appreciation of the basic economics underlying the sector is a major challenge.

While the basic laws of supply and demand are readily understood, the understanding of the price and income elasticities of demand while very much present in the old Common Agricultural Policy are apparently absent in the most recent models.

Within the space of a generation, European agriculture has gone from serious deficit to comfortable surplus. The potential and sometimes the actual weight of excess production has driven down farmer income and increased societal expectations of not just what can be produced but how it can be produced in terms of a whole range of what used be regarded as peripheral issues.

This capacity to produce is at the base of the challenges facing the sector.

The Nitrates Directive may have been the most contentious in a series of environmental expectations on societies part but EU Agriculture is also expected to be “sustainable” and “competitive”.

Yet GM technology is essentially denied to the EU farm sector and plant protection product legislation is becoming not just restrictive but in cases bizarre as might be expected when food sufficiency is taken for granted; the major challenges are becoming political. The power of codecision with the European Parliament will intensify the dilemmas.

The opportunities lie in the increasing awareness that growing numbers of people and regions are vulnerable to potential or actual food shortage. Europe itself is a bastion of not just political but also of productivity stability. The population is in the main well off with strong purchasing power which in times of stress is safeguarded by generous social welfare payments.

Even at the present stage of the WTO (World Trade Organisation) talks, the region’s Agriculture can be protected from damaging third country imports if the politicians ultimately responsible for setting the operational guidelines for the sector wish to implement the existing mechanisms.

Europe as a region is not the most competitive producer of any single commodity, milk can be produced more cheaply in New Zealand than in Ireland, sugar more cheaply in Brazil, beef more cheaply in Argentina but Europe can produce a range of high quality products that have the potential to be in demand as wealth grows in China and hopefully in Africa as well as satisfying domestic EU demand. European food and drink products like our cars are already occupying prime positions in the US and other high value outlets where quality is valued and paid for.

## Meeting the challenges of prioritizing land use – the role of agricultural science

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Concerns about the global security of supplies of food, water and energy have gone beyond lectures and articles and are now being taken forward in a raft of reports and initiatives. Agricultural science is centre stage in a way that would have seemed unthinkable to many of us even five years ago. It is appropriate that much of this research will address issues of productivity of the individual organism, through breeding and protection against pests and disease, seeking to ensure that production is achieved in a resource-efficient way, and is resilient to the increasing variability of weather and changing climates. Such research helps address how to get more agricultural production out of the land. However, it does not address the question of which land to use for agricultural production, nor does it address the question of how optimum patterns of land use (should they exist) be brought into being.

Optimisation and prioritisation of land use address the fact that there are different outputs from the land, with different values, that vary across space and time. These outputs include agricultural production of course, but also include other ecosystem services, such as climate regulation, flood management, cultural heritage and so on. They also include potential value for land transformation into and (more usually) out of agriculture into, for example, urban development, forestry and coastal set-back. A key question for agricultural science is, therefore, which ecosystem services we expect from agricultural land, and the extent to which their joint production is possible, as opposed to requiring segregation of land into different functions. It turns out that, for grassland systems at least, increasing levels of agricultural production tend to be associated with *decreasing* levels of many other ecosystem services. This gives three ways forward; either segregate land, go for less intensive production methods, or seek new methods of agriculture that avoid these conflicts. Such questions require a more holistic ecosystem science, in which agricultural research is but one component - as is being taken forward by the UK National Ecosystem Assessment.

Suppose we explore how to segregate land into different functions. In principle, it is easy to optimise land use by function according to environmental character. But this assumes that we know the value of each function. Such methods of valuation are being developed for ecosystem services, but it's worth recalling how transient some of these values are. Environmental quality is increasingly characterised in terms of carbon, very different to the emphasis on biodiversity in the 1990s. Also, different people place different values on different functions and different areas of land, making consensus difficult to achieve.

But even if such consensus could be achieved, would our national land prove capable of providing our needs anyway, no matter how well it is parcelled up? Current UK agri-food systems are extremely expensive in energy and water by the time they reach the consumer. So we need to start looking at agri-food systems that deliver human needs (perhaps needs as opposed to desires) within manageable global environmental footprints. For example, perhaps it is inappropriate to divert soya that could provide protein for people into animal feed. Our prioritisation of land use may involve prioritisation of human expectations.

If forecasts of population, social, economic and climate change are anything like robust, it will prove hard for us to adapt our land management to meet human needs in the coming decades. The evidence base will require the integration of agricultural science with the other developing sciences that address global change; this is a wonderful opportunity for a new kind of systems agricultural science, drawing on new farm-scale experimental facilities like the one being developed at North Wyke Research as well on latest developments of genomics.

But the science we need does not all have to be so grandiose. A great deal can be learned by combining information from many much smaller experiments, whether undertaken by scientists or by farmers as they adapt to changing conditions. Is there scope for evidence-based agriculture, as is already happening in medicine? If so, how should it be organised?

## Harnessing scientific developments in practice

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**Introduction** Adoption of innovations, be they new policies, technologies or products, is fundamental to the sustainable development of the agri-food industry in the UK and Ireland. This paper will review factors influencing the adoption of new scientific developments and, in this context, evaluate the linkages between research and the rural community in Northern Ireland. Information will be drawn from a range of sources, including a recent study of technology transfer on dairy farms (Morrison *et al.*, 2009).

**Farmer decision-making** Much work has gone into understanding and modelling farmer decision-making (e.g. Garforth *et al.*, 2004; Edwards-Jones, 2006). This has identified a large number of financial and non-financial factors associated with the level, and rate, of uptake of new innovations. The most important issues dairy producers in Northern Ireland considered when deciding on the adoption of research findings were, 'what are the financial rewards of the change?', followed by 'what is the cost of adopting the change?' and 'what is the labour/time/energy required to change?' (Morrison *et al.*, 2009).

Non-financial variables associated with the uptake of technology include: farmer characteristics (age, level of education, gender); household characteristics (stage in family cycle, level of pluriactivity, work patterns of spouse); farm business structure (farm type and size, fragmentation, land quality and debt to asset ratio); the wider social milieu (level of technology transfer activities, information flows, local culture, social capital, attitude of friends, the policy environment); characteristic of the innovation to be adopted (visibility, compatibility, similarity with existing technology) and the farmer's psychological make-up (farmer attitudes and beliefs). For example, technology adoption happens quicker when the individual is younger or less experienced, better educated, receptive to new ideas, self confident and in a position to access economic resources and make decisions; the farm system is large, profitable and linked to other businesses and knowledge networks; the innovation system is linked, or in contact with, farmers and more involved in management-intensive technology than in capital-intensive technology (Massey, 2004). Understanding these factors, and their interactions, is crucial in developing appropriate technology transfer programmes.

**Technology transfer models** Technology transfer models can be grouped largely into one of five major strategies or communication methods: (i) linear 'top-down' transfer of technology from scientific experts to farmers, (ii) participatory 'bottom-up' and group based approaches, (iii) one-to-one advice or information exchange (whether from farmer to farmer or from professional adviser to farmer and vice versa), (iv) formal or structured education and training and (v) new information technologies (particularly internet). It is recognised that no single model or strategy is likely to be sufficient by itself (Black, 2000) and in Northern Ireland a number of approaches are taken. In the area of sustainable livestock systems, large scale applied research programmes are undertaken on commercial farms across the region linked to more basic research programmes carried out within the Agri-Food and Biosciences Institute (AFBI). This type of model embeds technology transfer into research programmes and ensures the 2-way flow of information between the industry and research right from the outset of the work. Funding from farmers through AgriSearch (producer levy), along with the Department of Agriculture and Rural Development (DARD), has played a crucial role in the development of this work. Focus Farms have been established in Northern Ireland to promote good practice by example and monitor farms selected to provide a focus for farmer-driven discussion groups. DARD provides a business development service for farmers and growers providing one-to-one advice and has responsibilities in relation to education and training courses for the land-based and food industries and development programmes. Good linkages between research and education are crucial in establishing strong connections with new entrants to the industry and helping ensure new scientific developments are rolled out in a timely manner.

**Creating the right environment for adoption and change** To be effective, technology transfer programmes need to create the right environment for change to occur. Devenish (2006) reported the key factors are: (1) extensive knowledge of the problem, (2) working with farmers to identify and overcome barriers to adoption (3) involvement of a credible researcher, specialist or extension practitioner (4) experience in various communication methods and (5) funding to support research and development activities. Success might still be achieved if one key factor or process is missing, but if 2 or 3 factors are ignored or missing the programme is likely to fail as an effective means of promoting adoption and change.

**Conclusions** The effective adoption of scientific developments is a key determinant of the economic return achieved from investments in research, as well as being fundamental to the sustainable development of the agri-food sector. Central to its success is the inclusion of researchers at all stages of the process to help overcome barriers to adoption.

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## Perspectives on crop research: food vs fuel

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Arable farming was once a major component of Northern Ireland agriculture. In the 1850's 40% of farmed land was ploughed, some 400,000 ha. Potatoes, flax and turnips were important crops, but by far the greatest proportion of land was in oats, and most of the oats were fed to the work-horses that ploughed the land and carried out the cultivations. Until the advent of the tractor about 50% of ploughed land was essentially in an 'energy' crop, and not in the human food chain. These figures are a stark warning, if one is required, that to generate any meaningful quantities of biofuel and biomass for energy in Northern Ireland will require a huge commitment of land and a significant shift in the nature of our agriculture. However, the figures also indicate that if the 'climate' were right in all the senses of the word (physical, economic, political, social) there is a precedent to the devoting of a significant proportion of our country to 'energy crops' in the widest sense.

The debate on 'fuel vs fuel' has shifted because there is a greater understanding that the issue is not fundamentally 'fuel' but is being driven by the need to reduce greenhouse gas (GHG) emissions. We need therefore to look at the role Northern Ireland agriculture might have in achieving reductions in GHG emissions through the production of biomass and biofuel. Looking at the situation today, we have the lowest self sufficiency for animal feed in Europe, with 80% being imported. Furthermore the initial surge for the production of 1st generation biofuels (wheat to ethanol and vegetable oil to biodiesel) has now been shown to be of relatively little benefit in terms of reduction in fossil fuel use and GHG savings.

The food vs fuel debate was 'fuelled' by the increasing proportion of world cereal production being devoted to ethanol production, principally in the USA and currently at about 10%. In 2007 and 2008 when world grain reserves fell due to droughts, poor harvests, increasing demand and diversion of supplies to biofuel, commodity prices rose and penalised the world's poor disproportionately. However, as a result of record world harvests of cereals in 2008 and 2009, world cereal stocks have recovered, and in spite of devoting a proportion of cereals to biofuel, grain supplies have increased more rapidly than world population over the last 50 years. The scare of 2007/08 made people realise that food is a scarcer source than fuel, and its production has to be maintained on a continuous basis. Solar energy, the gravitational pull that generates the tides, nuclear power and even fossil fuels for all their failings are more consistent, predictable, and dependable than our sources of food production. Thus food production commands the greatest proportion of our efforts, and food use will out-bid fuel uses for scarce resources, pushing up the prices in times of shortage.

Any move from Northern Ireland to devote part our arable area into biofuel crops will result in an increase in imported feeds, and in a very small way reduce world cereal stocks while making very little impact on the reduction of GHG emissions. However we need to consider the impact of bringing grassland back into energy crop production. Grassland in NI supports the dairy, beef and sheep industries. Rough grazing and hill ground could be considered for forestation, but the more productive grass land that was, perhaps, once under the plough could have the potential for energy cropping in some form. The motivation for doing this might also be economic as the FAPRI-Ireland model (Breen, Hennessy and Thorne, 2008) indicated that 80% of beef farms in Ireland were not economically viable and the projection was that the position may worsen in the future.

While there is certainly an increasing market for meat particularly in the developing countries, changing land use from unviable animal production in NI to energy cropping will reduce imports of feedstuffs, release cereals for human consumption and therefore contribute to both more fuel and more food, a win/win situation. If ruminant GHGs also fall then it is a win/win/win situation!

Thus, if economically viable, possible options may be short rotation coppice (SRC) Willow and Miscanthus for dry biomass and the anaerobic digestion of fresh or ensiled grass. Of the 1000 or so hectares of SRC willows in Northern Ireland most has been planted in arable areas because it has been the more progressive forward thinking farmers who have taken up this opportunity. However, the real need is, through research, to develop economically viable SRC systems and markets for willow biomass in the predominantly grassland areas, and it will take community involvement to create local markets for biomass which cannot be economically transported great distances. Miscanthus has made little headway in Northern Ireland, principally through the lack of a market for this bulky material which is less suitable than wood chips for smaller scale markets.

The most exciting possibility for Northern Ireland is the potential to remain in grass production, build on the skill and experience of local farmers to grow and conserve digestible, high energy grass and use it to generate biogas methane. The technology is there to use this to generate electricity and heat, or even better to refine biogas into vehicle fuel. In Europe progress is being made into the gasification of dry lingo-cellulosic materials such as wood chips into vehicle fuels, but as yet only one such plant is operating in Europe and this is on a scale and requires capital investment that beyond our scope at present. Significant challenges therefore remain for the research community, government policy makers and for the agriculture industry if in Northern Ireland we are to see anything like to amount of biofuel grown than was the case in 1850.

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## Greenhouse gas emissions from Irish beef and dairy production systems

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**Introduction** In Ireland, agriculturally derived emissions account for 26% of total greenhouse gas emissions (McGettigan *et al.*, 2009). Emissions from agriculture reduced by 7% in 2007 relative to 1990 levels with a further 4% reduction projected by 2020 (McGettigan *et al.*, 2009). These projections relate to the levels of agricultural activity and thus, there is scope to reduce emissions further if production efficiency at farm level is also improved. Furthermore, new technologies that directly reduce the emissions of methane (CH<sub>4</sub>) or nitrous oxide (N<sub>2</sub>O) will, if adopted on farms, reduce the contribution from agriculture even further again (Beauchemin *et al.*, 2008; de Klein and Eckard, 2008). Systems modelling approaches have been used to investigate the GHG emissions from current and prototype agricultural production systems (Schils *et al.*, 2007). This paper reports on a number of studies which have attempted to quantify GHG emissions from Irish beef and dairy production systems.

**Modelling approaches** Whole-farm systems modelling determines the GHG emissions association with agricultural products by integrating the farm production profile with the relevant GHG conversion factors and converting the computed emissions to their global warming potential carbon dioxide (CO<sub>2</sub>) equivalent. Emissions from Irish livestock production systems have been investigated in this way (Foley, 2009; Foley *et al.*, 2010; Lovett *et al.*, 2006; O'Brien *et al.*, 2010). Foley (2009) and Foley *et al.* (2010) modelled GHG emissions per unit area and per unit product for suckler-beef and dairy-beef production systems. Input data was generated using a bioeconomic model of beef production systems thus permitting concomitant financial analysis (Crosson, 2008). A range of scenarios representing average Irish farm systems as well as incorporating data from research production systems differing in production intensity were investigated. O'Brien *et al.* (2010) linked a GHG model to a bioeconomic dairy systems model (Shalloo *et al.*, 2004) to evaluate GHG emissions and technical and financial performance of dairy production systems. This model has been used to investigate the effect of cow genotype, feed system, grazing season length and overall herd breeding index on dairy production GHG emissions per unit of product and per unit area.

**Findings** Foley *et al.* (2010) found that, for suckler beef production systems, emissions per farm and per unit area were substantially higher for research systems relative to average Irish farm systems due to higher stocking rates. However, per kg beef carcass, reductions in GHG emissions were in the order of 20% for research production systems. The main drivers of this reduction were level of beef carcass output, level of animal performance, efficiency of grass utilisation and efficiency of fertiliser utilisation (Foley *et al.*, 2010). Similarly, for dairy-beef production systems, emissions were approximately 30% lower for research production systems relative to average Irish farm systems (Foley, 2009). For both suckler and dairy beef production systems, reduced emissions per kg product were associated with higher levels of profitability.

In the case of dairy production systems, O'Brien *et al.* (2010) found that low concentrate systems and selecting cows based on both production and fertility traits produced the lowest GHG emissions per kg of milk solids. These results agree with the findings of Lovett *et al.* (2006) who also showed that cows selected solely for increased milk production potential increase GHG emissions per unit of product. Furthermore, the results of these studies (Lovett *et al.*, 2006; O'Brien *et al.*, 2010) indicated that profit can be increased and emissions per unit of product decreased by adopting available technologies. Thus, there is large potential to reduce the national average GHG emission for dairy production systems per unit of product without eroding farm profitability. The main technologies available to decrease national dairy emissions are improved fertility, early calving date, increased grazing season length, higher stocking rates and improved milk composition and yield.

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## **What industry requires from the application of research from equine science**

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The equestrian industry contributes approximately £3.4 billion to the UK according to the Henley Report (2004), it is responsible for 5 million consumers (11% of the population) declaring having an active interest in some form of the industry. This is also made up of 50,000 people directly and 150,000 – 250,000 indirectly employed in the equestrian industry. It is frequently subjected to criticism for relying on traditional approaches to solving management problems. The transfer of knowledge between equestrian generations often relies on authoritative transfer of knowledge, rather than a critical empirical approach. However, alongside this over-reliance on authority equestrian experience has been developed from observation and questioning of observed behaviour both directly with the horse and within its environment. In fact for the equestrian coach these skills in observation and reflection are an integral part of coaching practice. The wealth of knowledge that resides within the equestrian industry professional therefore should not be underestimated.

A lot of the knowledge of an equestrian professional is tacit, and therefore difficult to articulate. This is compounded by belonging to a community of speech practice that has a detailed and complex 'argot'. The potential for miscommunication with individuals outside this community is therefore great and can lead to assumptions that the equestrian professional has little knowledge to offer outside their direct environment. The integration of UKCC qualifications and the recognition that a 'riding instructor' may also be a skilled coach, communicator and professional has led to greater reflection within the equestrian industry. The increased focus on how the equestrian industry is perceived externally and the recognition that external benchmarking can be utilised to increase its status has opened the path for interdisciplinary communication and study that may not have been previously available.

Much of the research that has been undertaken in equestrian science to date has been establishing fundamental knowledge, often about physiological or performance based dilemmas about which a lot of comparative information is known. However when we consider more complex questions, especially those incorporating dyad relationships, e.g. horse and rider, there is very little, or in some cases no, comparative information to draw on. In these cases if research does not utilise the tacit knowledge within the equestrian industry researchers have to spend a great deal of time and effort establishing the reliability of information that an equestrian professional would consider so basic as to not be worthy of mention. The presentation of this information as new research can do untold damage in the name of equestrian research as professionals often regard it as simplistic, condescending and ultimately 'a waste of time'. Whilst this research can be valuable it must be communicated at a level appropriate to its impact within the industry, and to its audience.

Research should therefore be applied to the industry, and communication of this fundamental knowledge should be kept to those that it would benefit. There is a huge opportunity for collaboration and two way learning, to integrate best practice and work together to solve many of the challenges that face the equestrian industry. In this way research would increase its status, its funding and also its value and application to both riders and lay professionals who can have high kudos within their chosen field.

## Are there potential opportunities for industry, colleges providing equine science courses and primary research departments to work even more closely together?

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In an ideal world all the products, techniques, equipment etc. that we employ in relation to the horses under our care would have undergone adequate and appropriate testing to ensure that they are safe and have a high probability of fulfilling the claims made for them. Unfortunately, with respect to many of the non-pharmaceutical registered products, few have any proven science, especially not in the horse to back their claims. According to the OED: '*Prove = verb (past part. **proved** or **proven** ..) : demonstrate by evidence or argument the truth or existence of...*' In a scientific context I am taking *proven* to mean that studies showing efficacy have been published in at least one peer reviewed journal – either as a result of a primary 'research study' or as part of clinical evidence. It is important to note that such a level of proof is not always required. However, there are many reasons why such science is not carried out, but perhaps two of the most relevant to this paper are *cost* and *expertise*. An appropriate equine study which has been carried out with sufficient numbers of horses for the appropriate length of time will be expensive and time consuming. The profit margins within the equine field do not tend to be large and therefore there is often little money available for such work. Publication in highly respected journals requires a good study design, use of appropriate statistical methods, an ability to write scientific papers and a good knowledge of the subject area. The potential purchaser ideally should have sufficient knowledge to be able to distinguish between a study that actually shows some efficacy from one which just appears to. We therefore have :-

- An 'Industry' which either wants or should want to undertake, when appropriate, good quality research to provide support for certain products – but they cannot afford expensive projects. When there is no 'pull' from the end-users or regulatory pressure, then it is difficult to justify the cost of strongly scientifically based product support. Certain projects would be generic in their appeal and therefore are even more difficult to justify in today's market.
- Colleges/Universities providing equine degree programmes, as part of their course, usually require undergraduates to undertake small research projects. Colleges often have access to relatively large numbers of horses housed locally. Increasingly, such institutes are employing lecturers with research expertise. However, the supervisors cannot be experts in all topics/areas, there is often little free internal money to support any projects and often teaching commitments are large. The students are obviously not experienced in research techniques, the time they have to undertake their projects is often relatively short, and some are just not interested. The research commonly needs to be non-invasive in nature etc.
- Researchers working within departments/institutes, where one of their main objectives is to undertake and publish good quality research, are often limited by horse/people costs and availability although they may be experts in one or more areas. They may want to undertake industry focussed research but either cannot obtain sufficient funds, do not have the contacts or may be discouraged by the unsubstantiated concern voiced by some that 'industry linked research = biased research of an inferior quality.!

There are already good examples where the various groups are working well together e.g. WALTHAM's collaborative projects and the secondment of degree project students for specific projects with researchers expert in their topic area; BUT are there opportunities to develop even more effective working collaborations?

- Could we perhaps harness the student power from the colleges to obtain valuable information on a UK wide basis? Are there projects that would benefit from information being collected from several regions within the UK? Could this be centrally co-ordinated?
- Could colleges work more closely with 'industry' and 'researchers' to develop programmes, that help to answer key questions, which are additive and develop over a series of years – with data being collected via a series of small projects rather than one large one?
- Could 'researchers' help to 'mentor' college project supervisors where appropriate?
- How can we best educate the 'end-user' /purchaser re the value of good science?

Is this valuable, desirable, and achievable? What are the barriers to success and is the potential prize worth it?



## Equine exercise physiology: a challenge for the twenty first century

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The study of equine exercise physiology blossomed in the late twentieth century but we must not suppose that it is an entirely new science. As long ago as 1350 B.C., the Hittite Kikkuli text described methods of training horses reminiscent of interval training (McMiken 1990) and more recently the use of a treadmill for the measurement of equine respiration during exercise was reported in 1898 by Zuntz and Hagemann (Hörnigke *et al.* 1983). Gait analysis too, has a relatively long history. In the nineteenth century, Ellenberger used bells attached to the limbs of galloping horses to record footfall, Marey used pneumatic accelerometers to study gait and Muybridge recorded equine locomotion using a series of cameras (Barrey 1999). In the late twentieth century, a number of technological developments, for example, the high speed treadmill and the force plate, permitted great progress in a number of branches of equine exercise physiology, furthering our understanding of the responses of the horse during exercise and sometimes questioning some of our long held views on the way in which we should manage competition horses.

A challenge for exercise physiologists in the twenty first century is to define suitable research aims. It was stated a few years ago that, '*Unfortunately, at this time the elusive test to predict the future performance of the young and unproven horse does not exist*' (Hendrickson 1996), I would contend that such a test is highly undesirable as it would likely result in the best horses being bought by a few wealthy owners reducing opportunities for others. Rather than attempting to improve our ability to predict performance, I suggest that the prime objective of equine exercise physiology should be to improve the welfare of competition horses.

Equine safety and welfare in equestrian competition has advanced considerably but there is still great room for improvement. For example, in National Hunt racehorses, injuries or medical events were reported to occur in 2.88% of starts (Pinchbeck *et al.* 2004), tendon and suspensory ligament injuries being frequent and in younger, flat racehorses, Verheyen and Wood (2004) found an incidence of nontraumatic fractures of 1.15/100 horse months; 78% of fractures occurred during training rather than racing. Numerous studies have demonstrated the high prevalence of gastric ulceration in competition horses (see, for example, Jonsson and Egenvall 2006).

Serrano *et al.* (2002) suggested that many eventing horses are not appropriately trained. A high proportion of athletic injuries in the horse occur to the skeletal system and may frequently arise from a mismatch between the exercise levels and training adaptation (Smith and Goodship 2008). There is evidence that quality and quantity of exercise in the young horse may have an important effect on later susceptibility to injuries (Firth 2006). We should aim to determine optimal methods of training to reduce the predisposition to injury of competition horses, investigate ways in which equestrian sports can be conducted to minimise the risk of injury, and attempt to develop better methods for the sub-clinical diagnosis of competition-related disorders at an early stage.

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## Animal Science - Recent progress and future challenges

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Animal science spans a broad compass. It tends to be considered as the study of animals (nature, function, productivity etc) that are under the management of man in some way. This differentiates it from zoology, excludes wildlife and provides boundaries with at least some aspects of veterinary and biomedical sciences that can be rather indistinct. It covers both the understanding of animals as biological systems and the role of animals as components of broader systems ('Animals as systems; animals in systems'). By being allied to animals that are under management, I take it as implicit that any consideration of progress in animal science needs to include not only progress in intellectual understanding, but also progress in the utility of that understanding in practice. This does not deny the value of animal science to inform broader biological understanding.

There has been considerable scientific effort and increases in understanding both on animals themselves and on their roles in systems, although rather more in the former than the latter. Equally there are future challenges both at the level of the animal as a phenotype and for animals in systems of management. In each case the great challenge is not simply to understand the behaviour (in its broadest sense) of the animal or system, but to be able to predict it.

"Recent" is a word that is open to some interpretation. The rate at which some new techniques can help to generate data in research can make it appear that rates of progress are accelerating. 'Recent' progress in such areas might be considered to span small numbers of years. But a broader perspective is needed to recognise some of the major areas of progress.

Progress in any science is driven by a mixture of imagination to generate the ideas that are worth pursuing, and the acquisition of appropriate techniques to allow those ideas to be tested. Sir John Hammond was a fount of ideas on reproduction, growth and development in the inter-war years and through the 1950s. His insights and techniques led the way in practical improvements in reproduction and his concepts of growth and development have generally stood the tests of time. Like Hammond's waves of growth progress in animal science has been influenced by waves of technical development. The advent of chromatographic techniques in the early post-war years allowed access to rapid analytical methods that underpinned progress in nutrition and physiology especially. Isotopic tracing techniques gave access to more quantitative understanding of metabolism as well as facilitating analysis. More recently molecular techniques have opened avenues to understanding that were previously closed. And advances in computational and statistical techniques have not only supported the rapid delivery of other methods but have also allowed the realisation of ideas that, in some areas (perhaps especially genetics) were formed a significant time ago but were inaccessible for want of computational power.

Progress in genetics has helped to develop animals whose rates of productivity is dramatically increased although sometimes with negative associated consequences – which are now being corrected by adjustments to selection approaches. The promise of molecular approaches to replace more conventional quantitative methods has not been fully realised, although the prospects of using genome-wide selection are considerable. Understanding of nutrition and nutritional biochemistry has yielded rationing schemes that work tolerably well and allow some control over the qualities of products that animals produce. Our understanding of reproductive processes has grown substantially, but too often the application of new knowledge has been in the 'catch-up' mode, aiming to correct problems introduced by other 'advances'. Research on pregnancy and lactation for all mammalian species, including our own, has, though, been considerably advanced through the animal sciences. Our understanding of the processes of disease have been enabled through the animal as well as the veterinary and medical sciences, and increasingly methods of control can be expected to include broad-based approaches that will call on the products of a range of animal sciences. The welfare of animals, as sentient beings, is better appreciated and open to assessment and improvement. But the prediction of phenotypic expression (what will an animal actually do given a knowledge of its genotype and its environment?) is still an aspiration rather than a reality.

On broader fronts the impacts of animals on their environment are better understood now than they were. Management of waste to reduce pollution or of grazing to achieve biodiversity goals is more possible. Animals also influence the social, or operating environments. For example systems of animal management can be important for social cohesion and are important sources of work power or of equity.

The impacts of animals on the environment are a source of much current concern, though. Some of this relates to issues around climate change, others to the management of pollution and of managing biodiversity. Yet other concerns are to do with perceived negative contributions of animal products to the healthiness of the human diet. At the same time the growing size of the human population and its ability to afford more animal products in the global diet has, and is likely to continue, to increase the global demands for animals and their products.

This is the big driver that creates the grand challenges for animal science to address. The need is to produce animal products in appropriate abundance, that are beneficial (or at worst not detrimental) to the healthiness of our diets, that are produced with minimal waste (by efficient use of resources and minimisation of losses through disease and reproductive failure especially) no negative impacts on, and preferably benefits to, the environment and in socially acceptable ways. To achieve these ends, if such is possible, I would expect to see the next stages of progress in the animal sciences being more integrative and predictive. Synthesis of understanding to enable the prediction of functional behaviour of animals as systems and animals in systems (and the behaviour of those systems) as well as continuing lines of discovery to enable this to happen should be to the fore. A sharp eye to the outcome, and not simply to the understanding, is merited. Sir John Hammond was an outstanding scientist – but his goal was science to enable efficient animal production. We still need that.

## Research needs for an efficient livestock industry

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This paper addresses the research requirements of the dairy, pig, beef and sheep industries and reflects the collective views of the three relevant sectors (DairyCo, EBLEX and BPEX) of the Agriculture and Horticulture Development Board (AHDB) that are charged with providing technical support in the form of funding for research and knowledge transfer.

Research priorities are summarised under the headings of: breeding, feeding and health with the objective of enabling higher levels of production to be achieved per unit of greenhouse gas emitted. This high level objective is likely to be a major driver for research and knowledge transfer in the UK industry into the foreseeable future.

Along with other components of the food chain, the UK livestock sector is charged with reducing emissions of greenhouse gases (GHG) ( $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{CO}_2$ ) in line with the targets set by the 2008 Climate Change Act and the subsequent Low Carbon Transition White Paper published in July 2009. In the period to 2020, an 18% reduction in emissions ( $\text{CO}_2$  equivalents) over 2008 levels is required and, for England alone, this has been equated to 3 million tonnes of  $\text{CO}_2$  (equivalent) per annum.

It has been calculated that UK agriculture is responsible for 7% of the total UK GHG emissions made up as follows:  $\text{N}_2\text{O}$  (3.5%),  $\text{CH}_4$  (2.8%) and  $\text{CO}_2$  (0.7%). Reducing emissions of  $\text{CH}_4$  from ruminant enteric fermentation and manure heaps as well as  $\text{N}_2\text{O}$  from use of slurry and nitrogen fertilisers on grassland have become a major focus for future attention. At the same time however, there is increased demand for food production in general, and animal products in particular, as the UK population grows towards a projected 70 million by 2050. Achieving emissions targets by simply scaling down livestock and milk production is not an option given that this would simply pass the problem elsewhere in the world and result in an increase in imports at a time when recent government policy encourages greater reliance on home-based production.

The livestock and dairy industries are committed to increased efficiency which can best be measured in terms of increased units of production (Kg of meat or milk solids) per Kg  $\text{CO}_2$  equivalents. It follows that there is a need for well-targeted genetic improvement of the national herds of cattle, sheep and pigs coupled with improved health and nutrition alongside increased grassland productivity and better manure management. For the most part, increases in efficiency, defined in these terms, is entirely compatible with improved profitability and return on investment.

### Breeding

Genetic selection for conversion efficiency in the case of pigs and production volume in the case of dairy has had substantial impact on the efficiency of pig meat and milk production. However, genetic interactions with health and quality related traits continue to require elucidation. In the case of beef cattle and sheep, there is still much progress in terms of genetic improvement for feed conversion that could be made with significant benefit in all species. The exploitation of genomic tools provides the promise of greatly increased selection efficiency. Research to elucidate further the genetics of key traits underlying growth rate, conversion efficiency, disease resistance, fecundity, fertility and meat quality together with provision of closely associated markers is of continuing high priority. At the same time, in cattle and sheep, there is an urgent need to exploit the genetic basis for observed differences in methane emissions and to understand better the genotype x nutrition interaction in this regard such that selection of reduced  $\text{CH}_4$  emissions is conducted under the appropriate nutritional regime. In this context, selection for gut length, gut enzymes and transport functions and control over rumen microflora are all areas requiring further research.

### Nutrition

Diet formulation for pigs and dairy cattle are well advanced but there is scope to investigate alternative ingredients and particularly greater use of “co-products” from within the food chain that might otherwise have been classified as waste. There is likely to be pressure to reduce the quantity of cereals fed to pigs and cattle (in competition with biofuel production) and, in this context, alternative protein sources for pigs and increased productivity of grassland production, including more efficient use of slurries and fertilisers (to reduce  $\text{N}_2\text{O}$  losses due to de-nitrification) will assume increasing priority. Interactions between nutrition and the animal’s immune system and the way in which this impacts on animal health is an area requiring increased research effort.

### Health

Production animals can divert around 6% of available net energy supplied towards immune functions and a challenged animal expresses greater requirements for amino acids in the daily diet. It is axiomatic therefore that a healthy animal will utilise its in-feed nutrients in a far more efficient way and there will be associated benefits in terms of environmental impacts. For beef and sheep endemic disease control and reduction continues to be a high priority with further development work required on bio-security measures between farms as well as specific diagnosis and control. Recent developments in the genetic control of innate immunity offer new opportunities to reduce disease in herds exposed to pathogens.

## **With all those profits...Do retailers care about animal science?**

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Supermarkets – Do they really care?

The last 20 years have seen the meteoric rise of the multiple retailers and supermarkets in UK food retailing, to a position of their dominance in the food retail market place. Over the same period of time, livestock farmers and in particular producers of meat and milk have been challenged with abolition of quotas, reductions in direct subsidies, animal health challenges such as BSE and FMD, and rising costs of production, all of which have put pressure on farm gate prices and the viability of many farming businesses.

Rationalisation has happened in all the livestock sectors. In the dairy industry, three dairy farmers are still giving up every day. Suckler cow, ewe and sow numbers have all fallen, average herd and flock sizes have grown and production from individual animals has also risen. This increased efficiency in UK livestock agriculture has undoubtedly been driven by the prices farmers receive for their products and certainly hastened by supermarket buying strategies.

In all this, it is easy to think that supermarkets have little regard for animals and in the supporting science that generates the improved health, welfare and production traits, that generates the efficiencies that they demand. However, quite the contrary exists.

Supermarkets have grown their businesses by listening to their consumers and farm animal health is an area that consumers often feel passionate about.

This consumer concern has led most major supermarkets to adopt specific contracts, schemes and direct supply chains where excellence in animal health and welfare is paramount to their purchasing requirements. In each case, the application of the latest developments in animal science has been required by the supermarkets buying contract or promoted by the retailer. Recent examples include risk management strategies for reducing lameness in dairy cows, or vaccination schemes to reduce disease in neonatal calves.

Retailers clearly understand the benefits of healthy livestock in not only helping them generate healthy profits, but also to re-assure their customers of their provenance in delivering food of animal origin, from high welfare systems. A healthy animal science industry therefore is a key part of the current and future role of supermarkets in meat and milk retailing in the UK.

## Challenges in meat industry - impact on animal science research

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**Introduction** The meat industry in the UK faces a number of challenges centring on ongoing supply, cost of production and acceptability of product. Any successful business must focus itself on the end consumer and perhaps the biggest challenge facing farming, processing and research in the UK is to ensure that what we do moves from being production focused to become consumer focused. This single change has many implications for animal science in the UK and further afield.

**Key Questions:** When producing or researching for a market a number of key questions must be asked. These are;

- 1) What does the consumer want?
- 2) What will the customer want?
- 3) Does my current product meet expectations?
- 4) Could my product be changed to better meet customer expectations?
- 5) What would I need to change to do this?
- 6) How do I effect these changes and what would be the overall implications?
- 7) How do I verifying the change for the consumer?

**Considering the questions** Each of the above questions carries research implications. Question 1 involves simple analytical procedures to judge customer perception of what they currently receive. This should generate a list of likes and dislikes. The second question builds on the first and uses subjective judgement to assess what the customer is likely to require in the future. This type of survey will involve demographic, financial, social and technical considerations and will result in a series of predictions which should be used to guide (rather than slavishly dictate) the direction in which the producer must go. The list of likes, dislikes and expectations can then be used to focus future development, improving the good characteristics and eliminating the bad. In general, the elimination of the poor characteristics is the most cost effective of the two options, but both are necessary.

The possibility of alteration of the product is one of the most technical of the questions for consideration because it implies a solid understanding of the characteristics of the product and the factors which influence them to begin with. If the influencing factors are not fully understood, this then gives a base at which the next phase of research should commence. If changes are to be made, then a sound basic understanding of the components which may be changed is essential.

**What does the consumer want?** Each and every one of us is a consumer and we tend to have a natural understanding of what is expected once we think about it. We want a product we can trust, one we enjoy, one which is consistent in eating quality, healthy, lasts for an adequate length of time and has a good appearance. Above all else, it must offer value for money. A product which is consistently good will gain a loyal following and generate strong sales. One which is variable in taste, tenderness or appearance is immediately at a disadvantage.

**What is required from animal scientists?** Research must focus on a number of clear areas

- 1) Developing the production process to improve efficiency or welfare
- 2) Development in understanding of the key factors influencing eating quality and healthiness
- 3) Development of the product to improve eating quality and healthiness
- 4) Development of longer life products
- 5) Online verification of product integrity

**Summary** Research should only take place when at least one of the following questions can be answered positively.

- 1) Can we produce this product more efficiently?
- 2) Can we improve the method of production?
- 3) Can we improve the product itself?

**Conclusions** Research must aim to provide solutions to problems. These problems can be wide ranging, from consumer concerns through to high costs of production, but all is ultimately linked back to the overall saleability of product. We must never forget that ultimately, the consumer must be the focus of all work that we do because it is only through satisfying the consumer that Agriculture plc can truly develop and maintain a sustainable business model.

## Quantitative and Molecular Genetics, a love story

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Once upon a time, in research centres, universities and breeding companies around the world, there lived two types of scientists, the quantitative geneticist and the molecular geneticist.....

**The early years** Animal breeding based on the mating of animals of excellent phenotypes has been practiced for many centuries but major advances in animal breeding occurred with the development of BLUP. Although largely unappreciated at the time, Gregor Mendel is credited with providing the genetic understanding of heredity in 1866. DNA was sequenced for the first time in 1977 and in 2001 the first draft of the human genome was released with the first draft of the bovine genome being publicly available in 2006. Throughout this period both disciplines more or less pursued their own paths, each oblivious to the developing love story.

**Courtship** Flirting between the two disciplines intensified in the early 1990s as animal breeding changed focus from traditional quantitative methods to molecular genetics. Molecular geneticists were to identify regions of the genome associated with performance traits, commonly known as quantitative trait loci (QTLs), and quantitative geneticists were to develop the statistical methodology for incorporating such information into genetic evaluations. The latter step became known as marker assisted selection (MAS). Rumours of “genotype building” circulated where animals would be screened for QTLs and their effects summed to generate true additive breeding values. Quantitative geneticists became anxious when molecular geneticists seemed to imply that they would soon be dumped. However, MAS was not without its shortcomings. It was obvious that the successful exploitation of genomic information in animal breeding needed a stronger relationship between the two disciplines.

**Marriage** At the start of the millennium, the concept of genomic selection was introduced. Genomic selection is based on relating genetic markers, from dense marker maps of the genome, to accurately recorded phenotypes in a training population. The output is an estimate of the association between each marker and the phenotype under investigation. Animals without phenotypes are then genotyped and their marker effects summed to generate an estimate of their genetic (genomic) merit. This is similar to the “genotype building” alluded to previously except that the markers used in genomic selection are not necessarily causative mutations. The concept of genomic selection became a reality with the first draft sequence of the bovine genome in 2006. This is where the tables were turned and molecular geneticists feared being dumped! They were led to believe that it was no longer necessary to identify the gene or to understand the molecular processes underlying complex traits. All that was needed was to be able to reliably relate the desired phenotype with the genetic markers. The uptake of genomic selection was rapid, and caused a paradigm shift in animal breeding as we knew it. Genomic evaluations in dairy cattle are currently underway in most international genetic evaluations.

**The in-laws** Molecular geneticists belong to the large family of systems biology. This family is teaching quantitative geneticists the complexity of organisms, including gene regulation and expression. However, to date most quantitative geneticists are ignoring systems biology and are continuing to analyse genomic data from a predominantly statistical perspective. Conversely, quantitative geneticists are trying to teach systems biology researchers the importance of accurately phenotyping large numbers of animals. The quantitative geneticist needs to visit the systems biology household more often to discuss how the technologies and expertise developed can be better harnessed in animal breeding. A cup of tea and a chat should reap great rewards.

**Wedding anniversary** An anniversary is not only a time of celebration but also a time for reflection. With experience comes wisdom and, with the benefit of hindsight, it is easy to recognise that the complex organisms we are selecting are unlikely to be controlled by just a few major genes. At subsequent anniversaries we will still need to sit back and reflect on what we have learned. One particular lesson to be learned is that in the flush of youth, a development must be rigorously tested to determine whether it becomes a leader or a ‘has been’. The early hysteria of genomics is slowly being replaced by a more sober and realistic assessment of its true commercial value and how it fits into existing breeding programs. Has genomic selection really been tested rigorously? Rigorous testing is important to maintain credibility with industry. Poor concordance between reality and promises made may hinder future scientific endeavour in this area.

**Divorce?** A continuing and long-term relationship between molecular and quantitative geneticists is vital for sustainable genetic gain for several reasons: 1) linkage disequilibrium exploited between markers on available arrays and functional mutations will break down over generations so greater marker density is required, 2) smaller scale molecular experiments will be needed to elucidate the genetic architecture of difficult to measure traits to provide valuable “prior” information for statistical models, 3) elucidating interactions between genotype and environment may be more easily deciphered with smaller-scale experiments coupled with detailed gene expression analyses, which may be particularly important if marker effects are estimated in nucleus-herd type environments, 4) the impact on prevailing breeding goals on those traits not routinely measured may be more easily quantified using molecular genetics, 5) resolving the phenomenon of the “missing heritability” where the QTL detection studies to date have been unable to explain all the heritability of a given trait. Therefore, this is not the end of this love story, but merely the beginning.....

## Genomic selection in poultry and pig breeding: a breakthrough technology?

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**Introduction** Contrary to programs for most other species, breeding and multiplication programs for poultry and pigs are using hybridization to benefit from line specialization and heterosis. The introduction of crossbreeding created a breakthrough in poultry breeding in the 1950's and –later and less markedly so- in pig breeding. A second breakthrough technology in pig breeding –not so much in poultry breeding- was the application of BLUP breeding value estimation procedures. These technological breakthroughs have prompted the extinction of small, pure line and private farm based breeding programs and a consolidation among breeding organisations. Today very few (poultry) or some dozens (pigs) of breeding programs remain and these are exclusively (poultry) or increasingly (pigs) owned by private companies. Genomics is now truly coming of age when it comes to applications in animal breeding and this paper discusses the impact of this new development.

**Genomics of poultry and pigs** The first decades of genomics research (until 2005) produced very little in terms of tools and technologies that were applicable to animal breeding programs. Applications were mostly limited to genotyping for major genes that were largely discovered on the basis of homology with human and mouse sequences. Only after the genome assemblies of the species were completed (chicken, 2004, pig, 2009, turkey, 2009), large numbers of SNPs had become available (chicken, 2004, pig, 2008) and technologies had developed enough to allow relatively cheap high throughput genotyping, could marker assisted selection approaches really be developed. That is where we are today and we are all wondering if we are or are not at the brink of a new era that is to be heavily influenced by yet another breeding technology.

**Major genes and the infinitesimal model** Breeding programs deal with only a small number of traits that are determined by single or very few genes and selection for these is dealt with independently. Genetic evaluation for such traits is based on phenotypic testing or DNA based genotyping. Breeding value estimation systems for the far majority of (so called quantitative) traits are based on the infinitesimal model: every trait is assumed to be influenced by an infinite number of genes each of them with a small effect. The success of current breeding programs proves that this assumption cannot be totally wrong.

Indeed marker assisted selection addressing only a small number of genes underlying quantitative traits has not been successful up to now. The possibility to select for many loci combined into a single marker based breeding value may totally change the use of genomics technologies in breeding programs.

**Genome wide marker assisted selection (GWMAS)** The paper of Meuwissen *et al* (2001) showed the way towards application of GWMAS in breeding programs and this approach is now being applied or tried in its original or modified form in many animal breeding programs. Managers of successful commercial breeding programs can only realistically vary two parameters that influence genetic progress: accuracy of estimated breeding value and generation interval. Breeding programs for layer chickens and pigs may not always have minimum generation intervals e.g. because (crossbred) performance of offspring is used for breeding value estimation of selection candidates. In such cases GWMAS has the potential to cause dramatic increases of genetic progress through shortening of generation interval. Fast track programs such as applied in broilers, turkeys and pigs, which already have minimum generation intervals may suffer from less accurate breeding values at the time of selection. GWMAS has the potential to increase these very significantly. A third use of GWMAS is to increase the accuracy of breeding values for traits that are currently not or poorly measured because phenotyping is very expensive or impractical.

**Evaluation and optimization** Scientists in academia and industry are currently evaluating various options for use of GWMAS and MAS. At least a couple of generations of Genomic Selection are required to prove its effectiveness and to address many uncertain issues such as persistency of SNP effects. Just as challenging is the issue of optimization of commercial breeding programs: the cost of large scale genotyping is very high and cost of genotyping – with many options for fewer or more markers- of individual selection candidates needs to be balanced against the accuracy of breeding values provided, number of candidates genotyped, either or not after various options of pre-selecting these, to ultimately arrive at a design that yields the best competitive position (=profitability) in the mid to longer term for the company that operates the breeding program.

**Breakthrough** Our current estimates of additional genetic progress that may be obtained by application of GWMAS in commercial breeding programs for layer chickens and pigs make us believe that these are as large as for the previously introduced technologies of hybridization and BLUP breeding value estimation. Therefore: yes, the landscape of commercial breeding of poultry and pigs will change significantly in the next five years because of genomic selection.

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## Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes

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Soil physical factors interact in complex and intimate ways with the biological processes responsible for the production and consumption in soils of greenhouse gases. In many situations the physical condition of the soil can be the major factor controlling the level of emissions. Lowering the water table by drainage generally increases the release of soil carbon as  $\text{CO}_2$ , as a result of improving the supply of oxygen to aerobically respiring microorganisms – the gaseous diffusion coefficient varies by 4 orders of magnitude between well-drained conditions and saturation. This process is especially important in peats and peaty soils generally; the surface of peat soils under long-term cultivation, e.g. in the Fens and in the Netherlands and Scandinavia, has fallen by up to several metres over time. Conversely, the need for flooded, or near-flooded, conditions is an essential prerequisite for significant emission of methane,  $\text{CH}_4$ . Ebullition (bubble release) and diffusion of  $\text{CH}_4$  through the aerenchyma channels in the stems and leaves of rice plants, and of natural wetland plants, are both important pathways contributing to  $\text{CH}_4$  emissions. A strong inverse relationship is often observed between the mean depth to the water table and the rate of methane emission. Aerated soils, on the other hand, are a sink for atmospheric  $\text{CH}_4$ , through microbial oxidation. The main control on oxidation rate is gas diffusivity, and well-drained conditions promote the entry of atmospheric  $\text{CH}_4$  to where the methanotrophic organisms reside in the soil. The temperature response is small, because of the controlling influence of diffusion. Nitrous oxide,  $\text{N}_2\text{O}$ , is the third long-lived greenhouse gas produced in soils. Emission of  $\text{N}_2\text{O}$  depends on the presence of mineral N forms (ammonium and nitrate), but is also greatly influenced by physical conditions: emissions increase markedly with increasing temperature, attributed to increases in the anaerobic volume fraction, brought about by an increased respiratory sink for  $\text{O}_2$ , causing increases in the rate of microbial reduction of nitrate to  $\text{N}_2\text{O}$  – the denitrification process. Increases in soil water-filled pore space also result in increased anaerobic volume, because of the diffusion barrier provided by the water, limiting the ingress of  $\text{O}_2$ ; again, the outcome is often an exponential increase in  $\text{N}_2\text{O}$  emission. However, when soils are nearly or completely flooded,  $\text{N}_2\text{O}$  emissions decrease to low levels – the diffusion barrier presented by the water acts now to prevent the diffusive escape of  $\text{N}_2\text{O}$ , and it is reduced by microbial enzymes to harmless dinitrogen,  $\text{N}_2$ . This phenomenon is being explored with a view to using deliberate denitrification in riparian zones to remove nitrate from field drainage water, and thus minimize water pollution, without the “pollution swapping” that would result from replacing nitrate leaching by enhanced  $\text{N}_2\text{O}$  emissions.



## **Fate and transport of nutrients and pathogens applied to the land**

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Land application of biosolids and manure is generally considered beneficial. It adds nutrients and organic matter to the soil. It was especially beneficial early in the nineteenth century when chemical fertilizers were expensive and in short supply. However, currently most agricultural soils have sufficient nutrients and application of additional nutrients may lead to ground water pollution or eutrophication of surface waters. In addition there is a concern about the offsite movement of pathogens

Once land applied, fate of the pathogens and nutrients depends partly on the hydrology of the watersheds. The hydrology of the watershed in turn depends on the depth of the restricting layer for water movement in the soil profile and the slope of the hillsides. Effectively managing and reducing nonpoint source pollution should take the hydrology and consequently the type of landscape into account.

In general, watersheds that have shallow restrictive layers have limited base flow during the summer, high peak flows during the wet periods and short residence times for water. The soils in these watersheds are periodically saturated due a perched water table on top of the restrictive layer. These saturated areas are the source of the surface runoff and can transport dissolved and particulate phosphorus and pathogens to the stream. At the same time these saturated areas are ideal for removal nitrate from the water by denitrification. Thus watersheds with shallow soils are characterized by low nitrate and elevated P concentrations in the surface waters. Although traditionally it was recommended that land applications should occur on the flattest land (located usually near the stream), current nutrient management practices on the watersheds with shallow restricting layers (such as the New York City drinking water source watersheds) consists of land application on the hillsides during wet times. Hillsides are unsaturated and rain water infiltrates before it exfiltrates down slope as interflow and becomes surface runoff in the saturated areas. In the New York City source watersheds, where nutrient management practices were implemented that avoided manure spreading on wet areas, dissolved phosphorus concentration have been decreased significantly.

When the depth of the restrictive layer increases, more water can be stored in the landscape, summer base flows increase and peak flows decrease. The watersheds with deep soils and no restrictive layers generally have a permanent ground water and saturation (if any) is limited to near stream areas. Preferential flow can transport small quantities of land applied chemicals rapidly to the permanent ground water. When these leached chemicals are toxic at low concentrations, (i.e., pathogens and pesticides), groundwater can become polluted and in some cases unsuitable for drinking water when more than 0.1% of the amount applied leaches. For other chemicals such as nitrate the amount transported via preferential flow paths is not sufficient to bring the groundwater concentration above the drinking water limit. In this case, nitrate concentrations in groundwater are exceeded when the nitrogen is applied in excess of crop needs. Phosphorus concentrations in groundwater are usually small because the phosphorus will be adsorbed to the soil. However, dissolved P concentration in tile lines can be elevated when P leaches via preferential flow path from the surface to the tile lines. Structural best management practices to prevent groundwater pollution due to both matrix and preferential flow are not generally available and the best way to prevent groundwater pollution is by applying less at times when groundwater is being recharged.

## Global challenges: land use for food and energy – 2020 and beyond

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Until recently, there has been a widespread working assumption in many rich countries that problems of food production have been solved, and that food security is largely a matter of distribution and access to be achieved principally by open markets. The events of 2008 challenged these assumptions. Today, for the first time in almost a quarter of a century, food is back on the political agenda. There are many reasons for this including:

- the increased demand for food, especially meat, in the rapidly growing economies of Asia;
- the demand for land to grow biofuels;
- drought and severe flooding in diverse countries, and awareness that water for crop and pasture production is limited;
- diseases associated with the food consumed in rich countries; and
- the increasing awareness that changing climate may profoundly affect the zones of major food production globally.

The imperative to achieve food, energy and water security for an increasing global population with an increasing demand for meat is further complicated by environmental changes such as altered land use, modified biogeochemical cycles and changed and/or more variable climate. The growth in the human population from about 3.0 billion in 1960 to 6.6 billion in 2008, coupled with increased income and changes in diet, has been accompanied by substantial increases in crop and animal production (2.67-fold for cereals, 1.64-fold for roots and tubers and 4.02-fold for meat). This increase will need to be maintained if the projected population of 9 billion by about 2040 is to be sustained. Past increases in crop production have occurred as a result of both extensification (altering natural ecosystems to produce products) and intensification (producing more of the desired products per unit area of land already used for agriculture or forestry). Of the world's 13 billion hectare land surface, only about 3 billion ha is suitable for crop production and about one half of this is already cultivated (1.4 billion ha in 2008). The remaining potentially cultivatable land is currently beneath tropical forests but it would be undesirable to convert this to arable land because of the effects on biodiversity conservation, greenhouse gas emissions, regional climate and hydrological changes, and because of the high costs of providing the requisite infrastructure. While extensification may contribute significantly to crop production in sub-Saharan Africa and South America, elsewhere intensification will be the dominant means of increasing production.

It is widely recognised, then, that only a small proportion of future increases in crop production will come from the cultivation of new land, but forest may continue to be cleared for ranching and grazing of animals. The main means of intensifying crop production will be through increased yields per unit area together with a smaller contribution from an increased number of crops grown in a seasonal cycle. As cereal production (wheat, maize and rice) has increased from 877 million t in 1961 to 2,342 million t in 2007, the world average cereal yield has increased from 1.35 t ha<sup>-1</sup> in 1961 to 3.35 t ha<sup>-1</sup> in 2007, and is projected to be about 4.8 t ha<sup>-1</sup> in 2040. Simultaneously, per capita arable land area has decreased from 0.415 ha in 1961 to 0.214 ha in 2007. Put another way, had the increases in yield of the last 60-70 years not been achieved, almost three times more land would have been required to produce crops to sustain the present population; land that, as indicated above, does not exist except by using some that is unsuitable for cropping. Continued intensification of crop and animal production systems is anticipated.

Climate change will bring opportunities for agriculture in some regions but enhance existing problems of food and water security elsewhere, particularly in communities that are poor and with limited capacity to cope with, or adapt to, environmental shocks. Food security is underpinned by effective food systems, which are a set of dynamic interactions between and within biogeophysical and human environments (Gregory *et al.* 2005; Ericksen, 2008). A substantial challenge now and beyond 2020 will be to achieve the levels of food, energy and fibre production required in ways that contribute to fair access and utilisation by all human communities sustainably.

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## Re-defining efficiency of food production by livestock

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Livestock, particularly ruminants, can eat a wider range of biomass than humans. In the drive for greater efficiency, intensive systems of livestock production have evolved to compete with humans for high-energy crops such as cereals. Feeds consumed by livestock were analysed in terms of the quantities used and efficiency of conversion of human-edible (“edible”) crops and crop co-products into milk, meat and eggs, using the United Kingdom as an example of a developed livestock industry.

Some 45 million tonnes of forage DM were consumed in 2008/9 by the UK ruminant livestock population, of which 70% was grazed pasture. Almost 13 million tonnes of raw material concentrates were used in the UK animal feed industry in 2008/9, of which cereal grains comprised 5.3 and soyabean meal 1.9 million tonnes. The proportion of edible feed in typical concentrate formulations ranged from 0.36 for milk production to 0.75 for poultry meat production.

Example systems of livestock production were used to calculate feed conversion ratios (FCR- feed per unit of whole milk, carcase fresh weight or total egg mass). FCR for concentrate feeds was lowest for milk at 0.27 and for the meat systems ranged from 2.3 for poultry meat to 8.8 for cereal beef. Differences in FCR between systems of meat production were smaller when efficiency was calculated on an edible input/edible output basis, where spring-calving/grass finishing upland suckler beef and lowland lamb production were more efficient than pig and poultry meat production. Despite the significant roles of grassland and crop co-products in the nutrition of UK livestock, with the exception of milk and upland suckler beef production, FCR for edible food protein into animal protein were greater than 1.0. FCR may be improved to give values less than 1.0 by substituting concentrates with high-quality grazed and conserved forages in lowland grass-fed beef systems and in lamb production. With the exception of cereal beef it was possible to achieve edible protein FCR of 1.0 by replacing cereal grain and soyabean meal with cereal co-products in concentrate formulations, highlighting the potential for reducing the proportion of edible ingredients in concentrate formulations to increase efficiency of edible feed use by livestock. Total water use was lowest for milk (746 litres/kg whole milk) and highest for beef (7952 litres/kg bone-in carcase). There is need for research to improve efficiency of supply, conservation and delivery of water to livestock units.

Greenhouse gas emissions per kg edible protein were highest for ruminant meat production and lowest for poultry meat. The potential of grazing in carbon sequestration in soil and in habitat conservation for enhancing biodiversity and landscape value should be recognised and factored into debates on future land use and rural development.

## Greenhouse gas emissions and Irish agriculture in 2020

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**Background** With agriculture contributing over one quarter of Ireland's total greenhouse gas (GHG) emissions, the Irish agricultural sector is relatively unique in the EU. The EU has set Member State targets for reducing GHG emissions. For Ireland, at a minimum, the target is a reduction of 20% by 2020 in overall GHG emissions from all sectors of the economy, relative to the 2005 level. The reduction target would increase to 30% if a successor agreement to the Kyoto protocol is achieved. It remains unclear to what extent the agricultural sector might be required to contribute to the achievement of Ireland's GHG emission reduction target. However, in the context of reducing agriculture's GHG emissions, those agricultural activities that contribute to GHG emissions and that are currently marginally economic or uneconomic (in the sense of their profitability) will almost certainly be the first focus of policy makers in their search for the least cost abatement policy.

While science and technology holds out the promise of a more carbon efficient agricultural sector, it should be understood that there are limits to what can be achieved within the short timeframe to 2020. The contribution to GHG abatement of the technologies that flow from agricultural production research programmes will first have to be accepted by the IPCC. Farmers will then have to adopt the technologies proposed. Experience suggests that neither of these processes is rapid or guaranteed.

A 30% GHG reduction target implies a level of emissions from Irish agriculture in 2020 of 13.29 Mt CO<sub>2</sub> Eq. (exclusive of emissions by agriculture from fuel combustion). Such a reduction in emissions could not occur overnight and would need to take place gradually in the period to 2020. In this paper we examine the likely future level of GHG emissions from Irish agriculture in the absence of technical abatement strategies.

**Methods** Future GHG emission levels from agriculture will be the product of emission factors and future levels of agricultural activity. Considerable work has been done to provide GHG emission factors which are specific to Ireland, notably the work by O'Mara *et al.* (2006). The other element of the future GHG emissions equation, are the future levels of agricultural activity. We need to use economics to establish the likely future level of activity.

In Ireland this economic contribution is provided by the FAPRI GHG model. It is a sister component of the FAPRI-Ireland agricultural sector model and the FAPRI EU-GOLD agricultural sector model described in Hanrahan (2001). The agricultural sector models allow projections of future levels of agricultural activity and the FAPRI GHG model then uses a mix of national and default emission factors to convert this activity to annual estimates of GHG emissions out to 2020.

While the primary concern of this research programme is to understand how new agricultural policies or new trade policies will impact on the level of future agricultural GHG emissions, this work also provides the set of agricultural GHG emission projections for Ireland which is used by Irish Government Departments and reported by Ireland's Environmental Protection Agency in fulfilment of Kyoto Protocol requirements.

**Results** Under the Reference scenario, GHG emissions from Irish agriculture decrease by 11 % from 18.9 Mt CO<sub>2</sub> Eq. in 2005 to 16.6 Mt CO<sub>2</sub> Eq. in 2020. Under the Reference scenario there is a decrease in drystock animal numbers, however, in the absence of milk quotas, the impact on GHG emissions of this reduction in activity is largely offset by increasing emissions per cow and an increase in the number of dairy cows and their progeny. Under the Reference scenario the total cattle population in Ireland declines by 10% between 2005 and 2020. The production of beef in Ireland also declines, with production in 2020 under the Reference scenario 14% lower than in 2005.

**Conclusion** Agricultural policy and market returns will lead to a reduction in GHG emissions from agriculture over this decade. However, even with such reductions, the level of emissions from agriculture in 2020 is likely to be well short of a 30% GHG emission deduction target. A further 3.5 million tonnes CO<sub>2</sub> eq. emission reduction would be required to meet the target by 2020. This would leave policy makers with a number of tough choices. Impose larger cuts in other non-emissions trading sectors or impose policies which restrict the size of the agriculture sector so that emissions are further reduced. The implementation of technical abatement measures in agriculture would limit the extent to which agricultural production would need to be reduced to meet possible agricultural emission reduction targets.

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## Faeces: A model for hindgut function?

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**Introduction** The horse is a non-ruminating monogastric herbivore that has evolved over millions of years to a grazing and browsing existence, in which it has adapted to graze on high fibre, low energy fodder by the aid of a complex microbial community (Milinovich *et al.*, 2006). However, domestication of the horse has led to this natural feeding pattern being disturbed, and consequently gastrointestinal disease is the single most important cause of mortality in the domestic horse (Daly *et al.*, 2001). Nonetheless, despite its importance, the microbial community of the equine hindgut has received relatively little attention. An understanding of the microbiology of the equine gastrointestinal tract (GIT) is essential in improving our understanding of digestive processes, and for the prevention and treatment of disease involving the GIT. While microbial diversity has an important role to play in hindgut function and disease, its effect on the ability to degrade certain feedstuffs is of equal importance. Therefore, the capacity to assess the effects of feedstuffs on the large intestinal environment is essential to further our understanding of normal and disease processes within the GIT of the horse. However, many studies investigating microbial diversity and fermentation characteristics within the equine hindgut typically used animals specifically euthanased for the purpose, or surgically-modified. This is expensive, and highly invasive, and there is an urgent requirement to replace and refine these methods, with more cost-effective, welfare-friendly alternatives.

**Assessment of microbial populations in the hindgut of the horse** Current knowledge of gut microbial ecology and diversity is almost exclusively based on the use of classic culture-based methods that are often laborious, time consuming and may only recover a fraction of the microbial diversity present within the gut (Daly *et al.*, 2001). However, advanced modern molecular methods, such as real-time semi-quantitative PCR (Q-PCR), are culture-independent tools for accurate and sensitive quantification of individual bacterial species, as well as total bacterial numbers (Nadkarni *et al.*, 2002). Published data on the identification/quantification of intestinal bacteria using Q-PCR technology, which is a more accurate and sensitive alternative to conventional end-point PCR-based methodologies has been applied to study diet-dependent shifts in the bacterial populations of the rumen (Tajima *et al.*, 2001), infant gut (Haarman and Knol, 2005) and, more recently, to the hindgut of the horse (Hastie *et al.*, 2008). The latter study was conducted to determine the abundance of candidate cellulolytic (*R. flavefaciens*; *F. succinogenes*) and non-cellulolytic (*S. bovis*) bacteria in frozen and lyophilised lumen contents from the caecum, ventral and dorsal colon, and rectum of healthy horses. Results showed frozen and lyophilised samples to contain similar levels of *R. flavefaciens*, *F. succinogenes* and *S. bovis* relative to total bacterial load in luminal contents obtained from the dorsal colon and rectum, indicating that, similar to other monogastric animals (Whitehead and Cotta, 1993), equine faecal material could reflect the microbiological characteristics of the distal colon. Furthermore, data from the frozen luminal contents indicated similarities between the three bacteria in the ventral colon, dorsal colon and rectum, potentially allowing faeces to be used as a model for the whole colon. This would subsequently allow faeces to act as a model for the distal colon facilitating accurate determination of changes in gut microflora without the need for surgically modified animals or the use of slaughter material, which allows for no information on the animal's health or dietary management.

**Assessment of fermentation in the hindgut of the horse** In recent years the *in vitro* gas production technique of Theodorou *et al.* (1994) has been used to assess the effects of feedstuffs on large intestinal environment, using caecal fluid (McLean *et al.*, 1997) and more recently faeces (Murray *et al.*, 2005) as the source of inocula. This technique has also been used to assess the fermentative capacity of equine faecal inocula obtained from individual ponies, as an indicator of hindgut microbial activity (Murray *et al.*, 2006). It has been hypothesised that there may be differences in fermentative capacity between individual horses as a result of microbial diversity, and that these differences may be particularly evident in animals with a history of diseases affecting the GIT, such as laminitis. However, recent results (Murray *et al.*, 2009) show no difference between the ability of faecal inocula obtained from ponies with or without a history of laminitis to ferment grass hay, starch or inulin. Nevertheless, this work needs to be expanded to further validate the use of faecal inocula in the gas production technique as a model of hindgut function. In particular, there is a need to determine inter-animal variability in terms of hindgut regional variation in inocula source.

**Conclusion** By establishing a model of hindgut function using non-invasive techniques, further research can explore the role of key bacteria in different stages of gastrointestinal disease, and not just at the terminal stages following euthanasia. If a conclusive link can be established in healthy horses using faecal material to give an indication of bacterial community structure, then faecal material could potentially become a non-invasive tool to accurately monitor changes in the colonic bacterial populations in response to diet and other environmental factors, and allow for the accurate measurement of potential disease-causing bacteria in the colon.

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## Horse trials and tribulations: Balancing breeding, performance and welfare metrics

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**Introduction** Man has domesticated many animal (and plant) species primarily for the benefit of human culture in one form or another. As distinct from animals bred predominantly for the provision of food or those designed purely to function as companions or pets, the horse is generally bred to participate in one or more competitive sporting activities (Murphy & Wall, 2009). While others fulfil the role of companions, pets and/or workmates and even partners in some instances, the horse is commonly referred to as an athlete. Indeed, equine athletes are often the primary attraction in popular spectator sports such as horse racing, showjumping, polo and dressage among others, where humans participate as riders, drivers and trainers (Murphy & Arkins, 2007). The equestrian sports regularly attract very significant financial investment, ownership status, sponsorship arrangements and the opportunity to win or earn what is often very lucrative prize money, kudos and iconic status for man and beast.

There is however, a sector of society that espouses the view that these purpose bred sporting creatures are little more than entertainers or animal gladiators. This particular societal view contends that the animals' participation in the chosen sport is at best ambiguous. Their involvement on a truly voluntary basis may be suspect where there is easy justification for the ready disposal of animals when they have passed their 'sell by date'. Some thirty years ago, Jeffcott *et al.* (1982) published the initial assessment of British Thoroughbred breeding and racing, which examined the reasons why racehorses failed to fulfill their potential to train and race. Indeed, the term 'wastage' has been used to describe the phenomenon of unwanted surplus due to failure to compete in several subsequent equine studies (Rossdale *et al.* 1985; Bailey *et al.* 1997; Olivier *et al.* 1997). These 'wastage' studies, which detailed the causes and extent of horses failing to compete, have been useful in terms of evaluating athletic performances and reproductive success of the horses. But little work has been undertaken to address the increasingly important issues of over-breeding, under-performance and welfare concerns within the wider horse industry. Indeed, there now appears to be a growing issue of public concern aimed at the wider industry in terms of what happens to horses when they fail to train and/or perform (Murphy & Wall 2009). In reality, there are several different circumstances and conditions that could lead to athletic performances deemed as lack lustre or insufficient, or when what were previously successful careers decline due to the occurrence of injury/disease or some other issue that either radically reduces or terminates the animal's value or utility worth for owner/breeders.

In keeping with the Roman gladiator analogy, the success of some animal field sports (for example game birds) depends upon killing the animal in the pursuit of sport. Although not the case in any horse related activity, death happens on occasion and such fatal accidents are often reported as high profile incidents in the media. In fact, these events and the dearth of reliable data (including valid scientific studies on risk factors affecting retirement) on the fate of horses when they leave their sporting careers alive is often highlighted by societal indifference to or condemnation of many horse sports. For example, Animal Aid actively campaigns against horse racing through their official website ([www.animalaid.org.uk](http://www.animalaid.org.uk)) and provides statistics of fatal horse injuries that occur across UK racecourses. As in any formal breeding programme, breeding equine athletes or other animal gladiators is a game of skill and chance involving basic animal science principles. Generally, the primary goal is to breed horses that will win races or succeed in competition. However, the question must be asked if the human owner rather than the horse *per se* is the real winner? There may well be a fundamental assumption on the part of the breeder at the outset that his/her horses will always be well cared for and be useful and adaptable athletes. Every horse breeder dreams of breeding the horse with great potential for high level performance as an athlete in some equine career. However, as is the case with all normal distributions, all horses simply cannot be elite and most horses are merely average in terms of ability with only very few truly talented horses in any of the equestrian disciplines. This issue now poses a serious threat and presents an immediate challenge to the horse racing authorities and other equine governing bodies/federations. These institutions must all share in the application of proper scientific techniques to benefit the horse by reducing over-production and unnecessary wastage and improving welfare provisions.

**Conclusion** Humans have capitalized on the evolution of the horse to fulfil many of mans' sporting pleasures. The horses' innate motivation to gallop and ready acceptance of training has provided kudos, enjoyment and financial reward for countless breeders, owners and handlers. While the elite equine athletes are much admired individuals – often achieving legendary status, current societal standards demand immediate action to balance some indiscriminate breeding objectives, performance demands and appropriate welfare for the good of the horse.

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## Nutritional management of captive cheetahs; is the domestic cat an appropriate model?

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The nutritional requirements of captive cheetahs (*Acinonyx jubatus*) are unknown, and dietary regimes employed by zoological institutions are typically based on domestic cat (*Felis catus*) requirements and/or field studies of free-ranging cheetahs. Where data is lacking in regards to the specific nutrient requirements for cheetahs, information from studies in the domestic cat is often extrapolated to the cheetah. A review of the nutritional and reproductive anatomy and physiology of cheetahs and domestic cats was undertaken in order to evaluate the use of the domestic cat as a model species for the captive cheetah. Evaluation of reported anatomical features of domestic cats and cheetahs revealed that, relative to body weight, the internal organs of both species are remarkably similar. In fact, the cheetah appears to bear closer resemblance to the domestic cat in this regard than it does to other large felid species. The shared carnivory and highly conserved genome of the Felidae also appears to have resulted in a number of analogous nutritional metabolic pathways. For example, there is evidence that the cheetah and domestic cat possess equivalent metabolic activity for essential fatty acids, phenols, and xenobiotics. However important differences were detected in values reported for maternal milk composition, vitamin A transport in the serum, as well as growth and developmental features.

A series of experiments were conducted which enabled the investigation of potential inter-specific differences in cheetah growth, the efficacy of using milk replacers formulated for use in domestic cats to hand-rear cheetah cubs, as well as the serum metabolites produced following exposure to a dietary isoflavone. In the first study, the nutrient composition of two milk replacers (one from South Africa, and one from North America) which are used in hand-rearing cheetah cubs was determined. These diets were fed to cheetah cubs along with an indigestible marker (titanium dioxide) during a 14-day feeding trial. Faeces from cubs consuming each of these diets (n=4 on formula 1, and n=2 on formula 2) was collected on the final day. Faeces were then analysed for crude protein, fat, amino acids and dry matter in order to calculate the digestibility of each nutrient. Mean apparent faecal digestibility for both formulas was > 90% for all nutrients analysed. However, the total crude fat content of both formulas was lower than reported for maternal cheetah milk and both formulas were deficient in at least one of the essential fatty acids  $\alpha$ -linolenic, linolenic and/or arachidonic acid. Both formulas were low in the majority of essential amino acids and one formula contained an excessive carbohydrate fraction, at the expense of its protein content. Where data was lacking for cheetah maternal milk, comparison with domestic cat milk revealed excess concentrations of a number of minerals (K, Fe, Zn and Cu), while vitamin D<sub>3</sub> was not detected in one formula and only present in concentrations below the minimum requirement for domestic cats in the second formula. Therefore, despite their apparently high digestibility, neither formula was complete or balanced relative to maternal cheetah milk, and/or the requirements established for domestic cats. The results of these dietary analyses indicate that these milk replacers may not provide optimal nutrition for growth in cheetah cubs when used for extended periods. Of particular concern is the finding that these formulas were deficient in one or more essential nutrient, such as vitamin D<sub>3</sub> and arachidonic acid.

In a further study, daily body weight, feed and energy intake data was collected from 18 hand-reared cheetah cubs. Growth was approximately linear ( $R^2 = 0.95$ ) prior to weaning, but over the entire age range it exhibited a sigmoidal shape with an asymptotic plateau averaging 57 kg. Energy intake associated with the pre-weaning rate of growth was approximately twice the basal energy requirement calculated from mammalian energetic equations. Regression analysis determined a relationship between metabolic body weight, daily weight gain and metabolisable energy intake, which may be useful in predicting energy intake requirements for hand-reared cheetah cubs. However, comparison with an equation developed previously for suckling kittens indicated that maintenance energy requirements in the cheetah may be proportionally higher than the domestic cat, while the energy for gain factor calculated for cheetah cubs was lower than reported for the domestic cat at a similar age.

In a third study, 4 captive adult cheetahs and 6 adult domestic cats were provided with a single oral bolus of the isoflavones genistein and daidzein. These isoflavones are found naturally in some commercially prepared diets used to feed captive cheetahs in international facilities, but are known to have biological activity in other species (including perturbation of the reproductive system). Five juvenile cheetahs were also included in the study since the milk replacer they were consuming was found to contain these isoflavones. Pharmacokinetic analysis revealed that both species absorbed and excreted isoflavones in the urine and faeces, and both are capable of metabolising genistein and daidzein to alternative forms in the blood, which may represent de-toxification. However, the capacity of the cheetah to conjugate genistein and daidzein appears lower than that of the domestic cat. Furthermore, conjugation appeared reduced in juvenile cheetahs, which may result in greater susceptibility to isoflavone-induced physiological changes.

These findings indicate that differences in nutrition and physiology exist between the domestic cat and cheetah, and as such care must be taken when attempting to use the domestic cat as a model for the cheetah. In general, the assumption that nutritional guidelines developed for the domestic cat are applicable to the nutrition of captive cheetahs requires further validation. While a number of similarities exist between adult domestic cats and cheetahs, many nutrients or metabolic pathways are yet to be investigated. Likewise, the similarities identified between the cheetah and domestic cat cannot necessarily be extended to the rest of the Felidae family. Importantly, the domestic cat does not appear to provide an appropriate model for growth in cheetahs.

## Increasing milk production from grassland with reduced environmental impact

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Several reports, directives, regulations and initiatives challenge dairy systems at the environmental level. At the same time, the dairy sector must face other challenges: greater volatility of milk prices, reduction in non renewable resources and the consequences of global climatic change. Today great variety exists in dairy farming systems, not only between but also within countries/regions. In this context, dairy systems maximising grassland utilisation have many opportunities to combine and maximise economic and environmental performance. From the economic point of view, comparisons made at world level show that dairy systems maximising grass utilisation appear highly competitive. On the environmental level, the various roles of grassland in providing regulating and supporting services are widely recognized although the interaction and balance between these different functions differ between regions in Europe. The objective of this paper is to review existing knowledge for developing productive, efficient and environmentally friendly dairy systems based on grassland utilisation.

**Ideal forage** Net energy and metabolic protein content of fresh forages are high but intake is low compared to total mixed rations. Consequently fresh grass alone prevents high genetic merit cows fully expressing their milk potential. However several trials have shown that relatively high milk production (i.e. 7000 kg/lactation) is achievable with high genetic merit cows on grass based systems. The challenge is to increase fresh forage intake at grazing. Increasing leaf blade mass by appropriate grazing management in early season plays a major role in increasing herbage intake over the entire grazing season. Using grass-legumes mixtures also increases intake compared to pure grasses, the higher the clover content the higher the difference. Because voluntary DM intake of legumes is 10 to 15% greater than that of grasses of similar digestibility, pure legume silage and legume dominated silages increase milk yield compared to pure grass silage when cows are fed indoors. From an agronomical point of view there is interest in extending herbage growth season. Many results have shown that the first N application is important to stimulate the early growth of grass. New perennial rye grass varieties look also very promising. The difficulties in maintaining well balanced grass-legumes mixtures, the difficulties of legumes silage conservation and the slow growth rate of clover in early spring remain the main reasons for the preference of pure grass swards and required further research.

**Ideal cow** The improvement of genetic merit for milk production no longer appears to be a priority. Cows having high longevity are required. Inflated replacement rate, that it is due to infertility or other reasons, reduces efficiency because first lactating animals produce less than adult cows and thus the number of lactating animals and heifers as well as the amount of forage required to feed the herd increase for a given amount of milk. Older cows are also more efficient in converting forage into milk because intake capacity increases with the rank of lactation. Several trials have shown that cows selected solely on milk are not well suited for seasonal grass based systems that required a 365 day calving interval. For these systems highly fertile cows are required. Infertility problems are less acute when compact calving is not required as for example when high quality forage is available all around the year. Here, lengthening of lactations offers several advantages such as reducing non productive periods and limiting the inherent risks at the beginning of lactation but this strategy requires cows having a high persistency of lactation. The most efficient cow is that which produces the maximum per kg of forage intake or per kg of live weight. Thus Jersey cows appear to be very efficient but heavier cows generally produce more milk and it is probable that in practice the effect of the weight is not very relevant for animal effectiveness. It is also advisable to reconsider, at least in marginal zones, the interest of dual purpose breeds which make it possible to produce 6 to 7.000 kg of milk by lactation and one calf per year, primarily from grass, and to ensure a greater stability because of the double source of income (milk and meat). All the more interesting to have a system that is frugal in concentrate use.

**Ideal system management** With regards to food security and high land prices, maximising milk yield per unit area is more than ever a challenge for research. In low input systems this requires a need to convert the whole of forage produced into milk thus avoiding losses at grazing and during forage conservation. Given the high feeding value of fresh forages there is interest to extending the grazing season as much as possible. Several trials conducted in Northern Ireland, in Ireland and in France have shown there are considerable opportunities to extend the grazing season in early spring and/or in late autumn (at least in West Europe) thereby reducing costs associated with indoors feeding systems, increasing milk yield and consuming almost the full amount of grass that is produced. From an environmental point of view, nitrogen losses under grazed grasslands remain moderate for fertilization applications lower than 250 kg of mineral N/ha/year. However in regions with pasture-arable crop rotation the ploughing of grazed pasture is followed by rapid N mineralization. Using catch crops during winter is recommended as this contributes to reduced nitrate leaching compared to bare soil while providing forage for animals. Recent life cycle assessments indicate that forage legumes can contribute to reduced global warming potential and the consumption of non renewable energy per kg of milk compare to N-fertilized grass based systems and to conventional intensive systems using high amount of concentrates. However data in the literature are still relatively scarce and further investigation is warranted to better quantify the benefits and risks of grassland based systems relevant to their management.

**In conclusion** Forage legumes will undoubtedly constitute an important pillar for the development of future dairy systems with high environmental and economic performances. Selection on functional traits for more robust cows and adaptations of lactation and management systems will constitute others pillars.



## Increasing food production from grassland with reduced environmental impact - progress in red meat production from grassland

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**Introduction** This paper will review recent research into the impacts of grazing on biodiversity and environmental characteristics in the hills and uplands, strategies to reduce environmental impacts of lowland grazing systems', mitigation strategies to reduce greenhouse gas emissions (GHG) from grass-based systems and how grazing can impact on the nutritional quality of red meat.

**Impact of grazing in maintaining biodiversity in the hills and uplands** Reducing sheep grazing intensity has been found to lead to rapid increases in sward height and herbage mass, but only slow changes in structural diversity and plant species and no appreciable short-term increases in biodiversity (Holland *et al* 2008). Consequently, environmental policies which involve simple reductions in numbers of livestock may not lead to increased biodiversity. Reports suggest that suckler cows selectively graze grass species such as *Molinia caerulea* (Critchley *et al*, 2005) implying that management options which increase structural diversity such as seasonal grazing or mixed grazing may be more effective. As well as animal species, breed effects may be relevant as research with sheep (McCloskey *et al* 2009) and cattle (Umstatter, personal communication) has demonstrated that choice of breed has an impact on grazing patterns in hill environments (McCloskey *et al* 2009). These factors may affect the land manager's ability to manage biodiversity.

**Strategies to reduce nutrient losses from lowland grassland systems** Nutrient losses from grazing systems can be reduced by appropriate management of fertiliser applications and adopting new techniques for spreading slurry (Frost *et al* 2007). However, the main challenge facing grassland systems is to reduce nitrogen (N) losses resulting from urine deposition. Evidence that N losses from mixed grass/clover swards are lower than pure grass or pure clover swards (Loiseau *et al* 2001) suggests this may be one mechanism by which losses can be reduced.

**Mitigation strategies to reduce GHG emissions from beef & sheep production systems** The key mechanism by which carbon footprint of beef and sheep production can be reduced is to improve efficiency i.e. reduce dry matter intake/kg output (Dawson *et al* 2009). As well as improving overall feed efficiency, Hyslop (2008) has shown that using rapid finishing systems significantly reduces the overall GHG emissions from suckler beef production systems compared to longer duration finishing periods. For conserved forage-based diets this can be achieved through improving diet quality (Yan *et al* 2009). However, conflicting reports between quality of grass-based diets and methane emissions has been obtained (Hart *et al* 2008; Yan and Mayne (2008). The potential benefits of grazing forages containing condensed tannins (Woodward *et al* 2001) or with a high proportion of clover and high sugar grasses (Lovett *et al* 2004) as a strategy to reduce methane emissions is also recognised.

**Nutritional quality of meat produced from grassland** A number of research studies have demonstrated the beneficial effects of offering fresh grass relative to conserved forages or concentrates on fatty acid composition of meat. In addition, there is evidence that meat produced from botanically diverse pastures has higher concentrations omega-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) relative to ryegrass-based pastures (Moloney *et al* 2008).

**Conclusions** The appropriate selection of animal species or combination of species and breed will influence the ability to manage biodiversity in extensive areas of the UK and Ireland and must form an integral part of future strategies for these areas. Current research indicates that mitigation strategies to reduce the carbon footprint of red meat production systems involve high output rapid finishing systems using cereal inputs. The challenge is to achieve this from grazed resources through increased use of grass/clover swards and more efficient grass-based systems. In terms of meat quality, the benefits of grass-based systems of beef production are recognised and should be harnessed as a marketing tool to promote these systems.

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## Infertility in the high-producing dairy cow: Where is the light at the end of the tunnel?

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**Introduction** Dairy cattle have been traditionally selected for milk production and milk components (fat and protein). The traditional approach made sense because farmers are paid for milk. Dairy selection has changed in the past decade. Secondary traits, including reproduction traits, are being included in indices by specifically decreasing the emphasis on production. Greater emphasis on non-production traits reflects the industry desire for functional dairy cattle with greater fertility. The hope is that appropriate economic weightings are placed on secondary traits so that slowed progress in milk production is offset by improved farm economics created by more functional cows. Few will argue that high fertility has value. The real question is how much value (economic weight) does it have? This later question is particularly difficult when one considers that selection indices are designed for use by the national dairy herd that is comprised of increasingly diverse production systems.

**Postpartum body condition and fertility** Regardless of system, modern dairy cows are thinner than their ancestors. Given limited capacity to consume adequate dietary energy, dairy cows mobilize fat to support lactation. Improvements in milk production occur more rapidly than improvements in the capacity for cows to consume additional energy or the willingness of farmers to provide more feed. Thus, body fat is the obvious source of the additional energy. Once dairy cows begin lactation, they will migrate toward their body condition set point through the coordinated control of both feed intake and the depletion of adipose tissue. Their body condition during lactation affects their reproductive performance. Cows with a high body condition during lactation have high fertility whereas cows with a low body condition during lactation have low fertility. Reproductive tissues sense energy demands and function poorly during low body condition.

**Hormonal links between body condition and fertility** Growth hormone (GH) is an anabolic hormone that is increased during early lactation. It has the capacity to antagonize insulin action. Antagonizing the actions of insulin has a nutrient partitioning effect through which the production of milk is favoured. High producing dairy cows have high concentrations of GH and low concentrations of insulin. Dairy cows also suffer from insulin resistance (insensitivity to insulin manifested at the tissue level). The large increase in circulating GH concentrations during early lactation drives body condition loss. After this initial period of GH action, there is a second period where GH remains elevated. The long-term steady-state blood GH concentrations may ultimately determine the body condition for the individual cow because GH antagonizes lipogenesis. Many of the mechanisms that control reproduction are linked directly to the nutritionally-controlled hormonal milieu of the animal. Secretion of LH and FSH is controlled by GnRH from the hypothalamus. Postpartum cows will begin to cycle when energy balance improves and LH pulsatility reaches a critical level. Insulin and IGF1 concentrations gradually increase postpartum as well. Cows in negative energy balance have lower blood concentrations of insulin and IGF-I. Insulin and IGF-I stimulate GnRH secretion from the hypothalamus and LH secretion from the pituitary. The hormonal control of GnRH and LH, therefore, arises from the metabolic and nutritional status of the animal via insulin and IGF1. These peripheral metabolic hormones will act on the hypothalamus to convey information from metabolically important tissues. A variety of metabolites (glucose, nonesterified fatty acids, etc.) and other hormones may also be involved. The same metabolites and hormones that influence GnRH secretion and ultimately LH and FSH secretion may act directly on the ovary to influence the sensitivity of the ovary to LH and FSH. Thus, the effects of nutrition on reproduction are manifested at the ovary and at the pituitary and hypothalamus through metabolic hormones (GH, IGF1, and insulin) that are essential for nutrient partitioning. In addition to the follicle, the corpus luteum, uterus, and embryo respond positively to insulin and IGF1. Nutrient partitioning for greatest milk production occurs when blood GH is elevated and blood insulin and IGF1 are low. Improvements in reproduction occur under a contrasting hormonal milieu (i.e., high blood insulin and IGF1 concentrations). It is difficult, therefore, to consistently achieve good reproduction in cows that undergo extremes in nutrient partitioning and have low insulin and IGF1.

**Rethinking the lactation curve** An interesting question that could be raised is whether or not the dairy industry should attempt to change the lactation curve of the cow to relieve some of the body condition loss in early lactation. If peak milk production was less and persistency was greater then the overall level of production across the entire lactation may not change (loss of milk at peak being compensated by greater production in later lactation). Manipulating the lactation curve in this manner for the purpose of alleviating body condition loss is not a new concept and was proposed in 1985. Peak milk production, ascent to peak production, and persistency are traits with moderate heritability so the shape of the lactation curve can theoretically be changed. Recent publications have raised the possibility of increasing persistency and decreasing peak milk yield as a means to alleviate losses in body condition.

**Conclusions** Reproductive traits are being included in selection indices worldwide by decreasing the emphasis on production. Greater emphasis on reproductive traits reflects the industry desire for more functional and efficient dairy cattle. The traditional view of an "efficient cow" was one that mobilized body fat in early lactation so that peak milk production was maximized. This approach to efficiency has apparently antagonized reproduction through changes in metabolic hormones (GH, IGF1, and insulin) and metabolites (glucose and nonesterified fatty acids). Changing the shape of the lactation curve so that peak milk production is less and persistency of lactation is greater may improve reproductive function while maintaining total lactation yield.