ANIMAL BIOSCIENCE

mRNA expression of genes regulating oxidative phosphorylation in the muscle of beef cattle divergently ranked on net feed efficiency

Feed is the largest variable cost in beef production making feed intake and efficiency economically important traits. Net feed efficiency (NFE), an alternative measure of feed efficiency, is the difference between actual and expected feed intake based on size and growth rate of an animal. Mitochondria are responsible for producing 90% of cellular energy via oxidative phosphorylation. Recent evidence from poultry and rodent studies suggest that differences in expression of genes regulating mitochondrial respiration may be related to feed efficiency. However, despite its potential economic significance, there is a dearth of published information on this topic in cattle. The objective of this study was to evaluate the relationship between NFE and transcription of genes (i) involved in the respiratory chain complex and (ii) coding for transcriptional factors regulating mitochondrial biogenesis.

Following a 120 day individual feed intake and growth measurement period, NFE was calculated on 90 yearling Limousin × Friesian heifers. Muscle (*M. longissimus dorsi*) biopsies were taken from the 10 highest (feed inefficient) and 10 lowest (feed efficient) animals ranked on NFE. Total RNA was extracted using TRIzol reagent. RNA quantity and quality was determined. One microgram of each sample of RNA was reverse transcribed to generate cDNA. Primers were designed for the five principal enzymes of the respiratory chain complex namely NADH, succinate dehydrogenase, ubiquinol cytochrome c, cytochrome c oxidase (COX II), atpase, as well as the electron carrier cytochrome c and the transcription factors peroxisome proliferator-activated receptor coactivator 1-α (PGC1-α), proliferator-activated receptor gamma (PPAR-γ), proliferator-activated receptor alpha (PPAR-α), nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (mtTFA). Power SYBR Green quantitive real time RT-PCR reactions were performed to measure the relative expression of these genes. β-actin served as a housekeeping gene. All amplified PCR products were sequenced to verify their identity. Gene expression results were calculated using the $2^{-\Delta\Delta ct}$ method. Data were analysed using mixed models ANOVA (PROC MIXED, SAS 2006) and correlation methodologies (PROC CORR).

The mRNA expression of PGC1-α was 2.8 fold higher ($P < 0.01$) (Figure 1) and the relative expression values for PPAR-α, NRF-1, and mtTFA were numerically higher in low compared with high NFE heifers. No difference ($P > 0.10$) was observed between the high and low NFE animals for genes coding for the five principal enzymes of the respiratory chain complex or the electron carrier cytochrome c. Partial correlation analysis indicated that moderate negative correlations ($P < 0.05$) existed between the transcription factors PGC1-α and PPAR-γ with NFE, FCR, but not ADG ($P > 0.10$) (Table 1). Moreover, there was a trend towards a negative relationship ($P < 0.10$) between NFE and the level of transcription of *atpase*, a critical regulator of the ATP-synthesizing enzyme complex.

Table 1: Partial correlations of feed efficiency traits and genes involved in mitochondria biogenesis

Trait	ADG	FCR	NFE
NADH	-0.42	0.15	-0.12
Succ. dehydrog.	-0.20	0.47	0.29
Ubiquinol cyto c	0.22	-0.32	-0.21
COX II	-0.43	0.11	-0.12
Atpase	-0.26	-0.40	-0.44†
Cytochrome c	-0.05	-0.27	-0.29
PGC1-α	0.02	-0.65**	-0.63**
PPAR-α	-0.14	-0.41	-0.14
PPAR-γ	0.00	-0.56*	-0.56*
NRF-1	-0.00	-0.41	-0.28
mtTFA	0.00	-0.31	-0.26

†, <0.10; *, <0.05; **, <0.01

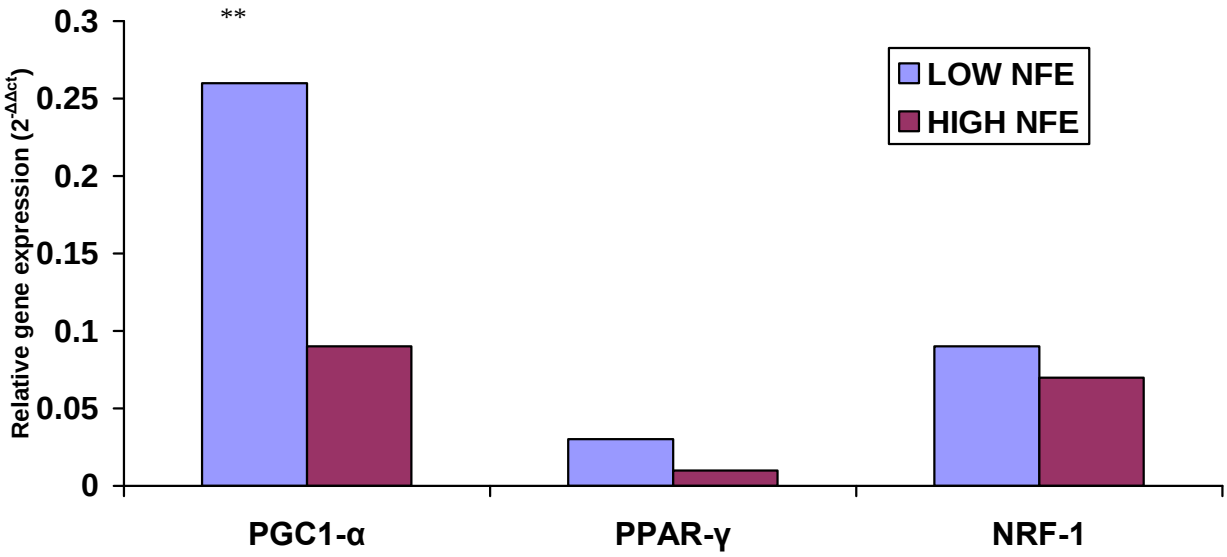

Figure 1. Relative gene expression ($2^{-\Delta\Delta ct}$) analysis of the transcription factors involved in mitochondria biogenesis.

This study provides evidence of an association between mitochondrial biogenesis and feed efficiency. For example, PGC1-α and PPAR-γ may be potential indicators for feed efficiency as both were independent of growth. Further investigation is warranted to determine if these results can be exploited as potential molecular markers for energetic efficiency in cattle.

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Single nucleotide polymorphisms in the bovine Neuropeptide Y5 Receptor gene and their predicted role in neuropeptide Y mediated feed intake behaviour in cattle

In Ireland enteric fermentation by the ruminant contributes ~14% of the total greenhouse gas emissions. For a 'sustainable beef production system' there is a requirement to select livestock with high feed efficiency and consequently a reduction in enteric methane emissions. Neuropeptide Y (NPY) is a key molecule that regulates appetite and energy homeostasis in animals and humans. The physiological functions of NPY are mediated through a numbers 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. Single Nucleotide Polymorphisms (SNP) in the bovine *NPY5R* gene are likely to influence feed intake behaviour in cattle and may act a potential genetic markers 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 their functional role in the protein product.

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 were determined using ProtParam software (Gasteiger et al., 2005).

Based on the alignment of the 2.1 kb sequence, a total of 17 SNPs were identified (Table 2). Of these SNPs, 4 were non-synonymous and 13 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 am 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 NPY5R molecule. This stop codon leads to a premature termination of the NPY5R peptide. The predicted changes in the physico-chemical properties of the NPY5R (Table 3) suggest important physiological consequences due to the presence of this SNP.

Table 2: Genotype 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 3: Properties of wild and truncated NPY5R protein

Physico-Chemical Properties	Wild NPY5R	Truncated NPY5R
Number of amino acids	446	364
Molecular weight	50775	41282
Theoretical pI	9.19	9.31
Extinction coefficient (M^{-1} cm^{-1})	56225	46005
Formula	$C_{2303}H_{3628}N_{610}O_{620}S_{31}$	$C_{1864}H_{2950}N_{498}O_{515}S_{22}$

There is high degree of genetic variation (1SNP/123 base) present in the bovine *NPY5R* gene. The non-synonymous SNPs, premature termination, SNPs in regulatory regions and changes in physico-chemical properties are likely to play vital physiological roles in the NPY mediated energy homeostasis in cattle. Hence, genetic association of the SNPs identified with the feed intake traits of the animals is currently being investigated.

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mRNA expression of genes regulating the insulin-like growth factor axis in the muscle of beef cattle divergently ranked on net feed efficiency

Recently, data showed differences in the transcription of some key genes regulating oxidative phosphorylation in muscle tissue. Protein synthesis and degradation within skeletal muscle are energy expensive processes with significant between animal variation. Insulin-like growth factors (IGF) stimulate proliferation and differentiation of myogenic cells as well as stimulating protein synthesis rate and suppressing protein degradation. It is currently believed that locally produced IGF-I in skeletal muscle plays a predominant role in supporting normal muscle growth. Furthermore, a family of high-affinity binding proteins (IGFBPs 1 to 6) affect IGF activity by binding to both IGF-I and -II and either inhibiting or enhancing their ability to bind to the type I IGF receptor that is responsible for many of their biological effects. However there is a dearth of published information on the influence of the IGF system in energetic efficiency of muscle tissue in cattle. The objective of this study, therefore was to evaluate the relationship between NFE and expression of genes controlling the IGF system in skeletal muscle tissue of cattle.

Following a 120 day individual feed intake and growth measurement period, NFE was calculated on 90 yearling Limousin × Friesian heifers. They were ranked on NFE and muscle (*M. longissimus dorsi*) biopsies were taken from the 10 highest (High; inefficient) and 10 lowest (Low; efficient) heifers. Total RNA was extracted using TRIzol reagent. RNA quantity was determined spectrophotometrically and quality was assessed using the Agilent Bioanalyzer. One microgram of each sample of RNA was reverse transcribed to generate cDNA. Primers were designed for the following genes: IGF-1 IGF-2, IGF-1, receptor (IGF1R), IGF-2 receptor (IGF-2R), IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5 and IGFBP6. Power SYBR Green quantitive real time RT-PCR reactions were performed to measure the relative expression of these genes. β-actin served as a housekeeping gene. All amplified PCR products were sequenced to verify their identity. Gene expression results were calculated using the $2^{-\Delta\Delta ct}$ method. Blood was sampled via jugular venepuncture for plasma IGF-1 analysis (RIA) on the day of biopsy. Data were analysed using mixed models ANOVA (PROC MIXED) and correlation methodologies (PROC CORR) (SAS, 2006).

There was no difference (P = 0.71) between NFE phenotypes in plasma concentrations of IGF-1 (481.60 ± 84.898 v 435.27 ± 84.898 ng/ml, for High and Low respectively). Similarly, there was no effect of ranking on NFE on the mRNA expression of any of the genes measured with the exception of a strong tendency (P=0.05) towards a higher level of expression of IGFBP6 in the more energetically efficient animals (Table 4). There was no relationship between systemic concentrations of IGF-1 and the mRNA expression of any of the genes measured. The results of this study suggest that it is unlikely that the IGF system plays a significant role in the local regulation of energetic efficiency in skeletal muscle. Although knowledge on the specific effects of IGFBP6 in muscle metabolism is poor, this protein is known to preferentially bind to IGF-II, inhibiting the proliferative actions of the latter. It is also thought to have IGF-independent actions. The reason for the tendency towards higher transcription of this gene in the energetically efficient animals, here is unclear.

Table 4: Effect of phenotypic NFE on differential mRNA expression of genes involved in the IGF system in muscle

	NFE group			
Gene	High	Low	Fold change	P-value
IGF-1	0.064	0.070	1.17	0.67
IGF-2	0.519	0.555	1.08	0.79
IGF-1R	0.010	0.010	-	0.95
IGF-2R	0.282	0.260	1.08	0.67
IGFBP1	0.000	0.000	-	0.92
IGFBP3	0.059	0.069	1.17	0.54
IGFBP4	0.180	0.132	1.38	0.31
IGFBP5	0.376	0.547	1.45	0.31
IGFBP6	0.016	0.021	1.31	0.05

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Examination of endometrial gene expression in heifers of high and low fertility using microarray technology

Cow conception rate is a major component of reproductive and production efficiency in beef and dairy herds. 70-80% of reproductive wastage is attributed to early embryo loss (within the first two weeks of pregnancy). There is evidence of repeatable differences between cows in their ability to become pregnant and other Irish Researchers have reported a repeatability estimate of 0.18 for early embryo survival in heifers. Furthermore, it has been suggested that uterine, rather than ovarian, factors influence these differences. In the cow the embryo enters the uterus from the oviduct on day(s) 3-4 and utero-embryo interactions at this early stage are detriment to the subsequent viability of the conceptus. Therefore, optimal uterine function prior to this implantation period is of critical importance. However, there is little information available on the molecular mechanisms regulating fertility in the bovine uterus. Thus, an experimental model consisting of two distinct herds of cattle; high and low fertility was established. The objective of this study was to explore the gene interactions causing this genetic bias toward increased embryo mortality.

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 oestrus. 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). Oestrous cycles were then synchronised. Animals from both high and low groups (n=12) were slaughtered on days 7 and uterine endometrial tissue (ipsilateral to *corpus luteum*) was harvested and stored at -80ºC in RNAlater solution. RNA was extracted using TRIzol® reagent and purified using RNeasy clean-up kits (Qiagen). RNA samples were biotinylated and hybridised to the Affymetrix 23,000 bovine master gene chip according to manufacturers' protocol. All arrays were scanned and quality control checked in the Bioconductor *affycoretools*. Normalisation and statistical analysis was carried out using the PUMA method in Bioconductor, R. Furthermore Ingenuity Pathway Analysis (IPA) was used to associate changes in gene expression with key biochemical pathways involved in global biological function in the bovine uterus.

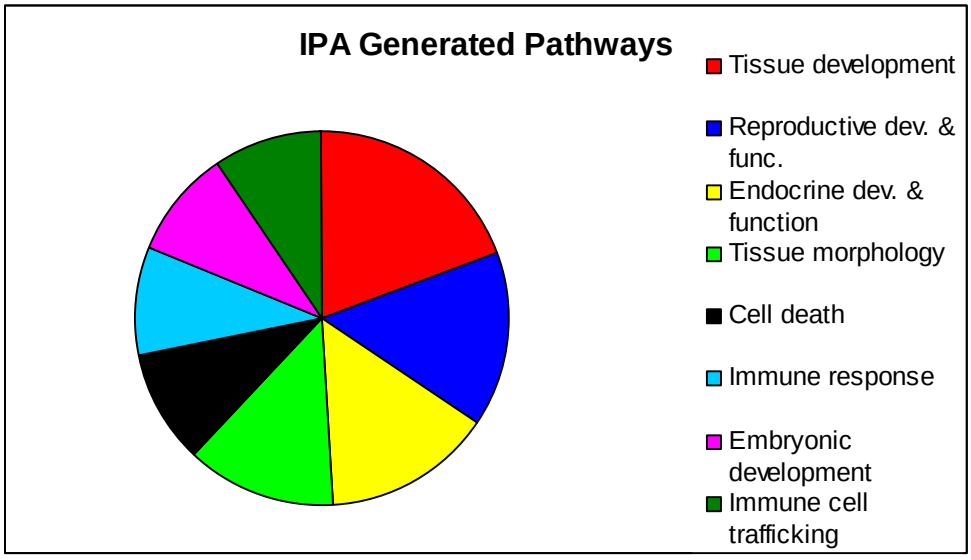

Figure 2: Key biological pathways differentially expressed [-log (P-value)] in bovine endometrial tissue related to reproductive function.

Following normalisation and statistical analysis using PUMA a pool of 5787 annotated genes were mapped to the IPA database. 5294 genes were mapped to networks. 126 genes were found to be differentially expressed ($P<0.05$) in the low relative to the high fertility group, of which 85 were up-regulated and 41 down-regulated. Genes mapped were implicated in an array of biological pathways including reproductive function (FST, PTGIS, SFRP1, PPARA & GJAI), tissue development (TPM2, ACTC1, ACTA2, & ACTB), immune response (C1R IFI6, CXCL2, CXCL3 & IL33), cell-cell signalling (GJA1, LPHN2 & RCAN2), energy coupling (ESSS, NDUFA10, UCP3 & DCXR) and cell death (TP53I11, CAP1 DAP & RAB3B) (Figure 2). Statistically significant biochemical pathways associated with reproductive development/function and embryonic mortality included VEGF, calcium and Wnt/β-catenin signalling and eicosanoid synthesis. Follistatin (FST) was down-regulated in low fertility heifers ($P<0.05$) and is suspected to play a central role in endometrial gland morphogenesis. Prostacyclin synthase (PTGIS), which was down-regulated ($P<0.05$), is critical for endometrial decidualisation and embryo implantation. Prostacyclin acts by binding to nuclear receptors such as PPAR-α which was found to be up-regulated ($P<0.05$). GALNT6 was up-regulated 7.5 fold ($P<0.05$) and has been implicated in the synthesis of oncofetal fibronectin. Oncofetal fibronectin is a protein found in human plasma and cervicovaginal secretions in abnormal pregnancy states.

Quantitative Real-time RT-PCR analysis was performed on a list of genes with roles implicated in reproductive function and maintanence of embryo survival using the ABI 7500 Fast Real-time PCR System with SYBR green master mix (Applied Biosystems, Warrington UK) to validate Microarray results (Table 5). Q-PCR data were normalised using Ubiquitin as a housekeeping control and statistical analysis was carried out on data using MIXED procedure of SAS (SAS 9.1).

Table 5: Comparison of candidate genes across both platforms; 23K Affymetrix Genechip® and ABI 7500 v2.0.1

Gene	Microarray	P value	Q-PCR	P value
FST	-1.37	<0.05	-1.32	0.640
LRAT	1.354	<0.05	-2.04	0.301
TGFB1I1	-1.24	<0.05	-2.25	0.002
NMB	-1.73	<0.05	-2.59	0.020
IL-33	1.35	<0.05	1.71	0.117
SFRP1	-1.45	<0.05	-3.32	0.047
PPARA	1.38	<0.05	1.04	0.291
DONSON	1.26	<0.05	1.17	0.052
MOSC2	1.13	<0.05	1.40	0.073

In conclusion expression of genes involved in key biological pathways including reproductive processes are differentially regulated in animals of high versus low fertility. This has been further confirmed by Q-PCR.

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To compare the ruminal microbial populations in cattle divergent for net feed efficiency using PCR-DGGE microbial community analysis

Cattle with poor energetic efficiency have a higher cost of production and consequently a greater environmental footprint. There is evidence that selection for energetically efficient ruminants may lead to inherent improvements in ruminal fermentation processes and consequently greater nutrient absorption. This would allow additive and lasting reductions in greenhouse gas (GHG) emissions with the added benefit of more profitable animal production. Despite its importance as a significant contributor to global warming, relatively little is know about the methanogen populations in the rumen. A number of molecular-based community approaches have been developed which allow rapid profiling of microbial community change.

The objective of this study was to compare the ruminal microbial populations of cattle divergent for net feed efficiency, across contrasting diet types (high forage v high concentrate) using PCR-DGGE microbial community analysis.

Ninety Limousin × Friesian yearling heifers, 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 diet (on a DM basis). Ruminal fluid was collected on four separate occasions (in sequential order) (i) at the end of a six week period (Time 1) on grass silage (ii) twice during an eight week period (Time 2 and Time 3) at pasture and (iii) at the end of a five week period (Time 4) on a 30:70 maize silage:concentrate TMR. In this report it is proposed to look at the two most extreme groups, time 1 (grass silage diet) V time 4 (30:70 maize silage:concentrate).

Table 6: Information on sampled animals and their related RFI values

Animal ID (Tag No)	Breed	Mean age (days)	RFI Value	RFI Ranking	Rumen sample source
329	Limousin x Friesian	346	-1.10345	LOW	Fluid
375	Limousin x Friesian	346	0.5669	High	Fluid
532	Limousin x Friesian	346	-0.7917	LOW	Fluid
554	Limousin x Friesian	346	-1.0781	LOW	Fluid
559	Limousin x Friesian	346	-0.99459	LOW	Fluid
633	Limousin x Friesian	346	-0.45542	LOW	Fluid
655	Limousin x Friesian	346	1.50472	High	Fluid
712	Limousin x Friesian	346	0.70121	High	Fluid
739	Limousin x Friesian	346	-0.67022	LOW	Fluid
759	Limousin x Friesian	346	-0.63298	LOW	Fluid
763	Limousin x Friesian	346	0.73338	High	Fluid
968	Limousin x Friesian	346	0.93088	High	Fluid
1264	Limousin x Friesian	346	0.67139	High	Fluid
1453	Limousin x Friesian	346	0.5618	High	Fluid

Total microbial DNA was isolated from rumen microbes, in ruminal fluid, from animals with low RFI (**LRFI** (n=7) most efficient) and high RFI (**HRFI** (n=7) least efficient) (Table 6). DNA quantity was determined spectrophotometrically. PCR-DGGE analysis of the total detectable bacteria was performed using universal bacterial HDA primers. PCR products (c.200bp) were amplified and were subjected to run on a 8% acrylamide gel with 35-55% gradient using the Bio-rad DCode universal Detection System. For the purpose of sequencing, to verify their identity, ethidium bromide stained bands of interest were excised and DNA was eluted from the polyacrylamide.

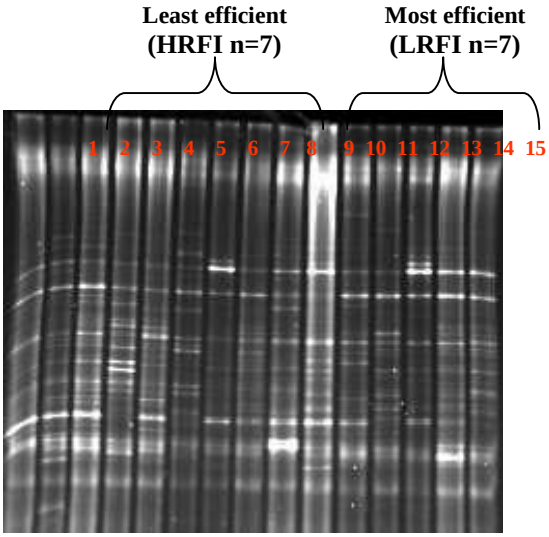
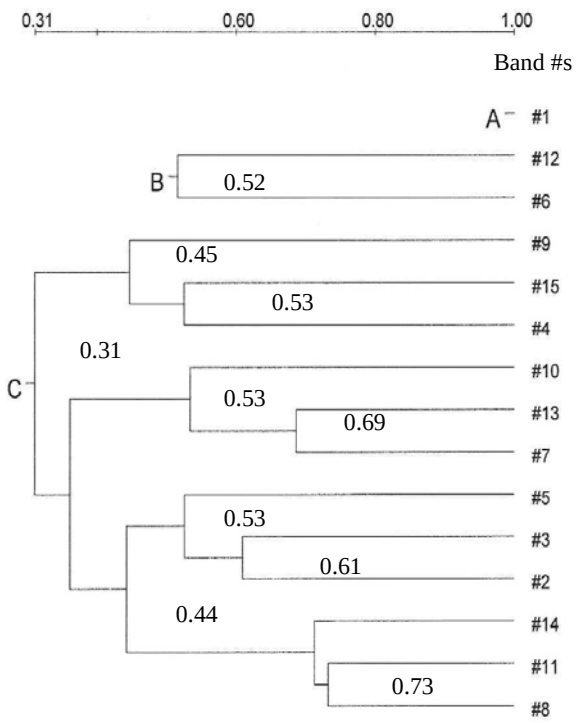


Figure 3. PCR DGGE analysis of DNA samples from rumen fluid taken at time point 4. Lane 1; ladder, lanes 2-8; HRFI animals, lanes 9-15; LRFI animals.

The analysis of detectable bacterial PCR-DGGE profiles (Figure 3) showed that the profiles generated from efficient animals clustered together (Figure 4) and were clearly separated

from those obtained from inefficient animals, indicating that specific bacterial groups may be present only in efficient animals.



Band No.	RFI Ranking
1	Ladder
2	High
3	High
4	High
5	High
6	High
7	High
8	High
9	Low
10	Low
11	Low
12	Low
13	Low
14	Low
15	Low

Figure 4. UPGMA (Unweighted pair-group method using arithmetic averages) dendogram generated from bacterial denaturing gradient gel electrophoresis (DGGE) profiles.

In conclusion, significant differences were found in the rumen microbiota which may be associated with feed efficiency (RFI) in beef cattle. To our knowledge this is the first study in Ireland to link rumen microbial PCR-DGGE profiles and their fermentation products with feed efficiency in cattle. This study, on the identification of potential associations between rumen microorganisms and cattle feed efficiency trait will assist in the development of potential strategies to improve the economic and environmental sustainability of beef production in Ireland.

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Effect of dietary n-3 polyunsaturated fatty acids on bovine uterine endometrial and hepatic gene expression of the insulin-like growth factor system

Fertility rates are decreasing in high producing dairy cows. Nutrition plays a key role in reproduction and there is evidence suggesting that dietary long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) may improve reproductive performance in cattle. n-3 PUFA, particularly eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), are known to be

regulators of gene transcription in many tissues. The majority of circulating insulin-like growth factor-1 (IGF-1) is produced by the liver and increases in systemic IGF-1 concentrations have also been associated with improved fertility. Although mainly of hepatic origin IGF-1 is also produced locally in the endometrium and localised IGF signalling may play a significant role in influencing uterine function and in turn, fertility. The endometrium is the inner lining of the uterus and plays a vital role in uterine-embryo interactions. Despite its critical role to reproductive success there is little published information on the effect of n-3 PUFA intake on the gene expression of the IGF system in the liver and uterus. Thus, the objective of this study was to examine the effects of dietary supplementation of n-3 PUFA on the expression of genes involved in the IGF signalling system in the bovine uterine endometrium and liver.

Reproductively normal crossbred beef heifers (n = 24) were individually fed a straw and barley/beet pulp based concentrate diet and supplemented with a rumen protected source of either saturated fatty acid (palmitic acid; Low PUFA) or high n-3 PUFA (High PUFA 273g) per head per day for 45 days. Following slaughter on day 17 of a synchronized estrous cycle, uterine endometrial tissue was harvested from all animals. Total RNA was extracted using TRIzol reagent, from endometrial tissue of animals offered High (n=7) and Low (n=7) PUFA diets. RNA quantity was determined spectrophotometrically and quality was assessed using the Agilent Bioanalyzer 2100. Samples were DNase-treated with RQ1 RNase-free DNase. RNA was reverse transcribed to generate cDNA. Primers were designed, using the on-line Primer3 software package, to amplify specific fragments of genes involved in the IGF/GH signalling axis. The relative expression of each gene was analysed using a real time RT-PCR approach. Ubiquitin was used as a reference gene for endometrial samples following evaluation of a number of 'housekeeping' genes, while glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as a reference gene for liver samples. Gene expression data was calculated using the $2^{-\Delta\Delta CT}$ method. Statistical significance of differences between the groups was tested using the Proc GLM procedure of SAS (2002).

Uterine endometrial IGF-1, IGF-2, IGF-1 receptor (IGF-1R), IGF-2 receptor (IGF-2R), IGF binding proteins (IGFBP) 2, 3 and 6 genes were found to be differentially ($P<0.05$) expressed in the HIGH compared with CON group (Table 8). Expression of mRNA for the other four candidate genes, IGFBP-1, IGFBP-4, IGFBP-5, and growth hormone receptor (GHR) were not differentially expressed between animals fed the HIGH PUFA or CON diets in endometrial tissue. Hepatic IGF-2R, IGFBP-5 and GHR-1A genes were found to be differentially ($P<0.05$) expressed in the HIGH compared with CON group (Table 9). Expression of mRNA for IGF-1, IGF-2, IGF-1R, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6, acid labile subunit (ALS) and GHR were not differentially expressed between animals fed the HIGH PUFA or CON diets in liver tissue.

Table 8: Endometrial differentially expressed genes of high PUFA vs. low PUFA

gene ID	high PUFA vs. low PUFA	fold change	p-value
IGF-1	down-regulated	6.1	0.003
IGF-2	up-regulated	1.5	0.028
IGF-1R	up-regulated	1.8	0.046
IGF-2R	up-regulated	1.5	0.041
IGFBP-2	up-regulated	2.9	0.018
IGFBP-3	down-regulated	11.0	0.0006
IGFBP-6	down-regulated	4.1	0.005

Table 9: Hepatic differentially expressed genes of high PUFA vs. low PUFA

gene ID	high PUFA vs. low PUFA	fold change	p-value
IGF-2R	up-regulated	2.8	0.00003
IGFBP-5	up-regulated	2.3	0.004
GHR-1A	down-regulated	1.7	0.046

These results indicate that dietary supplementation with LC n-3 PUFA can alter the expression of the IGF system in both the bovine uterus and liver. However, the gene expression profiles were tissue dependent and changes in gene expression may have major implications but the response is highly dependent on tissue. IGF1 and IGF-2 are both associated with key reproductive events such as preimplantation and placental development. IGF-1R mediates the actions of both ligands, while IGF-2R only binds IGF-2 and acts as a "clearance receptor" preventing IGF-2 signaling. IGFBPs can have both a stimulatory and inhibitory effects. Differential gene expression of IGF -1 & -2, IGF -1R & -2R and IGFBPs in the endometrium and IGF-2R, IGFBP-5 and GHR-1A in the liver may positively influence reproductive efficiency by providing a more suitable environment for embryo survival.

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Effect of breed and genetic merit on muscle gene expression relating to muscle growth and development in Aberdeen Angus and Belgian Blue crossbred steers

The objective of this study, using two contrasting beef cattle breeds, was to examine the effect of breed and genetic merit potential on muscle gene expression for muscle growth and development in the *Longissimus dorsi* (LD) muscle. At present little information is available on the molecular mechanisms regulating LD muscle growth and development.

Crossbred Aberdeen Angus (AA; n=16) and Belgian Blue (BB; n=16) steers, born to Holstein-Friesian dams were used in this study. Within breed, animals were classified as either high or low genetic merit based on sire predicted differences (PD) for carcass growth.

Samples of *longissimus dorsi* muscle were harvested from the animals within 30 minutes of slaughter, washed in sterile DPBS, and placed in RNA*later* at 4^0C for 24 hours before being transferred to -20^0C freezer. Total RNA was isolated from the muscle tissue using TRIzol reagent. RNA quantity was determined by measuring absorbance at 260 nm on a Nanodrop spectrophotometer and the 28s/18s ratio and RNA integrity number, an indicator of RNA quality, was assessed using the Agilent Bioanalyser with the RNA 6000 Nano LabChip. Samples were treated with RQ1 RNase-free DNase and total RNA was then reverse transcribed, using random hexamers, to first strand (c)DNA using the reverse transcription method according to the manufacturers instructions. The converted cDNA was quantified by absorbance at 260 nm, diluted to 50ng/µL working stocks and stored at -20^oC for subsequent analyses. GAPDH was determined as the most stabile reference gene using geNorm software. Primers were designed to measure expression of genes involved in the IGF-1 and Myostatin pathway in muscle using the Primer3 software programme. Each real-time PCR reaction was carried out in a total volume of 20 µL with 1 µL of cDNA, 10 µL of SYBR Green I master mix (Applied Biosystems, Ireland), 1 µL of forward and reverse primers and 8 µL of nuclease-free H_2O. Real-time RT-PCR was performed using the Applied Biosystems Fast 7500 v2.0.1 with the following cycling parameters: 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 60 s, followed by amplicon dissociation (95°C for 15s, 60°C for 60 s, 95°C for 15s and 60°C for 15 s. Dissociation curves were examined for the presence of a single PCR product and the gene expression results were calculated using the delta delta cycle

threshold (2−ΔΔCT) method. The 2−ΔΔCT method was used to determine mean fold changes in gene expression between the various breeds.

Table 10: Differentially expressed genes in the *longissimus dorsi* muscle

Gene of interest	Breed effect	Genetic effect
Collagen type 2	P < 0.001	No
IGF-1	P < 0.05	P < 0.001
IGFBP-3	P < 0.001	No
Follistatin	No	*P* < 0.001
Growth Hormone Receptor	P < 0.05	P < 0.001

The genes coding for Collagen type II, IGF-1, IGFBP-3, Follistatin and Growth hormone receptor (GHR) were found to be differentially expressed in *Longissimus dorsi* muscle of genetically high and low Aberdeen Angus and Belgian Blue steers (Table 10). A breed effect was noted as mRNA levels of collagen type II were higher in Aberdeen Angus animals compared to Belgian Blue ($P < 0.001$); however, there was no genetic effect for this gene in this study. Although several of the genes involved in the IGF system remained unchanged, expression of IGF-1 varied between breed and genetic groups with genetically low animals having a higher expression ($P < 0.001$). IGFBP3 reported a significant difference between breeds ($P < 0.0001$). Follistatin showed a significant difference between genetic groups but having no breed effect.

Aberdeen Angus animals are renowned for their high levels of fat in the muscle commonly known as a 'marbling' effect. The higher levels of Collagen type II are possibly a result of this fat accumulation. Follistatin results in this study agree with research which states that follistatin in muscle is a possible antagonist to the myostatin gene. The myostatin gene inhibits muscle growth. In this study genetically high animals showed higher mRNA levels for this gene.

The genes studied to date show significant differences for both breed and genetic groups. Further research is ongoing with this project.

RMIS No. 5759

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Effect of breed and compensatory growth potential on plasma analytes and on lumbar muscle and fat growth in beef cattle

Compensatory growth (CG) is a complex physiological process whereby animals, following a period of under nutrition, undergo accelerated growth upon realimentation in order to achieve their inherent growth capacity (Hornick *et al*., 2000). The objective of this study, using two contrasting beef cattle breeds, was to examine the effect of breed and CG potential on muscle and fat deposition and on systemic metabolic indices in beef cattle, with a view to developing an animal model to study the effects of breed and CG on muscle gene expression.

Crossbred Aberdeen Angus (AA; n=22) and Belgian Blue (BB; n=22) yearlings, born to Holstein-Friesian dams, and with a mean (sd) bodyweight of 171.5 (29.2) kg were used. Within breed, animals were classified as either high or low genetic merit based on sire predicted differences (PD) for carcass growth. Animals were blocked within breed and sire

PD to either a high (H) or low (L) energy diet (D) in a 2 (breeds; B) x 2 (diets) factorial design. All animals were offered grass silage *ad libitum*. Animals on L received 1 kg of supplementary concentrates daily while those on H received 3 kg daily. At the end of the winter period (P1), animals were turned outdoors, offered high quality pasture *ad libitum* and were managed as one herd. Ultrasonic muscle (MD) and fat (FD) depths were measured at the 3rd lumbar vertebra on three occasion's *viz.* end of the winter period (P1), two months later (P2) and again a further four months later at housing (P3). Blood samples were also taken, via jugular venipuncture, once per period (P), for the determination of plasma concentrations of insulin, insulin-like growth factor 1 (IGF-1), glucose, urea, betahydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Bodyweight was measured twice monthly. Data were analysed using ANOVA (SAS) with fixed effect terms for breed, diet and period as well as all two by two and three-way interactions initially included in the statistical model. Sire was included as a random effect.

The effect of breed, diet and period of measurement on post-turnout average daily gain (ADG), MD, FD and plasma concentrations of metabolic indices is shown in Table 11. There was a breed x period interaction for MD with values statistically higher for BB in P1 and P3. Overall, BB had greater muscle depth (P <0.001) and deposition (P<0.05) across all periods. Furthermore, there were latent effects of diet (P<0.01) and period (P<0.001) on MD with MD higher (P<0.01) on H compared with L animals and increasing with period post turnout (P <0.001). Fat depth was higher in AA (P <0.001) and in animals on H diet (P<0.05) and increased with P (P<0.001). There was little evidence of either B or D effects on the plasma analytes measured. In general all responded in a quadratic fashion to the duration since turnout. The model to examine the effects of breed and CG potential on muscle gene expression needs further development.

Table 11: Effect of breed, diet & period of measurement on plasma analytes & muscle & fat deposition

	Breed (B)			Diet (D)			Period (P)				Significance				
Variable	AA	BB	SED	H	L	SED	1.00	2.00	3.00	SEM	B	D	*P*	BxP	BxD
ADG (kg)															
P1-P2 (kg)	1.33	1.38	0.03	1.32	1.40	0.03					0.05	**			NS
P2-P3 (kg)	0.44	0.44	0.02	0.41	0.46	0.02					NS	*			0.01
Blood analytes															
Insulin (µIU/ml)	5.49	7.00	1.15	7.06	5.43	1.07	5.46	5.76	5.52	0.74	NS	NS	*	*	NS
IGF-1(ng/ml)	623.70	616.90	39.17	632.20	608.40	42.33	566.70	695.90	598.70	23.86	NS	NS	***	NS	NS
Glucose (mmol/L)	4.35	4.27	0.06	4.39	4.24	0.06	4.56	4.48	3.90	0.05	NS	*	***	NS	NS
Urea (mmol/L)	4.40	4.41	0.20	4.43	4.38	0.11	6.50	2.81	3.89	0.14	NS	NS	***	**	NS
BHB (mmol/L)	0.30	0.33	0.01	0.32	0.31	0.01	0.32	0.39	0.23	0.03	0.05	NS	***	NS	NS
NEFA (mmol/L)	0.29	0.28	0.03	0.28	0.29	0.02	0.29	0.39	0.18	0.02	NS	NS	***	*	NS
Ultrasonic															
MD (mm)	52.32	57.11	0.92	56.42	53.01	0.92	47.59	52.75	63.80	0.69	***	**	***	*	NS
Δ MD (mm)	0.08	0.10	0.01	0.09	0.09	0.01	.	0.10	0.08	0.001	*	NS	NS	NS	NS
FD (mm)	1.95	1.06	0.12	1.61	1.40	0.08	0.75	1.15	2.60	0.11	***	*	***	NS	NS
Δ FD (mm)	0.01	0.01	0.002	0.01	0.01	0.001	.	0.01	0.01	0.001	***	NS	NS	NS	NS

* P<0.05, **P<0.01, ***P<0.001. NS = P>0.05, Δ=daily gain. No 3-way interactions were detected (P > 0.05), P1-P2 = duration between P1 and P2, P2-P3 = duration between P2 and P3

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Evaluation of an immunospecific technique for the depletion of albumin from bovine plasma

Proteomic analysis of plasma and many other biological fluids is made difficult due to the large range of protein concentrations. Highly abundant proteins such as albumin can mask low abundance proteins making them difficult to detect and identify during mass spectroscopy. Depletion, or removal of highly abundant proteins from the sample, allows the relative enrichment of low abundance proteins, which can then be further fractionated by methods such as 2D electrophoresis or liquid chromatography (LC) prior to mass spectroscopy. The objective of this study was to determine the efficiency of immunodepletion spin columns in removing bovine serum albumin (BSA) from bovine plasma.

Six aliquots of a bovine plasma sample were depleted successively on two spin columns (Beckman Coulter, USA) to give a total of three replicates per column. A volume of 9.6µl of bovine plasma was added to 490µl of Tris Buffered Saline (pH 7.4) and this was applied to each column. Columns were incubated at room temperature to allow albumin to bind to the IgY microbeads. The unbound proteins were then eluted from the column with a total of five washes with Tris Buffered Saline (pH 7.4). Proteins (albumin) that had bound to the column were then eluted using four washes of Glycine-HCl (pH 2.5). All washes were collected in 2ml collection tubes and frozen at -20°C prior to further analysis. The protein concentration of bovine plasma and each collected wash (bound and unbound) was determined using a bicinchoninic acid (BCA™) protein assay (Pierce, USA). The concentration of albumin in the unbound and bound washes was also determined using an ELISA (Bethyl laboratories, USA). Microsoft Excel was used to calculate the mean, standard error of the mean and the percentage albumin recovered in each fraction (Figure 5). Fractions were rehydrated on a 24cm 3-10cm pH non-linear Immobiline Drystrip gel and isoelectric focused for 64kVH. The second dimension was carried out on a 12% SDS-PAGE gel. Separated proteins were detected using coomassie staining and imaged using a CCD system (Figure 6).

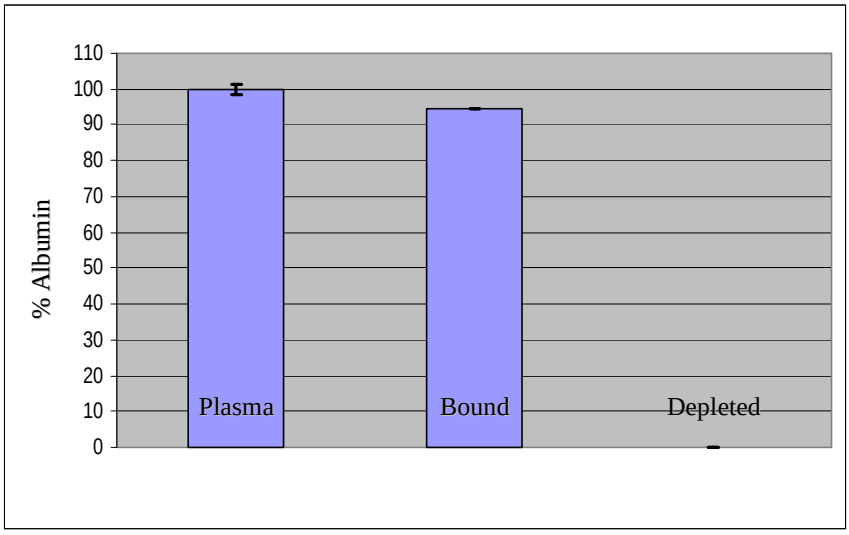

Figure 5. Percentage of albumin recovered in each fraction.

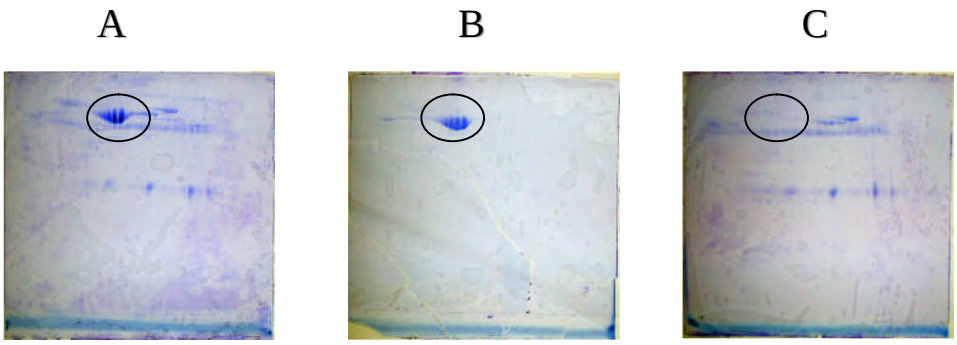

Figure 6. 2-DE gel of proteins from plasma (A) the bound (B) and the unbound (C) fractions. Spots corresponding to BSA are circled.

The original plasma sample contained 670.9µg protein and 249.7µg of BSA. The total protein in the combined unbound and bound washes was 328.9±12.82µg and 299.8±9.55µg respectively. Overall, 93.7±2.82% of the total protein applied to the column was recovered. The bound and unbound washes contained 235.9±8.80 µg and 0.06±0.012µg of BSA, respectively. The overall recovery of BSA applied to the column was 94.5±3.50%. The concentration of BSA in the depleted fractions was 0.02±0.001% (Figure 5). 2D-PAGE confirmed the absence of albumin in the unbound fraction (Figure 6). This study shows that BSA immunodepletion spin columns are an efficient and rapid method for the depletion of the highly abundant protein albumin from bovine plasma. The spin columns show high recovery of protein and high specificity for the removal of albumin

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Bioconductor Buntu: a web-based microarray analysis suite

The Bioconductor Project's suite of genomic analysis software is perhaps the best known and most widely used open-source data processing platform for a wide variety of data sources particularly for the analysis of microarray data. Its unique strength lies in the large dynamic community of developers and the constant evolution of novel analytical and statistical techniques, freely accessible to the user community the most recent of which include modules devoted to the processing of next generation sequencing data. Its R-based command line environment, however, can make Bioconductor software packages intimidating for many potential users. BioconductorBuntu is a user-friendly web-based microarray processing platform utilising relevant Bioconductor modules, facilitating its installation on a local server by bundling it into a custom Ubuntu linux port.

Following connection to the server via a web browser, each user must create an account on the system via the 'Register' link, inputting their relevant details. After registration, the user may upload raw data. Uploaded data are assumed to be in a '.zip' archive, whose data files are unpacked and stored server-side prior to analysis. Currently, a user has three options, to upload Affymetrix®, dual- or single-dye data. At the time of upload, users creating Affymetrix®experiments have the option of using Affyprobeminer's remapped chip definition (CDF) files instead of the default Bioconductor files. An entry containing all relevant information is created in the database for the new experiment. Logs of data upload and processing printed to

the GUI allow users to monitor this procedure in real time. Assignment of phenotypic information for the dataset follows. For Affymetrix® data, the GUI requests the user to specify the number of factors for the experiment and the number of levels of each of these factors and names for the levels and factors, and subsequently to assign the appropriate levels of each factor to each array. Design and contrasts matrices are created automatically from this information, allowing great flexibility in experimental design. For dual- and single-dye arrays, currently only experiments with two RNA sources are supported. The GUI then opens up a quality control page.

There are a number of visualisation options available such as boxplots, histograms, PCA and other diagnostic plots on normalised and non-normalised data, with the most commonly used normalisation options available. For dual- and single-dye data, the user may also select which spot types are to be included, if a spot type file was specified at upload, with additional support for within array duplicate spots. Based on these outputs, the user may decide if any of the arrays need to be redone or removed from subsequent analysis. The 'Data Select' link allows the removal of arrays from the dataset.The next step is differential expression analysis. Using Affymetrix® data, the user has the option of using the limma or PUMA packages to perform this analysis. Under limma a number of preprocessing methods, such as RMA, GCRMA, MAS5 and custom methods, specifying the steps taken for normalisation, background correction, PM correction and summarisation, are available. If the user opts to use the PUMA package, data are preprocessed using PUMA's multi-mgMOS method, which implements global median scaling normalisation. Differential expression is then calculated using the Probability of Positive Log Ratio method. Because this analysis can be a lengthy process, notification of completion is emailed to users upon conclusion.

For dual-dye analysis, the system incorporates all of normalisation methods that are available in the Limma package. There are a number of different options for normalisation between arrays, normalisation within arrays and background correction. All of these options are available at both Quality Control and Differential Expression stages. Single-dye data are normalised using VSN as this is the only viable option available to single-dye data within Bioconductor. Upon completion of differential expression, a ranked gene list, containing metrics such as fold change, adjusted *P*-values and *B*-statistic is output to the GUI. This list contains links to gene annotation information, which is retrieved using the Bioconductor package BiomaRt and is dynamically loaded using AJAX. This ranked gene list may also be downloaded as an Excel® file. Gene Set Enrichment Analysis has also been incorporated allowing the user to identify significantly dysregulated KEGG pathways based on the differential expression analysis of Affymetrix microarray data. The list of such pathways is outputted in tabular format with links to associated metadata in the KEGG database. All previous analysis, including all files generated, are stored in the users profile for future reference. The user also has the option to download the .Rdata file containing the complete R workspace environment for a particular analysis procedure. This file can be used if they wish to continue analysis from the command line, which may be more flexible in certain situations and useful to advanced users. Users may also manage their account by deleting previous experiments and analyses.

BioconductorBuntu is entirely based on open-source software which is free to redistribute, use and alter, under standard GPL terms. The server runs on the widely used Ubuntu Linux operating system, and is fully functional on conventional desktop PC systems. For example, an analysis of the Bioconductor supplied estrogen dataset at the +48 h timepoint between low/high-estrogen conditions using limma following pre-processing using RMA took 15.3 s on laptop equipped with an Intel Core2 duo 2.0 GHz processor and 2GB RAM. The distribution was customised using the open-source script remastersys, allowing modification and saving of a basic Ubuntu installation as a remastered install CD, or as a Debian upgrade for existing Ubtunu installations. All analysis

of gene expression microarray data are performed in R, specifically using Bioconductor. Pipelines facilitating the analysis of other microarray platforms are potentially available to the user community by coding additional functionality in R and Python. BioconductorBuntu facilitates such extensibility due to its generalised framework implemented to facilitate the current analysis pipelines. The CGI scripts, which run on the server are written in Python. These scripts handle input and output to and from the xHTML-, CSSand JavaScript-based user interface, as well as writing to and from the database and making calls to R and Bioconductor via the RPy interface. The Apache web server is used and the powerful open-source database MySQL is used to store and retrieve various forms of data. Exim is used as a mail transfer agent and Dovecot as an IMAP server. Imagemagick is used for image manipulation and particularly creating thumbnail versions of image and PDF files.

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Detection of numerous verotoxigenic *E. coli* serotypes, with multiple antibiotic resistance from cattle faeces and soil

Since the emergence of verotoxigenic *E. coli* (VTEC) as human pathogens, contamination of foods of animal origin has been a major public health concern. The present study compares the phenotypic and genotypic characteristics of unusual non-O157 VTEC strains, which were isolated from healthy cattle and soil.

Faeces and soil samples were collected from 6 unrelated farms in the months of July to November. One faeces sample (10 g) was taken from the rectum of each animal (n = 108) while soil samples (20 g), were obtained once from 5 paddocks on the same farm (n = 20). Each animal was sampled once. The soil samples were obtained from paddocks, which were empty of cattle at the time of sampling, although they had been grazed previously. There was no known contact of personnel or animals between the farms. Faeces samples were enriched in mTSB broth, (Oxoid, UK) containing streptomycin sulphate (1000 μg/ml) stomached in a Colworth Stomacher (Model BA 6024, A.J. Steward & Co. Ltd., London, UK), and incubated at 37 °C for 24 h. Aliquots were plated onto sorbitol MacConkey agar (Oxoid, UK) containing streptomycin sulphate (1000 μg/ml), SMAC-strep, and incubated at 37 °C for 24 h. Following incubation, a single colony was taken from each sample and stored on cryoprotective beads at −20 °C until required for biochemical and molecular testing (Protect, Technical Services Consultants, Lancashire, UK). The presence of any bacterial growth on the plates after enrichment was considered a positive sample.

Each isolate was Gram-stained and characterised using API 20E and API 50CH commercial kits according to manufacturers instructions (Biomerieux). After resuscitation from storage beads, template DNA was prepared from a single colony of each isolate. DNA was purified from the culture with a DNeasy extraction kit (Qiagen, Crawley, UK). All isolates were screened for virulence factors commonly associated with VTEC, *eae*, *hly*, *vt*1 and *vt*2 genes using a multiplex PCR method. The presence of virulence genes was also confirmed by independent PCR analysis by the Laboratorio de Referencia de *E. coli* (LREC, Lugo, Spain). Isolates positive for one or more of these genes were also screened for the presence of the rfb_{O157} gene and $fliC_{h7}$ gene. PCR products were separated by electrophoresis on a 2% agarose gel, stained with ethidium bromide and visualised under UV illumination. The PCR products of *eaeA*, *hly*, *vt*1 and *vt*2 genes were purified using a PCR purification kit (Nucleospin Extract 11, Machery-Nagel, Germany) and sequenced commercially in duplicate (MWG Biotech, Ebersberg, Germany). Sequences were

initially compared with the current GenBank sequence databases using the BLAST suite of programs. ClustalW amino acid sequence alignments were produced for comparison online at http://www.ebi.ac.uk/clustalw.

Isolates positive for *vt*1 or *vt*2 genes following PCR were tested for verotoxin production, using the commercial ELISA Premier-VTEC kit according to the manufacturer's instructions (Meridian, Bioscience). Serotyping was performed on isolates positive for virulence genes. The determination of O and H antigens was carried out by the LREC, (Lugo, Spain), employing all available O (O1–O185) and H (H1–H56) antisera. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the non-specific agglutinins. The O antisera were produced in the LERC and the H antisera were obtained from the Statens Serum Institute (Copenhagen, Denmark).

Isolates positive for virulence genes, were examined for susceptibility to 12 antibiotics using the Bauer–Kirby disc diffusion method. After incubation, the diameter (in millimeters) of the zone of inhibition of each antibiotic was measured.

Template DNA from seven serotyped isolates was examined by PCR for several β-lactamase (*bla*) encoding genes including; *tem, carb, shv, oxa* and *ctx*, as described previously. Variable regions containing the gene cassette(s) associated with class 1 integron structures were amplified, as described previously. The genes mapping to the 5′- and 3′-conserved structures of class 1 integrons, were also identified by PCR.

Amplicons of interest were extracted directly from agarose gels using a QIAGEN gel extraction kit. The recovered DNA fragment of interest was purified and quantified (as described above) prior to being sequenced commercially. Sequence text files were subsequently obtained and used to search the current GenBank databases using the BLAST suite of programs. CLUSTALW amino acid sequence alignments were produced for comparison.

From 128 samples collected, 103 isolates were obtained which grew on SMAC-strep, covering the plate with a sorbitol fermenting (pink), mucoid growth. Each sample was individually sub-cultured onto SMAC-strep from which a single colony was taken for subsequent testing. Upon sub-culturing, large (2 mm diameter), round, mucoid colonies were observed and Gram-negative rod-shaped bacteria were identified following staining. All isolates were negative for Voges–Proskaur, hydrogen sulfide, citrate, oxidase and urease production and were positive for indole and fermented glucose, mannitol and sorbitol. The isolates were confirmed as *E. coli* following analysis of API 20E and API 50CH strip results. The API results were confirmed by PCR amplification of the 16S rRNA gene (data not shown).

From the 103 *E. coli* isolates tested, 7 were positive for the presence of *vt*1 or *vt*2 genes as presented in Table 12**.** Of these isolates, 4 were detected from cattle in Farm A, one from soil in Farm A and 2 isolates were detected from cattle faeces taken in Farms B and D. All 7 isolates were negative for the presence of the rfb_{O157} and $fliC_{h7}$ genes. Two isolates were also identified positive for the presence of *eae* and *hly* genes. Both isolates were serotyped as O123:H2.

Table 12: Phenotypic and genotypic characteristics of VTEC isolates from cattle faeces and soil

Farm	Animal ID	Isolate origin	Serotype	*vt*	Verotoxin produced	*Hly*	*eae*	Antibiotic resistance pattern[a]
A	12	Dairy calf	O2:H27	*vt*2	VT2	+	−	A, S, S3, C, T
A	50	Soil	O149:H1	*vt*1	VT1	−	−	S
A	58	Beef cow	O26:H11	*vt*1	VT1	+	+	A, S, S3, C, T
A	80	Beef calf	O63:H$^-$	*vt*1	VT1	+	+	A, K, S, W, N, S3, C, T, CIP
A	84	Beef calf	O148:H8	*vt*2	VT2	+	−	S
B	92	Steer	ONT:H25	*vt*1	NT	−	−	A, K, S, W, S3, T
D	101	Calf	O174:H21	*vt*2	VT2	−	−	A, S, S3, C, T

[a] Anti-microbial agents tested: A = ampicillin; K = kanamycin; S = streptomycin; W = trimethoprim; S3 = compound sulphonamides; CIP = ciprofloxacin; C = chloramphenicol; T = tetracycline and N = nalidixic acid; ONT = untypeable; NT = not tested.

Isolates obtained from animals 92 and 80 were not included in the PCR product sequencing analysis as either the *vt*1 gene was lost following storage (isolate 92) or the isolate could not be resuscitated (isolate 80). BLAST searches showed that PCR products from positive *vt*1, *vt*2, *hly* and *eaeA* reactions were homologus to similar sequences in known bacteria carrying these genes. Deduced amino acid sequences from each PCR product were compared with selected known bacteria using ClustalW. The alignment demonstrated a similar amino acid homology across the regions examined. In summary it can be stated that the genes for *vt*1, *vt*2, *eae* and *hly* were present in the isolates examined. Verotoxin production was confirmed for each isolate positive for the presence of *vt*1 or *vt*2 gene.

Serotyping was performed and 7 different VTEC serotypes were identified; O2:H27, O149:H1, O26:H11, O63:H$^-$, O148:H8 and O174:H25 with 1 untypeable serotype ONT:H25 (Table 12). Multi-drug resistance (the resistance to three or more different antibiotic classes) was demonstrated in 5 of the 7 isolates. The AR profiles show that some of the isolates share a common resistance pattern. Serotype O174:H21 obtained from Farm D was resistant to the same 5 antibiotics as serotypes O2:H27 and O26:H11 isolated from Farm A. The *bla*-$_{TEM}$ gene was present in all 7 of the VTEC isolates with the exception of isolate 50 and 84. None of the other *bla* genes tested were found to be present. *E. coli* O63:H$^-$ was the only strain found to contain complete class 1 integron structures. The integrase1 (*int*1), quaternary ammonium compound resistance (*qacDE*1), and sulphonamide resistance (*sull*) genes in addition to 4 gene cassettes (of sizes including1.0, 1.2, 1.6 and kb) were detected after PCR. Sequence analysis of 2 gene cassettes 1.0 and 1.2 kb (Accession numbers EU938126 and EU938125) amplified from the E. *coli* O63:H−, gave 100% sequence identity to the aadA1 gene in *E. coli*. The remaining gene 1.6 kb gene cassette (Accession No. EU938127) contained two ORF's in the classical 'head-to-tail' orientation, with complete identity to a dfr1 gene and aadA1 gene in E. *coli*. The aadA1

(adenyltransferase) gene confers aminoglycoside resistance and the dfr (dihydrofolate reductase) gene confers trimethoprim resistance.

Findings from the current study suggest that targeted sampling and method development utilising the antibiotic resistant characteristics may reveal many more of these serotypes in the bovine population and possibly elsewhere. Antibiotic resistant *E. coli* are known to be disseminated through the food chain and, therefore, the bacteria from this study may represent a new group of clinically significant food-borne pathogens.

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The effect of pre-transport periods (0, 12 and 24 h) of food and water deprivation on the response to 12 and 24 h transport in yearling heifers

There is a paucity of data with regard to the interaction between pre-transport food and water deprivation and the response to transport, particularly to long-haul road transport. The objective of this study was to quantify the effect of pre-transport food and water deprivation on the response to transport in yearling cattle.

At CSIRO in Australia, eighty-four (261.7± (s.e.) 12.4 kg) *Bos taurus* composite beef breed yearling heifers were blocked by weight and assigned to treatment. A factorial design was used comprising three pre-transport food (pasture) and water deprivation treatments of 0, 12 and 24 (hour) h (restricted) and two transport duration treatments of 12 and 24 h, and these were replicated twice over a period of 4 weeks. The cattle were transported at a space allowance of (0.82 m^2/head) over a route that included highway and secondary roads. Each animal was blood sampled via jugular venipuncture on 6 occasions; prior to and on completion of the food+water deprivation (restricted) period and 0, 24, 48 and 72 h post-transport. Two blood samples were taken at each time point (6ml EDTA and 10 ml serum vacutainers). An aliquot of whole blood was analysed for red blood cells (RBC) and white blood cells (WBC) count, differential cell counts of neutrophils (NEU) and lymphocytes (LYM), and haematocrit (HCT) %. The concentrations of cortisol, total protein, albumin and creatine kinase (CK) were determined in serum samples. Urine was collected and specific gravity was measured with a refractometer. Body temperature was recorded using Thermochron iButtons attached to rectal probes which were removed at 72 h post-transport. IceTag™ behavioural monitors (IceRobotics) were secured to the left hind leg of each animal and these monitors recorded lying, standing or active (walking, moving). Data were analysed using the MIXED model procedure in SAS (SAS Institute Inc., Cary, NC, USA). A model comprising the terms restricted treatment, transport treatment, replicate and time (pre-transport, 0 h post-transport, 24 h post-transport, 48 h post-transport and 72 h post-transport) and interactions plus a random term for animal was used to quantify the main fixed effects of restricted and transport duration.

The rectal temperature profiles and the temperature humidity index (THI) during the transport phase for 12 h and 24 h transport treatments are presented in Figure 7. Transport invoked an increase in temperature followed by a reduction to more basal levels. A significant four-way interaction between restricted treatment × transport treatment × replicate × time was found for a number of measures (liveweight, urine osmolality and specific gravity, red blood cell count, haematocrit % and the serum concentrations of BUN, total protein and albumin) Table 13. This interaction generally showed that the key effect of interest, namely the magnitude of the

interaction between restricted treatment × transport treatment × time varied between replicates. Variation between replicates was expected. In view of this, and in the interests of simplicity, emphasis was given to the three-way interaction between restricted treatment × transport treatment × time. There was a significant difference in the pre-transport liveweights as a consequence of the restricted treatments. The significant interaction between restricted treatment × transport treatment × time ($P<0.05$) was largely influenced by the differences observed pre-transport and immediately post-transport. On arrival, the transport mediated losses in liveweight were inversely related to the restricted duration. For example, the liveweight lost over 24 h of transport was 26.6, 18.0 and 13.7 kg for the 0, 12 and 24 h restricted treatments, respectively. The liveweight differences between the different restricted x transport treatments at 24, 48 and 72 h post-transport were not significant. This indicates that although there were significant differences in liveweight on arrival between the treatment groups, this was rapidly attenuated during the 72 h recovery period. At the conclusion of the recovery period, the cattle had returned to 93 – 95 % of their pre-transport mean liveweight.

Several of the indicators of hydration status (serum osmolality, urine specific gravity and osmolality, Tables 13 and 14 were significantly influenced by the interaction of restricted treatment × transport treatment × time ($P<0.001$). The temporal changes over the transport and recovery phases were very similar for these measures (Figure 8 and 9). As the pre-transport period of food and water deprivation increased, these measures increased, although there were some inconsistencies and in some cases, the differences were not always apparent. The period of transport resulted in further concentration of both blood and urine indicating further dehydration. The combined effects of restricted and transport duration were generally additive, where urine osmolality and specific gravity and serum osmolality increased as the cumulative period of feed and water deprivation increased. In some instances, the differences were not always consistent or large in magnitude. For example, for urine osmolality immediately post-transport, only the 0 h restricted + 12 h transport group significantly differed from the other five treatment groups.

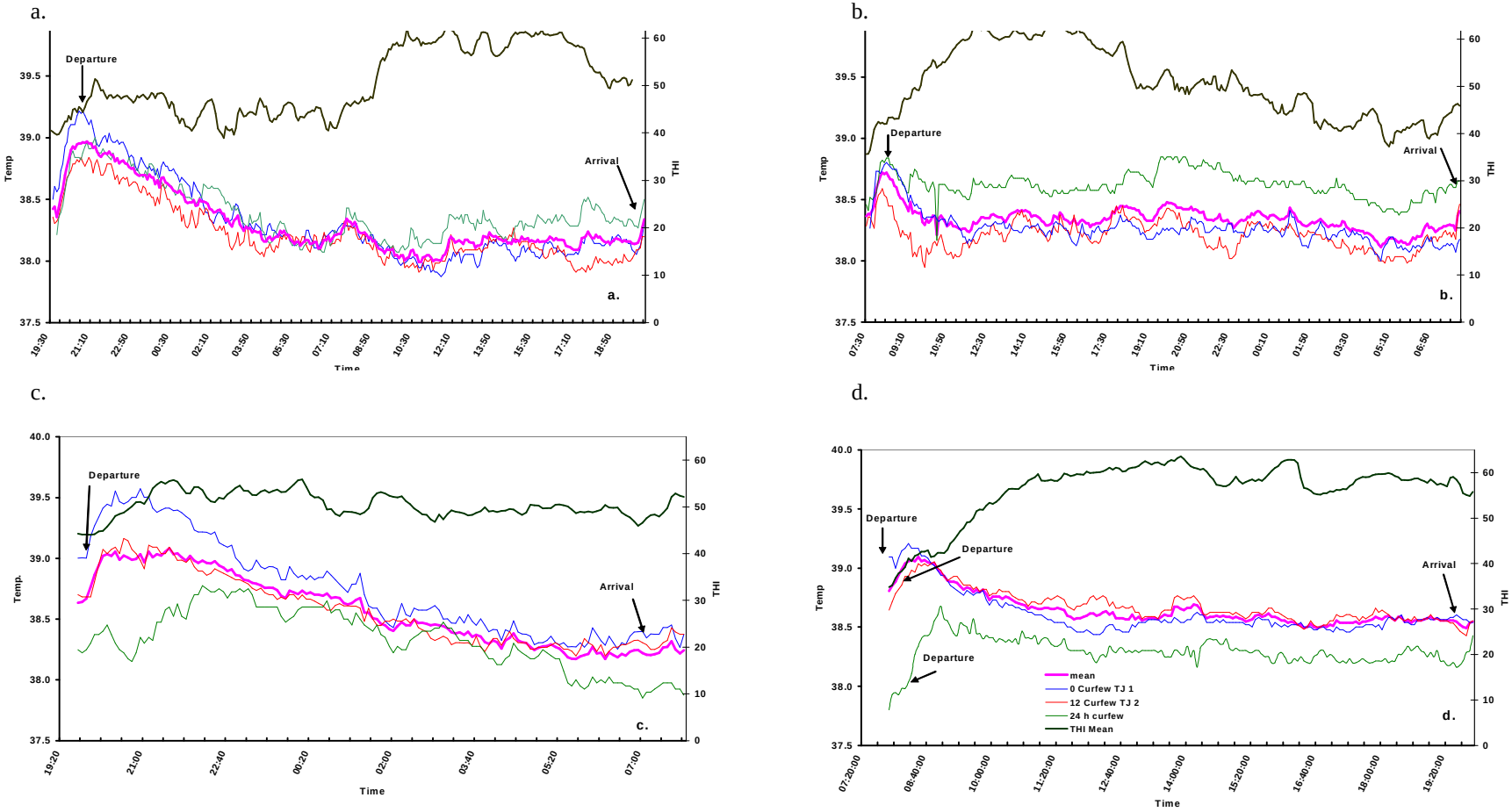

Figure 7. Rectal temperature profiles and the temperature-humidity index (THI) during: a. (Upper left) 24 h of transport (pm departure), b. (Upper right) 24 h of transport (am departure), c. (Lower left) 12 h of transport (pm departure) and d. (Lower right) 12 h of transport (am departure).

Table 13: Least square means ± sem for restricted treatment, transport duration, replicate and time and significance of the interactions for serum concentrations of blood urea nitrogen (BUN), total protein (TP), albumin (Alb), creatine kinase (CK), β-hydroxy butyrate (BHB), cortisol (Cort) and serum osmolality (Osmol)

Main Effects	**BUN** (mg/dL)	**TP** (g/L)	**Alb** (g/L)	**CK*** (U/L)	**BHB** (mmol/L)	**Cort*** (nmol/L)	**Osmol** (mOsmol/L)
Restricted duration (C)							
0 h	12.82	65.66	31.51	336.47	0.21	61.89	281.89
12 h	14.33	68.11	32.31	351.05	0.19	67.31	285.36
24 h	12.78	68.69	32.55	560.95	0.19	76.43	288.09
sem	0.30	0.61	0.40	-	0.007	-	0.70
Significance	*P<0.001*	*P<0.01*	*ns*	*P<0.01*	*P<0.05*	*P=0.07*	*P<0.001*
Transport duration (T)							
12 h	12.96	67.62	32.16	408.27	0.19	64.34	285.55
24 h	13.66	67.36	32.08	401.06	0.20	72.61	284.68
sem	0.24	0.49	0.33	-	0.006	-	0.57
Significance	*P=0.05*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Replicate (R)							
1	13.14	68.19	31.69	378.15	0.20	69.61	284.00
2	13.47	66.78	32.55	433.01	0.19	67.22	286.23
sem	0.24	0.49	0.33	-	0.006	-	0.57
Significance	*ns*	*P<0.05*	*P=0.07*	*ns*	*ns*	*ns*	*P<0.01*
Time							
Pre-transport	11.31	68.31	32.34	319.13	0.20	82.30	282.30
0 h post-transport	13.77	70.58	34.16	847.06	0.17	55.48	291.02
24 h post-transport	14.53	66.96	31.38	440.19	0.21	78.06	284.80
48 h post-transport	13.70	66.29	31.68	339.15	0.17	72.00	285.78
72 h post-transport	13.23	65.30	31.06	268.83	0.22	56.47	281.66
sem	0.23	0.42	0.28	-	0.008	-	0.81
Significance	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*	*P<0.001*
Interactions							
C x T	*ns*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*ns*
C x R	*ns*	*P=0.08*	*ns*	*ns*	*P<0.01*	*ns*	*ns*
C x Time	*P<0.001*	*P<0.001*	*ns*	*P<0.001*	*P<0.05*	*ns*	*P<0.001*
T x R	*P<0.001*	*ns*	*ns*	*ns*	*P<0.05*	*P<0.05*	*P<0.01*
T x Time	*P<0.001*	*ns*	*P<0.01*	*P=0.05*	*ns*	*P=0.08*	*P<0.001*
R x Time	*P<0.05*	*ns*	*ns*	*ns*	*P<0.001*	*ns*	*P<0.01*
C x T x R	*ns*	*ns*	*ns*	*ns*	*P<0.01*	*P<0.001*	*P<0.01*
C x T x Time	*P<0.001*	*ns*	*ns*	*ns*	*P<0.05*	*ns*	*P<0.001*
C x R x Time	*P<0.001*	*ns*	*P<0.05*	*P<0.01*	*P<0.05*	*ns*	*P<0.001*
T x R x Time	*P<0.001*	*ns*	*ns*	*ns*	*ns*	*ns*	*P<0.001*
C x T x R x Time	*P<0.01*	*P<0.001*	*P<0.01*	*ns*	*ns*	*ns*	*P<0.01*

*backtransformed least square means shown

The interaction of restricted treatment × transport treatment x time was not significant for all hydration measures, the notable exceptions being serum albumin and total protein and haematocrit %. However, although not significant, a similar temporal trend was observed where there was a general increase in these measures (i.e. haemoconcentration) as the cumulative period of feed and water deprivation increased. Significant interactions between restricted treatment × time (total protein and haematocrit % $P<0.001$) and transport treatment x time (albumin $P<0.01$) were also observed for these measures.

A significant interaction between restricted treatment × time ($P<0.001$) was found where the CK levels were higher in the 24 h restricted treatment at each of the post-transport time points (Figure 10). This was particularly evident immediately on arrival. The interaction between transport treatment x time was also just significant ($P=0.05$) and it showed that transport caused the serum CK levels to increase and that the magnitude was directly related to the transport duration (Figure 10). During recovery, the CK concentrations very quickly returned to their pre-transport levels and there was no sustained effect of transport duration. However, there was an indication of a sustained restricted duration effect where the CK levels were significantly higher ($P<0.01$) for the 24 h restricted group relative to the other two treatments, even after 24 h of recovery.

A significant interaction between restricted treatment transport treatment ($P<0.05$) was found for serum cortisol concentration. Overall, however, the changes in cortisol over time and indeed in response to either the restricted or transport treatments do not indicate any consistent or obvious trends. The temporal changes in the overall and differential white blood cell (neutrophils and lymphocytes) counts significantly differed between the restricted treatment × transport treatment groups. However, apart from the very obvious transport-mediated increase in white blood cell counts and neutrophil:lymphocyte ratio, there were no clear trends with regard to the interaction between restricted and transport duration.

The changes in red blood cell count (RCC) over time differed between the restricted treatments ($P<0.001$). The interaction restricted treatment × transport treatment × time was significant for BUN ($P<0.001$) and BHB ($P<0.05$). With the exception of the restricted 0 h + transport 12 h group, the serum concentrations of BUN increased in response to transport. The levels on arrival were generally reflective of the total cumulative time of feed and water deprivation. During recovery, the temporal changes were quite variable between the restricted × transport groups. For BHB, the interpretation of the interaction was difficult because of a lack of clear or consistent changes over time between the treatment groups.

Table 14: Least square means ± sem for restricted treatment, transport duration replicate and time and significance of the interactions for a subset of blood haematology measures (red cell count – RCC, haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lympocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), liveweight, and urine specific gravity (SG) and osmolality

Main Effects	**RCC** ($x10^{12}$/L)	**HCT** (%)	**WCC** ($x10^{9}$/L)	**NEU*** ($x10^{9}$/L)	**LYM*** ($x10^{9}$/L)	**NEU:LYM**	**Liveweight** (kg)	**Urine SG**	**Urine Osmol*** (mOsmol/L)
Restricted duration (C)									
0 h	7.80	34.89	7.81	1.25	3.75	0.90	254.76	1.031	914.53
12 h	7.83	35.60	7.91	1.33	3.78	0.95	251.67	1.031	943.08
24 h	8.05	36.94	7.74	1.33	3.56	0.95	247.16	1.035	975.85
sem	0.13	0.47	0.27			0.07	2.37	0.0008	21.70
Significance	ns	P<0.05	ns	ns	ns	ns	P=0.08	P<0.001	ns
Transport duration (T)									
12 h	7.82	35.50	7.66	1.28	3.53	1.00	251.91	1.031	914.08
24 h	7.96	36.12	7.98	1.32	3.87	0.87	250.49	1.033	974.89
sem	0.10	0.39	0.22			0.07	1.94	0.0006	17.73
Significance	ns	ns	ns	ns	ns	ns	ns	ns	P<0.05
Replicate (R)									
1	7.75	35.33	8.13	1.33	3.82	0.94	254.0	1.031	927.46
2	8.03	36.29	7.52	1.27	3.57	0.92	248.4	1.033	961.50
sem	0.10	0.39	0.22			0.07	1.94	0.0006	17.73
Significance	ns	ns	P=0.05	P<0.05	ns	ns	P<0.05	ns	ns
Time									
Pre-transport	8.03	35.93	7.07	1.08	3.89	0.68	257.19	1.033	895.13
0 h post-transport	8.03	36.22	9.14	2.11	2.96	1.89	240.44	1.045	1124.08

Table 14 (cont'd): Least square means ± sem for restricted treatment, transport duration replicate and time and significance of the interactions for a subset of blood haematology measures (red cell count – RCC, haematocrit – HCT, white blood cell count – WCC, neutrophils – NEU, lympocytes – LYM, neutrophils:lymphocytes ratio – NEU:LYM), liveweight, and urine specific gravity (SG) and osmolality

Main Effects	**RCC** ($x10^{12}$/L)	**HCT** (%)	**WCC** ($x10^{9}$/L)	**NEU*** ($x10^{9}$/L)	**LYM*** ($x10^{9}$/L)	**NEU:LYM**	**Liveweight** (kg)	**Urine SG**	**Urine Osmol*** (mOsmol/L)
24 h post-transport	7.95	36.33	7.55	1.09	4.02	0.64	252.31	1.028	887.70
48 h post-transport	7.86	35.97	7.65	1.17	3.87	0.70	252.43	1.027	909.92
72 h post-transport	7.59	34.60	7.71	1.27	3.79	0.77	253.62	1.027	905.60
sem	0.08	0.32	0.20			0.07	1.45	0.0008	21.50
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0001	P<0.001	P<0.001	P<0001
Interactions									
C x T	ns	ns	ns	P<0. 01	P=0.07	P<0.01	ns	ns	ns
C x R	ns	ns	ns	P<0.05	P<0.05	P<0.05	ns	ns	ns
C x Time	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001	P<0.001
T x R	ns	ns	ns	ns	ns	ns	P<0.05	P<0.01	P<0.05
T x Time	ns	ns	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001	ns	P<0.05
R x Time	ns	ns	ns	ns	P<0.01	ns	ns	P<0.01	ns
C x T x R	ns	P=0.07	ns	P<0.05	P<0.01	P<0.01	ns	ns	P=0.06
C x T x Time	ns	ns	P<0.001	P<0.001	P<0.001	P<0.01	P<0.05	P<0.001	P<0.001
C x R x Time	ns	ns	ns	ns	P<0.001	ns	P<0.001	P<0.05	P<0.01
T x R x Time	ns	ns	P<0.001	P<0.01	ns	ns	P<0.001	P<0.05	ns
C x T x R x Time	P<0.01	P<0.05	ns	ns	ns	ns	P<0.001	P<0.001	P<0.001

*backtransformed lensast square means shown

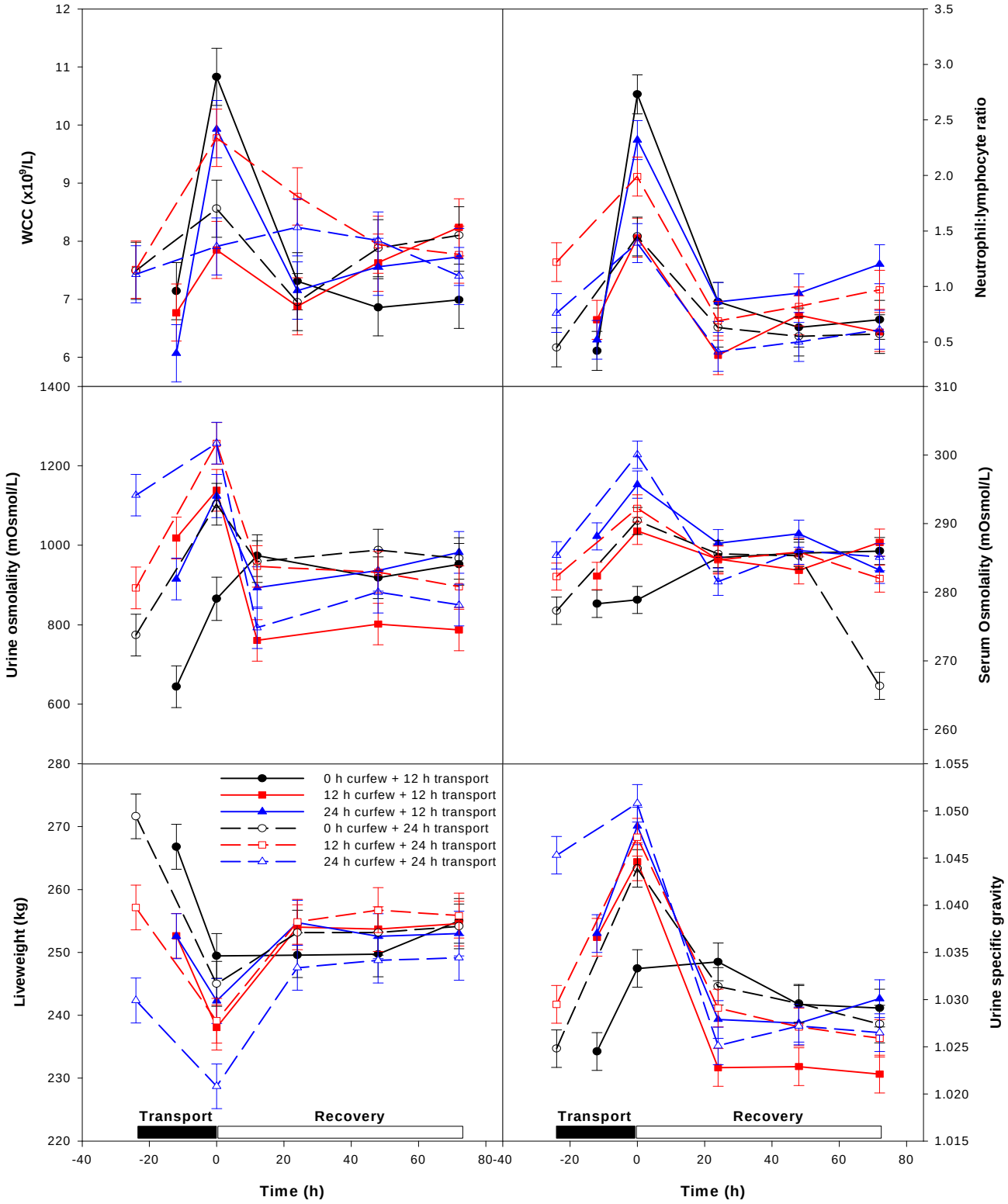

Figure 8. Least square means for white blood cell count (WCC), neutrophil:lymphocyte ratio, urine osmolality, serum osmolality, liveweight and urine specific gravity for the interaction between restricted duration × transport duration × time.

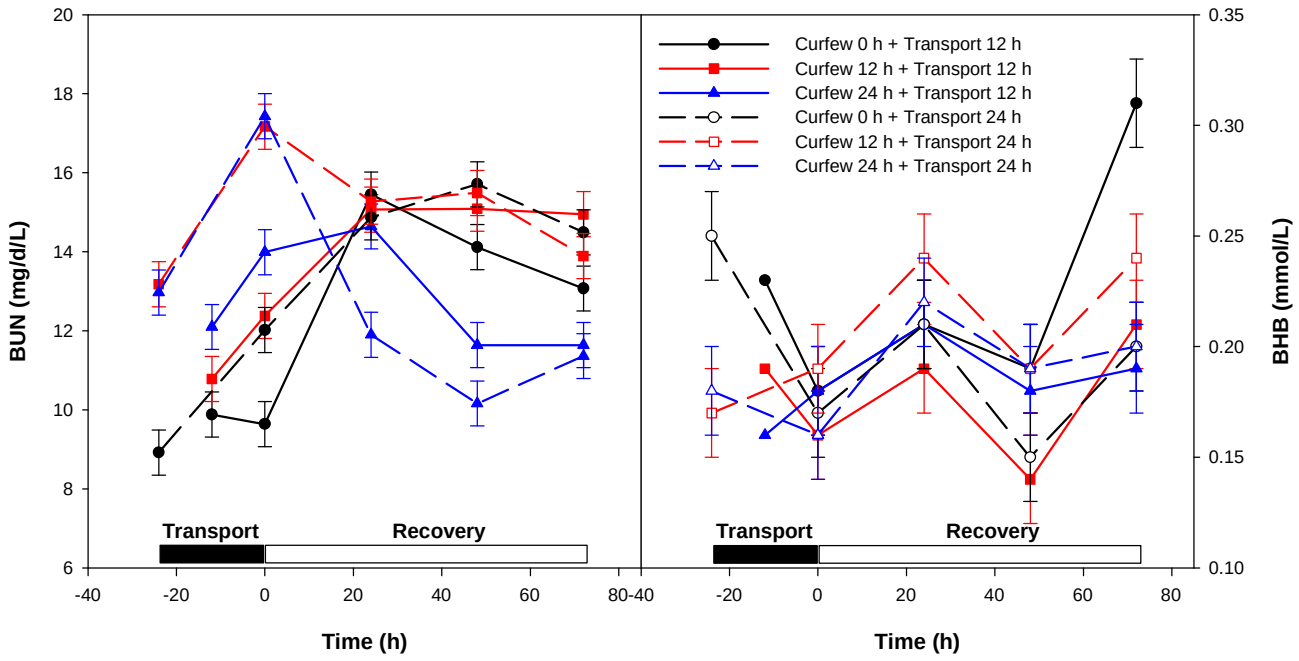

Figure 9. Least square means for blood urea nitrogen (BUN) and β-hydroxy butyrate (BHB) for the interaction between restricted duration × transport duration × time.

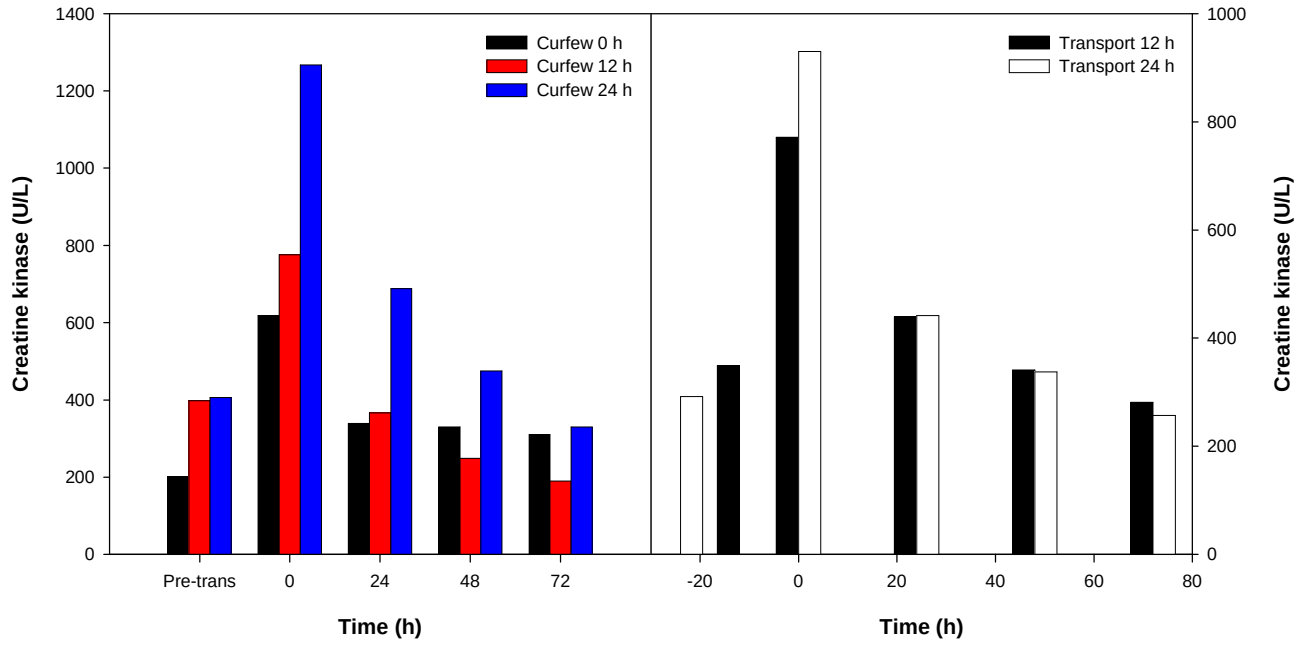

Figure 10. Least square means for serum creatine kinase concentration for the interactions between restricted treatment × time (P<0.001) and transport treatment × time (P=0.05).

It is concluded that during livestock transport, particularly over moderate to long durations (12 – 48 h), it is the physiological states of fatigue and dehydration that are of most concern from an animal welfare perspective. With regard to dehydration, the results based on the haemoconcentration and urinary measures indicate that the combination of the curfew and transport periods were additive in their effect on hydration status. Put simply, fluid loss, as indicated by these measures, increased commensurate with the total combined period of water deprivation. The water consumed by the treatment groups during the initial 3 h of recovery was also indicative of the total period of water deprivation, particularly at the extremes (i.e. 12 versus 48 h). It is worth noting that the treatment group differences in water consumption during this period were not significantly different. The combination of 24 h of restriction of feed and water followed by 24 h of transport, which represents the maximum time of water deprivation allowable under the welfare transport code, elicited the greatest changes in both urine and haemoconcentration measures. However, these changes were generally within the normal physiological limits.

Subjecting healthy, grass-fed cattle to pre-transport periods of food and water deprivation (12 and 24 h compared with 0 h controls) before transport for 12 or 24 h did not enhance the capacity of these animals to cope with transport. Furthermore, the results also emphasise that periods of food and water deprivation prior to transport are simply additive to transport-related periods of deprivation, and provide further evidence that healthy cattle under non-threatening environmental conditions can tolerate periods of up to 48 h of feed and water deprivation without undue compromise to their welfare.

RMIS No. 5475

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Effect of abrupt weaning on circulating leukocyte subsets and phagocytic activity of neutrophils in beef suckler calves

At weaning 16 spring-born, single-suckled, previously grazed calves (mean age and weight (s.d.): 227 (18.2) d and 310 (31.1) kg, respectively) of Limousin × Holstein-Friesian and Simmental × Holstein-Friesian dams, were either abruptly weaned (AW, n = 8) and housed in a slatted floor shed and offered grass silage *ad libitum*, or not weaned (control) (NW, n = 8) and housed with their dam and offered the same diet. Jugular blood was collected on day -7, 0 (weaning), 2, 7, and 14. Neutrophil number was determined in K_3EDTA anti-coagulated blood. Leukocyte subset were characterised in ACD anti-coagulated blood by flow cytometry. Percentages of positive-staining $CD4^+$, $CD8^+$, $WC1^+$ lymphocytes, MHC Class II^+ cells and $G1^+$ neutrophils were recorded. Surface expression of CD62L was recorded as mean fluorescence intensity (MFI) of neutrophils gated on the basis of their light-scattering properties. Phagocytic activity of neutrophils was examined in heparinised blood using a commercial assay. Data were analysed as repeated measures using PROC MIXED of SAS. The model included fixed effects of treatment (T), sampling time (S), and sampling × treatment interaction (T × S).

On day 2, neutrophil number increased ($P < 0.001$) in AW (6.7 ± (s.e.m) 0.36 × 10^3 cells/μl) compared with day 0 (2.6 ± 0.36 × 10^3cells/μl), whereas neutrophil number in NW did not differ ($P > 0.05$) from day 0 (2.9 ± 0.36 ×10^3cells/μl) or throughout the experimental period. On d 2, % $CD4^+$ and $CD8^+$ lymphocytes decreased ($P < 0.001$) in AW and did not differ ($P > 0.05$) in NW, compared with day 0 (Table 4). The % $WC1^+$ cells, CD62L MFI and phagocytic activity decreased ($P < 0.05$) on day 2 in AW and NW, however the decrease was greater ($P < 0.001$) in AW (Table 15, Figure 11). On day 2, % $G1^+$ neutrophils and MHC Class II^+ cells increased ($P < 0.01$) in both treatments compared with day 0, but the increase was greater ($P < 0.001$) in AW than NW.

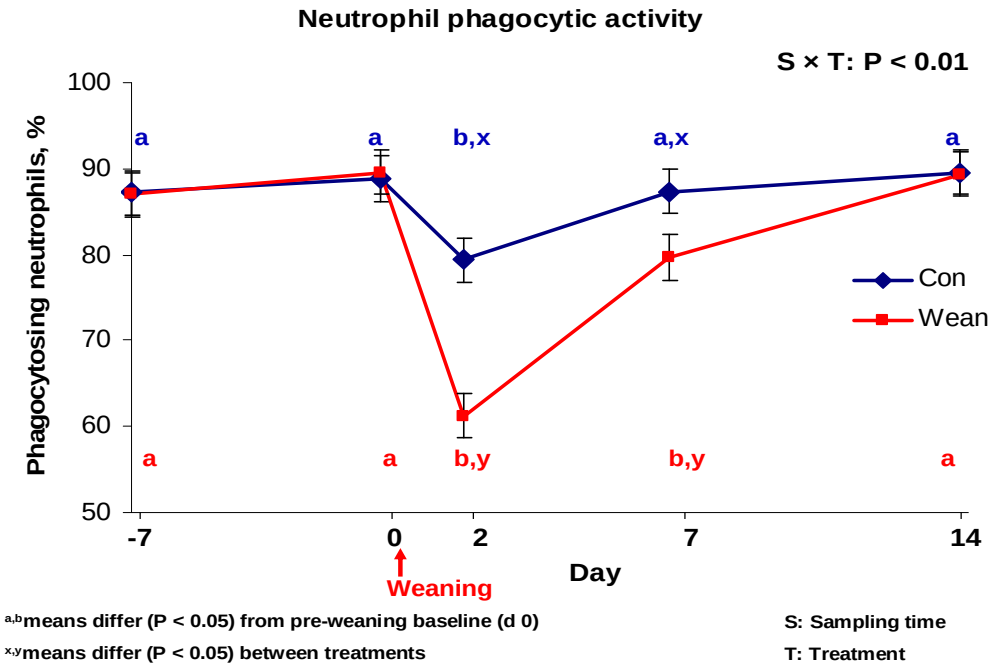

Figure 11. Percentage of phagocytosing neutrophils in abruptly- (wean) and non-weaned calves (controls) relative to day of weaning (day 0). Means not having a common superscript differ (P < 0.05) from d 0$^{(a,b,c)}$ and between control and weaned$^{(x,y)}$.

It was concluded that abrupt weaning resulted in increased neutrophil number and the functionality of these cells to potentially traffic and phagocytose efficiently was impaired. Together with the greater changes in lymphocyte subsets and % MHC Class II^{+} cells this suggests that there was a greater transitory reduction in immune function in abruptly weaned than non-weaned beef suckler calves.

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Table 15: Percentage (mean ± S.E.M) of $CD4^+$, $CD8^+$ and $WC1^+$ lymphocytes, MHC class II^+ cells, $G1^+$ neutrophils and neutrophil CD62L MFI in weaned and non-weaned calves

		Day						P value		
Leukocyte subset		-7	0	2	7	14		T	S	T × S
$CD4^+$ lymphocytes, %	NW	19.4	20.2	20.2^{x}	22.3^{a}	22.5^{a}	F value	NS	***	**
	AW	21.8	20.2	$15.1^{a,y}$	21.7^{b}	22.6	SEM	0.46	0.73	1.03
$CD8^+$ lymphocytes, %	NW	10.7	10.6	10.1^{x}	10.0	10.8	F value	NS	***	**
	AW	11.8	11.0	$8.0^{a,y}$	9.9^{b}	11.4	SEM	0.19	0.3	0.43
$WC1^+$ lymphocytes, %	NW	23.1	24.0	$20.0^{a,x}$	23.5^{b}	24.0	F value	**	***	***
	AW	24.3	24.1	$12.3^{a,y}$	23.9^{b}	23.7	SEM	0.3	0.48	0.68
MHC Class II^+ cells, %	NW	13.4	14.3	$21.9^{a,x}$	16.0^{b}	12.6	F value	**	***	***
	AW	14.1	13.7	$30.3^{a,y}$	16.8^{b}	13.6	SEM	0.48	0.77	1.08
$G1^+$ neutrophils, %	NW	37.6	37.3	$45.0^{a,x}$	40.9	37.7	F value	NS	***	NS
	AW	38.9	37.4	$52.0^{a,y}$	41.3	38.1	SEM	0.94	1.49	2.11
CD62L, MFI^1	NW	57.1	58.3	54.5^{x}	61.7	61.5	F value	NS	***	**
	AW	59.5	59.3	$44.0^{a,y}$	56.6	62.4	SEM	0.95	1.51	2.13

^{a,b} means within row not having a common superscript differ ($P < 0.05$) from pre-weaning baseline (d 0). ^{x,y} denotes difference ($P < 0.05$) between control and weaned. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, NS = non significant, $P > 0.05$.

Effect of abrupt weaning and subsequent housing on leukocyte populations and acute phase proteins in beef suckler cows

Thirty-six first parity, spring-calving (mean date of calving March, 19, single-suckled Limousin × Holstein-Friesian (n = 18) and Simmental × Holstein-Friesian (n = 18) cows were used. Cows and their progeny were rotationally grazed on a predominantly perennial ryegrass (*Lolium perenne*)-based sward from early April until weaning on October 17. The pasture had a mean (s.d.) dry matter digestibility (DMD) of 750 (22.1) g/kg and mean (s.d.) crude protein (CP) concentration of 229 (8.7) g/kg DM. On the day of weaning (day 0), cows and calves were moved to a handling yard where the cows were abruptly separated from their calves. The calves had a (mean age (s.d.) of 212 (24.5) days; a mean weight of s.d. 279 (38.0) kg and were returned to the grazing area for a 35 day period. The grazing area was located at a sufficient distance from the handling yard so that vocalisations between the cows and calves could not be heard. On day 35, the cows were housed indoors in a slatted floor shed in 3 pens (n = 6 cows per pen). Each pen was equipped with automatic water drinkers and cows were offered grass silage *ad libitium* (mean (s.d) DMD 714 (17.7) g/kg; CP 144 (11.3) g/kg DM) plus 60 g of a mineral vitamin supplement daily. Blood samples were collected from the cows by direct jugular venipuncture to evaluate i) the effects of weaning (day 0 (pre-weaning baseline), 2, 7, 14, 21, 28, and 35) and ii) the effects of housing (day 0 (pre-housing baseline), 2, 7, 14 and 21). On day 0 for weaning and housing, blood samples were collected prior to the management practice to establish pre-weaning and pre-housing baseline, respectively. Blood (10 ml) collected into vacutainer tubes containing lithium heparin was used to determine concentrations of glucose, non-esterified fatty acid (NEFA), β-hydroxybutyrate (β-HB), haptoglobin, cortisol, dehydroepiandrosterone (DHEA), and for the determination of *in vitro* lymphocyte production of IFN-γ from a whole blood culture. Blood (4 ml) was collected into vacutainer tubes containing sodium citrate for the subsequent assay of plasma fibrinogen concentration. An additional 6ml of blood was collected into vacutainer tubes containing K_3EDTA for haematological determination.

Following abrupt weaning, total leukocyte number was increased ($P = 0.07$) on day 2 and decreased ($P < 0.05$) on d 7 compared with day 0 (Figure 12). Total leukocyte number was unchanged ($P > 0.05$) on day 14 to d 56 compared with day 0. On day 2, neutrophil number increased ($P < 0.001$) and subsequently was decreased on day 7 to day 21 ($P < 0.05$) compared with day 0 (Figure 12). Lymphocyte number decreased ($P < 0.05$) on day 7 compared with day 0. Following housing, neutrophil number increased ($P < 0.01$) and lymphocyte number decreased ($P < 0.05$) on day 37 compared with day 35 baseline; both returned to day 35 baseline by day 42. Concentrations of plasma fibrinogen increased ($P<0.05$) on day 7 to 35 and plasma haptoglobin increased ($P<0.01$) on d 7 to 28 compared with pre-weaning baseline (Table 16). Post housing, concentration of plasma fibrinogen decreased ($P<0.05$) on day 2 and subsequently increased ($P<0.001$) on day 14 compared with pre-housing baseline. Concentration of plasma haptoglobin was unchanged on d 2 and 7 ($P>0.05$) but decreased ($P<0.05$) on d 14 and 21 compared with pre-housing baseline (Table 16). Abrupt weaning did not affect plasma cortisol and DHEA concentrations in the present study.

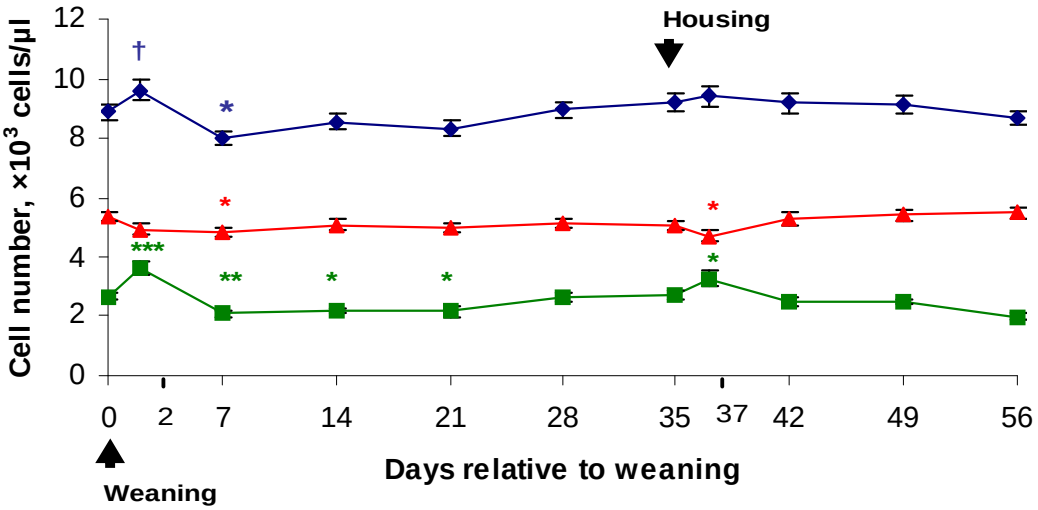

Figure 12. Total leukocyte (-♦-), neutrophil (-■-) and lymphocyte (-▲-) profiles in beef cows following abrupt weaning (d 0). * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

The present study has characterised the effect of abrupt weaning and subsequent housing on the extended physiological, haematological and immunological responses in beef cows. It is concluded that, following abrupt weaning, increased N: L ratio, as defined by transient neutrophilia and lymphopenia, coupled with reduced *in vitro* lymphocyte production of IFN-γ, elevated acute phase protein response and altered metabolic response indicates that a transitory stress response, similar to that exhibited by abruptly weaned beef calves, is also evident, although to a much lesser extent, in beef cows. These findings suggests that cows may be less sensitive to weaning stress than their calves. Post-housing, increased N: L ratio defined by transient neutrophilia and lymphopenia coupled with reduced *in vitro* lymphocyte production of IFN-γ were evident in beef cows however unaltered total leukocyte number and the complex acute phase protein responses observed suggest that housing elicited a less marked stress response compared to weaning in beef cows. Results indicate that there was a transitory reduction in immunity in cows immediately post-weaning and to a lesser extent post-housing.

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Table 16: Acute phase protein concentrations (NS means ± se for plasma fibrinogen and haptoglobin) least square means (s.d.) in beef cows following abrupt weaning and subsequent housing compared with pre-weaning and pre-housing baselines, respectively

	Days											
	Pre-weaning	Post-weaning						Pre-housing	Post-housing			
Variable	0	2	7	14	21	28	35	0	2	7	14	21
Fibrinogen	408	458	489	489	493	462	467	467	411	488	587	364
(mg/dl)	(15.2)	(16.9)[a]	(18.6)[a]	(20.7)[a]	(15.7)[a]	(12.8)[a]	(20.1)[a]	(20.1)	(20.1)[b]	(22.2)	(24.9)[b]	(27.2)[b]
Haptoglobin	0.33	0.43	0.53	0.72	0.46	0.57	0.68	0.68	0.74	0.68	0.49	0.54
(mg/dl)	(0.013)	(0.031)[a]	(0.025)[a]	(0.071)[a]	(0.016)[a]	(0.025)[a]	(0.038)[a]	(0.038)	(0.037)	(0.032)	(0.017)[b]	(0.069)[b]

[a]Within a row, means differ (P<0.05) from pre-weaning baseline
[b]Within a row, means differ (P<0.05) from pre-housing baseline

Immunological response of heifers divergently ranked for residual feed intake following a bovine corticotrophin releasing hormone challenge

Limousin × Friesian heifers (n=86) were ranked on phenotypic residual feed intake (RFI). The 15 highest and 15 lowest ranking animals were used for this study and assigned to high (H) and low (L) RFI groupings, respectively. All heifers were fitted asceptically with an indwelling catherer to facilitate repeated blood sampling. On day 0 two blood samples were taken at -120 and 0 min prior to an intramuscular administration of dexamethasone (20ug/kg). On day 1 samples were taken at -40 and 0 min and then animals were intravenously administered bovine corticotrophin releasing hormone (bCRH; 0.3ug/kg). Further samples were taken at 60, 120, 240, 350, and 410 min after bCRH. Blood samples collected in K_3EDTA were analyzed for lymphocyte (LYMPH), neutrophil (NEUT) and leukocyte (LUC) counts and haemoglobin (HGB), and haematocrit percentage (HCT) using an ADVIA haematology analyzer. Distributions of data were determined by UNIVARIATE (SAS v9.1.3), and appropriate box-cox transformations applied. A repeated measures ANOVA was conducted with the main effects of bleed time (T), group (G; high or low RFI), and T × G and the average of the two blood samples pre-dexmathasone was used as a covariate.

A significant time effect was found for LUC and HGB ($P<0.01$), and for HCT ($P<0.05$). There were no significant effects for G or T × G (Table 17). Results from the immunological parameters suggest that divergently ranking animals based on RFI does not impact on the animals' immune system. These results are consistent with previous studies at Teagasc, Grange which found that LYMPH and HGB were not affected by a social regrouping stress.

The data thus suggests that divergent selection for animals with low RFI will not compromise their immune function.

RMIS No. 5742

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Table 17: Immunological variables (L.S. mean ± s.e.) for high (H) and low (L) RFI groups (G) in response to bCRH

Variable	G	Bleed Time (min)							Significance		
		-40	0	60	120	240	350	410	T	G	T×G
LYMPH ($x10^3$/µl)	H	4.25 (0.23)	4.14 (0.23)	4.15 (0.23)	3.96 (0.23)	3.93 (0.23)	4.09 (0.23)	3.95 (0.23)	NS	NS	NS
	L	3.47 (0.23)	3.91 (0.23)	4.02 (0.23)	3.57 (0.23)	4.04 (0.23)	3.96 (0.23)	3.80 (0.23)			
NEUT ($x10^3$/µl)	H	13.28 (0.61)	13.52 (0.60)	12.96 (0.60)	12.94 (0.60)	12.99 (0.60)	12.56 (0.60)	13.13 (0.60)	NS	NS	NS
	L	12.80 (0.60)	12.26 (0.60)	13.00 (0.60)	12.11 (0.60)	12.46 (0.60)	12.16 (0.60)	12.22 (0.60)			
LUC ($x10^3$/µl)	H	0.06 (0.006)	0.06 (0.006)	0.05 (0.006)	0.07 (0.006)	0.07 (0.006)	0.07 (0.006)	0.08 (0.006)	**	NS	NS
	L	0.07 (0.006)	0.06 (0.006)	0.06 (0.006)	0.06 (0.006)	0.07 (0.006)	0.07 (0.006)	0.08 (0.006)			
HGB (g/dL)	H	11.55 (0.22)	11.39 (0.22)	11.21 (0.22)	11.23 (0.22)	11.21 (0.22)	11.19 (0.22)	11.19 (0.22)	**	NS	NS
	L	11.46 (0.22)	11.17 (0.22)	11.23 (0.22)	10.84 (0.22)	11.19 (0.22)	11.27 (0.22)	11.21 (0.22)			
HCT (%)	H	30.38 (0.62)	29.91 (0.62)	29.45 (0.62)	28.89 (0.62)	28.76 (0.62)	29.04 (0.41)	29.27 (0.41)	*	NS	NS
	L	30.00 (0.62)	29.01 (0.62)	29.29 (0.62)	28.32 (0.62)	28.71 (0.62)	29.57 (0.41)	29.42 (0.41)			

* indicates $P < 0.05$ and ** indicates $P < 0.01$

Effect of banding or burdizzo castration of bulls on neutrophil phagocytosis and respiratory burst, CD62-L expression, and serum interleukin-8 concentration

Thirty-two Holstein-Friesian bulls (14 month old, 505 ± (s.e.) 7.8 kg) were blocked by weight and assigned to one of four treatments (n = 8 animals / treatment): 1) sham handled control (CON); 2) banding castration alone (BAND); 3) burdizzo castration alone (BURD); or 4) hydrocortisone i.v. infusion (CORT). On day -1, animals were jugular vein catheterised for intensive blood sampling. On day 0, each CORT animal received 16 mg hydrocortisone at 0 h (time of castration), 8 mg at 0.25 and 0.5 h, 4 mg at 0.75 and 1 h, followed by 2 mg at 1.5, 2, 3, 3.5, 5, 5.5, 6, 7 and 8 h relative to the time of castration in BAND or BURD animals. Jugular blood was collected into heparin anti-coagulated tubes on d 0, at -2 h, 0.5 h, 1 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h and 144 h, relative to treatment time, for neutrophil phagocytosis and respiratory burst assays using flow cytometry, and for plasma cortisol assay; acid citrate dextrose (ACD) jugular blood samples were collected for CD62-L flow cytometric assay; serum samples were collected for IL-8 ELISA assay. All data were analyzed using the PROC MIXED procedure of SAS. Statistical differences among treatments at different times were determined by ANOVA.

Following BAND and BURD castration and hydrocortisone infusion, plasma cortisol concentration increased ($P < 0.05$) at 0.5 and 1.0 h post treatment (Table 18). CORT bulls had greater ($P < 0.05$) cortisol concentrations than castrated animals at 0.5 h post-treatment. There was no interaction between treatment, time of treatment ($P = 0.51$), or treatment × time ($P = 0.97$) on the percentage of phagocytosing neutrophils (Phago %). Respiratory burst activity was similar ($P \geq 0.20$) among treatment groups at all time points except at 72 h post-treatment when BURD castrates had greater respiratory burst activity (8.2 ± (s.e.) 1.0%) compared with BAND castrates (5.4 ± (s.e.) 1.1%) ($P = 0.048$) and CON (4.6 ± (s.e.) 1.1%) ($P = 0.01$). There was no interaction between treatment group ($P = 0.70$), time of treatment ($P = 0.51$), or treatment group × time ($P = 0.75$) on the percentage of CD62-L positive neutrophils. Serum IL-8 concentration was lower in BURD (41.4 ± (s.e.) 11.5 pg/ml) castrates than in CON (80.0 ± (s.e.) 12.5 pg/ml) ($P = 0.026$) and CORT (77.6 ± (s.e.) 11.5 pg/ml) ($P = 0.035$) bulls at 2 h post treatment. Serum IL-8 concentrations were similar; between BAND (49.7 ± (s.e.) 14.0 pg/ml) castrates and BURD (41.4 ± (s.e.) 11.5 pg/ml) castrates ($P = 0.62$); between CON ($P = 0.07$) and CORT ($P = 0.10$) bulls at 2 h post-treatment.

Table 18: Mean plasma cortisol concentration (Lsmeans; ng/mL) of bulls banding castrated (BAND), burdizzo castrated (BURD), left untreated (CON), or injected with cortisol (CORT). [The P values for the effects of treatment, time, and treatment × time interaction, were, P < 0.0001, P < 0.0001 and P < 0.0001, respectively]

Time relative to	Treatment				Pooled s.e.
castration	CON	BAND	BURD	CORT	
– 2 h	6.9	8.0	11.1	7.5	3.61
0.5 h	6.7[b]	24.7[a]	32.2[a]	61.2[c]	4.15
1 h	5.0[c]	27.2[ab]	21.5[a]	31.0[b]	2.78
2 h	11.2	22.7	10.0	20.1	4.77
6 h	5.1	2.6	12.1	6.4	3.38
12 h	5.4[a]	8.0[ab]	8.5[ab]	16.0[b]	3.13
24 h	4.1[a]	3.5[a]	13.5[b]	8.0[ab]	2.68
48 h	7.0[b]	11.1[ab]	14.7[a]	8.6[ab]	2.40
72 h	8.4	8.7	10.1	8.4	2.42
144 h	7.7	12.0	7.8	11.2	2.22

[a, b, c] Means within a row that do not have common superscripts differ ($P < 0.05$)

[a, b, c] Means within a row that do not have common superscripts differ ($P < 0.05$)

It was concluded that neutrophil functioning in terms of phagocytosis and respiratory burst, and serum IL-8 concentration, were not compromised by banding or burdizzo castration and cortisol infusion. The results presented indicate banding or burdizzo castration does not affect the capacity of neutrophils to adhere to blood vessel endothelia, and hydrocortisone infusion on its own does not affect neutrophil trafficking. It is concluded that non-surgical castration is unlikely to induce a severe acute systemic inflammatory response in terms of neutrophil functioning.

RMIS No. 5475

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The effects of short-term dietary restriction on gene expression in key reproductive tissues of cattle

Previous studies in Athenry have shown that up to 50% of heifers become anoestrus following a short period of negative energy balance (NEB). The site(s) or the molecular mechanism(s) by which NEB induces anoestrus have not been established. The objective of this study is to identify genes that are uniquely regulated by NEB in key reproductive tissues.

A total of 28 Charolais-X heifers exhibiting regular oestrous cycles were used in the study. Heifers had their oestrous cycles synchronised using a CIRD (Controlled Internal Drug Releasing) insert for 8 days. During synchronisation all heifers were fed a diet supplying 1.2 maintenance (M). One day before device removal, heifers were allocated randomly to either a diet supplying 0.4 M (n=20) or a control diet (1.2 M; n=8). Following insert removal heifers were closely monitored for oestrus and ovulation. Ovarian follicle growth patterns were monitored, using utrasonography during the 1st follicle wave of the subsequent oestrous cycle. On the eleventh day after diet allocation and coincident with the presence of a dominant follicle (DF) on the ovaries of each heifer, all heifers received 2ml of synthetic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to induce luteolysis and allow heifers return to oestrus and ovulation. The fate of this DF was ultrasonically monitored. Twice daily blood sampling was performed every day of the diet restriction, and daily ovarian scanning was performed during each periovulatory period as shown in boxes (Figure 13). Heifers in which the DF failed to ovulate were classified as anoestrus. All heifers were slaughtered on day 17 – 19 after diet allocation and hypothalamic tissue, anterior pituitary, adrenal gland, liver and follicular tissue were collected immediately after slaughter and stored for subsequent molecular studies.

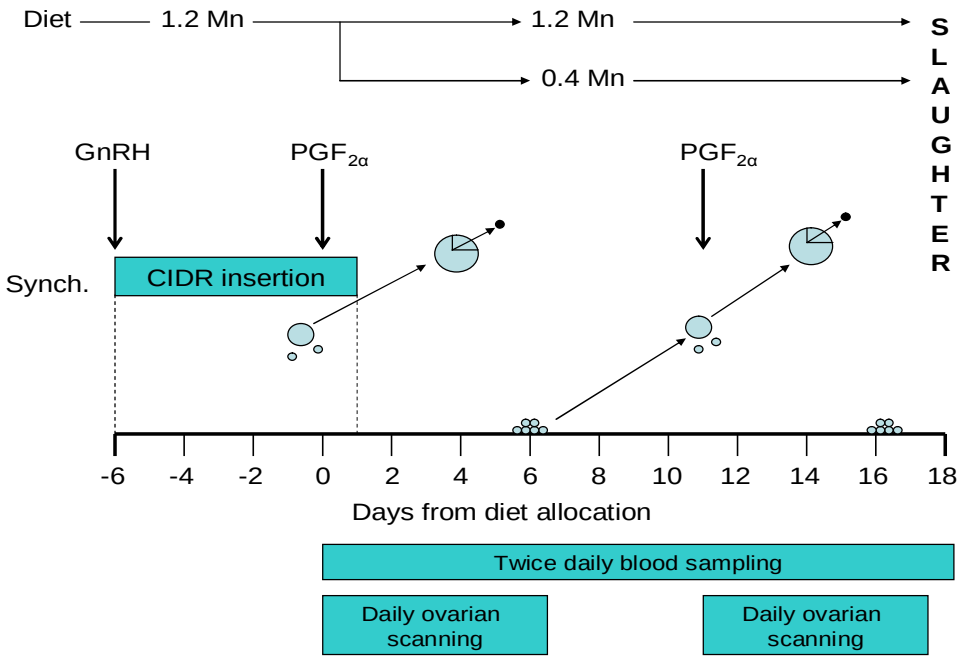

Figure 13. Diagrammatic representation of oestrous synchronisation and dietary treatments performed on beef heifers during the experiment and in relation to ovulation of the DF present at progesterone withdrawal (synchronised DF) and ovulation of the first DF of the subsequent of the oestrous cycle (first DF).

After 17 – 19 days of dietary treatment, heifers on the 0.4 M diet had lost more live weight (-35.2 ± 2.5 versus -4.3 ± 3.3 kg, respectively; $P < 0.0001$), and body condition score (-0.25 ± 0.06 versus +0.03 ± 0.08 BCS, respectively; $P < 0.05$) than heifers on the 1.2 M diet. The effect of diets supplying 0.4 M or 1.2 M on follicle wave dynamics in beef heifers when applied from one day before the removal of the CIDR is summarised in Table 19.

The size of the DF present on the ovaries of heifers allocated to either diet was similar on the day after diet allocation ($P > 0.10$). There was no significant effect of diet on any of the variables measured relating the synchronised DF. However, two heifers failed to ovulate the synchronised DF and became anoestrus within 5 days of the start of NEB. The diameter of the first DF wave of the oestrous cycle, at the time of PG injection on day 11 was smaller in heifers fed 0.4 M ($P < 0.05$) compared with heifers on the control diet. Similarly, the maximum diameter achieved by this DF and the growth rate were both reduced ($P < 0.05$) in heifers fed 0.4 M compared to control heifers on 1.2 M. Six heifers out of 18 fed 0.4 M failed to ovulate this DF while all 8 of the heifers fed 1.2 M ovulated ($P < 0.10$). The day of emergence of the second follicular wave after ovulation or regression of the first DF was not affected by diet. However, the number of follicles emerging was higher in heifers fed 1.2 M compared to restricted heifers ($P > 0.10$). After 17 – 19 days of feeding 8 of 20 heifers fed 0.4 M became anoestrous, compared to 0 out of 8 becoming anoestrous in heifers fed 1.2 M. The overall total incidence of anovulation was affected by diet ($P < 0.05$).

Table 19: The effect of diets supplying 0.4 M or 1.2 M on follicle wave dynamics in beef heifers when applied from one day before removal of the CIDR

	Nutritional level		
Parameter	**0.4 M**	**1.2 M**	**P**
No. of heifers	20	8	
Synchronised DF			
Diameter @ day 1 (mm)	9.7 ± 0.5	8.5 ± 0.8	ns
Maximum diameter attained (mm)	12.2 ± 0.3	11.9 ± 0.4	ns
Day of ovulation	3.3 ± 0.2	3.6 ± 0.2	ns
Interval: CIDR withdrawal to ovulation (days)	2.3 ± 0.2	2.6 ± 0.2	ns
No. of anovulatory heifers	2/20	0/8	ns
1st new follicular wave			
Day of emergence	5.6 ± 0.4	4.4 ± 0.6	ns
No. follicles emerging	4.2 ± 0.3	3.3 ± 0.5	ns
Interval: ovulation to PG (days)	7.7 ± 0.2	7.4 ± 0.2	ns
Diameter @ PG injection (mm)	8.6 ± 0.4	10.9 ± 0.7	< 0.05
Maximum diameter of DF (mm)	10.9 ± 0.4	12.7 ± 0.6	< 0.05
Day maximum diameter achieved	13.1 ± 0.4	12.8 ± 0.6	ns
Growth rate of DF (mm/d)	0.85 ± 0.05	1.06 ± 0.09	< 0.05
Day of ovulation	14.3 ± 0.2	14.0 ± 0.2	ns
Interval: PG to ovulation (days)	3.3 ± 0.2	3.0 ± 0.2	ns
No. of anovulatory heifers	6/18	0/8	< 0.10
2nd new follicular wave			
Day of emergence	13.8 ± 0.4	14.9 ± 0.7	ns
No. follicles emerging	4.8 ± 0.4	6.1 ± 0.6	< 0.10
Incidence of anovulation			
Total no. anoestrous heifers	8/20	0/8	< 0.05

Within the restricted NEB heifers there was a significant association (P<0.05) between maximum diameter of the 1st dominant follicle and its probability of ovulation (Odds Ratio=3.2 CI 1.208-8.266; R^2=0.34).

Throughout the experiment blood samples were taken for haematology studies on days -2, 0, 5, 9, 13, and on the day of slaughter (see Figure 13) and results are presented in Figures 14 and 15. These samples were analysed for neutrophil and lymphocyte numbers, both strongly related to stress within animals. A significant dietary treatment by day interaction was observed for both variables ($P < 0.01$).

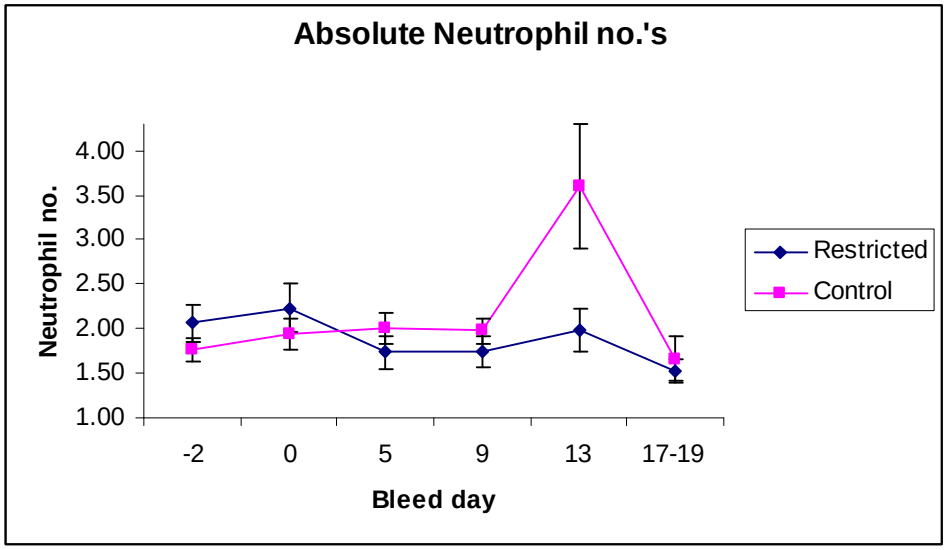

Figure 14. The effect of diet (Restricted -0.4 M or Control -1.2 M) on blood neutrophil numbers.

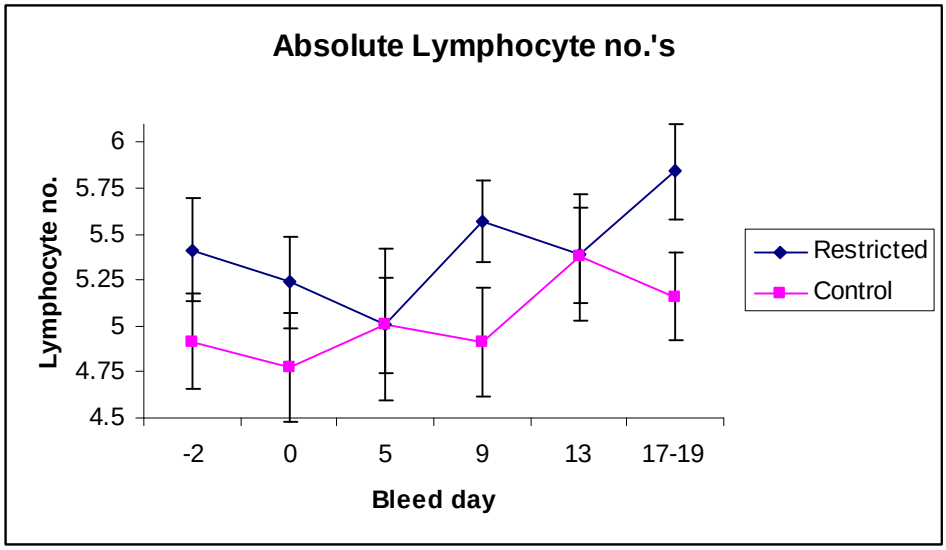

Figure 15. The effect of diet (Restricted -0.4 M or Control -1.2 M) on lymphocyte numbers.

RMIS No. 5756

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Optimisation of RNA extraction from hypothalamus and anterior pituitary

Optimisation of RNA extraction from hypothalamus and anterior pituitary tissue samples has been completed. The average RNA yields and RIN values for these tissues and liver samples as a comparison are shown in Table 20. Bioanalyser electropherograms are also displayed in Figure 16 for hypothalamus and anterior pituitary.

Table 20: RNA yields and RIN values for hypothalamic, anterior pituitary and liver tissues

Tissue	Yield (µg/mg)	RIN
Hypothalamus	0.29	7.2
Anterior pituitary	2.48	8.4
Liver	2.00	8.3

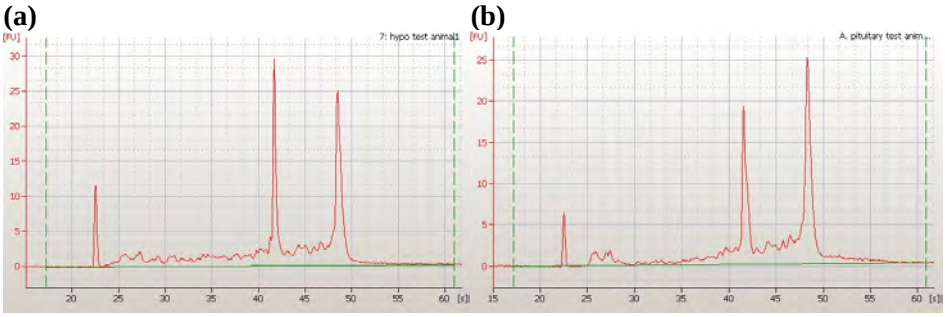

Figure 16. Bioanalyser Electropherogram displaying RNA quality of (a) hypothalamic and (b) anterior pituitary RNA sample.

The results to date agree with the hypothesis that some animals are more tolerant to NEB than others.

RMIS No. 5756

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Establishment of DNA Bank

The objective of the project was to develop a DNA bank of Irish AI beef sires, beef cattle and dairy animals with accompanying detailed phenotypic data from Teagasc research herds and commercial farms. The DNA bank is being used as a resource for genomic projects being undertaken in Teagasc. Currently it is being used to underpin the genomic selection programme for Dairy AI sires which was launched in January 2009 and will be used for the genomic selection programme anticipated to be launch for Beef AI sires in early 2010. It is also being used in studies to quantifying the frequency and the effects of discovered polymorphisms as well as quantifying the effect of candidate genes on important traits.

The expansion of this DNA bank to larger numbers of samples and the inclusion of sheep DNA will provide an invaluable national resource for many future projects that aims to identify genomic markers for traits of commercial importance in cattle or sheep. This will contribute significantly to improving the competitiveness of the Irish agricultural sector.

Currently there are a total of 2874 DNA samples stored which have been extracted from whole blood or semen. Of these over 2000 are have been extracted from semen from AI dairy bulls and 10 from AI beef bulls. There remainder are from blood from dairy and beef animals from Grange and one commercial farm.

The methods for the extraction of DNA from blood and semen were chosen after literature review and were compared and optimised and determined to give the best quality and quantity of DNA. The DNA extraction procedure is a proteinase K / salting out /ethanol precipitation protocol for whole blood. For DNA extraction from semen straws the Maxwell from Promega is used following and overnight proteinase K digestion. The quality and quantity of DNA sample is determined using the NanoDrop spectrophotometer (A260/280) and gel electrophoresis. Stringent quality control procedures are in place to continually ensure high quality DNA is obtained guaranteeing compatibility for future projects. Protocols for purification of DNA from ear punches are currently being established.

DNA samples are stable in TE buffer at -30^{o}C and this is the solution being used for long term storage. Screw capped 1.5ml tubes with rubber O ring detachable caps have been chosen as the most suitable storage vials. These vials are compatible with a robotic system to allow for alternative handling systems. The Brady IP Labelling System has been purchased to print labels that will withstand temperatures of -80^{o}C. This system also produces a 2D bar code that can be read by a hand held scanner. The tube and its cap are labelled with this system.

In the case of whole blood samples an aliquot of blood is also stored on FTA cards which are used for the long-term storage and purification of nucleic acids at room temperature.

The quantity of DNA available for every sample is carefully monitored and regulated to ensure long term future resource availability. Procedures are currently being developed to control DNA bank access. DNA authorised for removal from stocks are logged, so that the current stock of each DNA sample is known.

Standard Operating Procedures (SOP) have been written for all protocols and operations and a Quality Management System put in place. This will provide standardisation of the procedures and also tractability of raw materials and samples. Four -30^{o}C chest freezers for storage of the DNA samples have been purchased and are currently in use and contain the extracted DNA. A suitable inventory storage system was also purchased and is in place. Two of these freezers are in the Animal Bioscience Centre in Athenry with the backup freezers based in Grange. All samples will

be divided with one half remaining in Athenry and the other stored in Grange. All freezers in both centres have been alarmed with a call back alarm system. A database has been developed and is being employed to catalogue all sample information. The Phenotypic database is currently being developed and will be linked to those samples already catalogued.

RMIS No. 5678

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Identification and validation of SNPs in the IGF-1 gene in beef and dairy cattle in the Irish herd

IGF-1 influences cellular proliferation and differentiation and is involved in various aspects of reproductive biology and metabolism. Recent studies have shown positive associations between fertility and feed efficiency traits and circulating concentrations of IGF-1 in dairy and beef cattle. Given the importance of physiological IGF-1 there is the potential of using SNPs in the IGF-1 gene as indicators of production traits. The initial step in this process is the identification and validation of SNPs in the IGF-1 gene. Therefore, the objective of this study was to validate their presence in animals in the Irish herd and in the process identify novel SNPs in the IGF-1 gene.

A panel of 22 animals representative of 6 different breeds was assembled. These comprised of 6 purebred Holstein Friesians, 4 purebred Charolais, 4 purebred Simmental, 4 Angus x Holstein Friesians and 4 Belgian Blue x Holstein Friesians. Blood samples were collected form each of these animals and DNA was extracted using a modified standard proteinase K protocol. SNPs in the IGF-1 gene including its promoter regions were identified using the ENSEMBL website. From this 6 published SNPs were identified. Following identification, PCR primers were designed using the Primer3 website to amplify approximately 1000bp fragments of the gene flanking the SNPs of interest. PCR products from the DNA panel were sequenced and sequence data were checked for quality and analysed using BLAST on the NCBI webpage to confirm their identity. Sequence alignments, identification of SNPs and determination of their position on the chromosome were performed using Clustal W and MEGA software programmes. Results of the sequencing are summarised in Table 21. Analysis of the sequencing information validated the presence of 1 of the 6 published SNPs. This study has identified 9 novel SNPs present in the IGF-1 gene and validated their presence in animals in thee Irish herd. As a result of the findings of this study these SNPs can now be included detailed genotype association studies.

RMIS No. 5546

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Table 21: Details of SNPs present in the IGF-1 gene shown using their IUPAC nucleotide code

Breed	SNP 1	SNP 2	SNP 3	SNP 4*	SNP 5	SNP 6	SNP 7	SNP 8	SNP 9	SNP 10
Simmental 1	W	Y	A	A	T	A	T	G	G	C
Simmental 2	T	T	A	R	T	A	T	G	G	C
Simmental 3	T	Y	R	G	T	A	T	G	G	C
Simmental 4	T	T	A	R	T	R	R	G	G	C
Angus x Holstien Friesan 1	W	T	A	A	T	A	T	G	G	C
Angus x Holstien Friesan 2	W	T	A	A	T	A	T	G	G	C
Angus x Holstien Friesan 3	T	T	A	A	T	A	T	G	G	C
Angus x Holstien Friesan 4	T	T	A	A	T	A	T	G	G	C
Belgian Blue x Holstien Friesan 1	T	T	A	A	T	A	R	G	G	C
Belgian Blue x Holstien Friesan 2	T	T	A	A	T	A	R	G	G	C
Belgian Blue x Holstien Friesan 3	T	C	A	A	T	A	T	G	G	C
Belgian Blue x Holstien Friesan 4	T	Y	A	A	T	A	T	G	G	C
Holstien Friesan1	W	T	A	A	T	A	T	G	G	C
Holstien Friesan2	T	T	A	A	T	A	T	G	G	C
Holstien Friesan3	W	Y	A	A	T	A	T	G	G	C
Holstien Friesan4	W	T	A	R	Y	A	T	K	R	Y
Holstien Friesan5	T	T	A	A	T	A	T	G	G	C
Holstien Friesan6	T	T	A	A	T	A	T	G	G	C
Charolais 1	W	Y	R	A	T	A	T	G	G	C
Charolais 2	T	T	R	A	T	A	T	G	G	C
Charolais 3	W	T	A	A	T	A	T	G	G	C
Charolais 4	W	Y	R	A	T	A	T	G	G	C

* Denotes published SNP

SNPs in the IGF gene and their associations with fertility and production traits in dairy cows

Poor reproductive performance in the modern high yielding dairy cow is a major economic cost. The inclusion of fertility traits in breeding programmes is well established in Ireland. Fertility traits, however, have a low hereditability. Therefore, it can take a number of generations before significant differences can be seen at farm level. This has resulted in a number of studies examining the potential of physiological and molecular indicators of fertility. Previous studies from this laboratory have shown strong associations between circulating concentrations of IGF-1 during the early *post-partum* period and conception rate in dairy cows (see research report 2007 p147 – 149). The objective of this study was to determine what association SNPs in the IGF-1 gene have with fertility and milk production traits in dairy cows.

For this study 610 Holstein Friesian cows from 10 different co-operating dairy herds across the country were involved. These cows were selected as being representative of the breed with milk yields ranging from 3500 kg to 13500kg. All cows involved in the study received their first insemination between days 30 – 100 *post-partum*. Pregnancy diagnosis was carried out initially between days 30 – 50 post AI and confirmed with an end of year scan once the last cow had passed 100 days post AI. Blood samples were collected via the coccygeal vein into 10ml EDTA vacutainers. DNA was then extracted from blood samples using a modified standard proteinase K protocol. An aliquot was subsequently submitted for genotyping for 10 SNPs in the IGF-1 gene by Sequenom Ltd. This process is currently on-going.

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Identification of uterine proteomic and genomic differences in dairy heifers with different genetic levels of embryo survival rate

The aim of this project is to use heifers of proven high or low fertility with a view to beginning to identify 'fertility genes' or other biomarkers in cattle for future commercial use. Dairy heifers (n=120) of known genetic background and normal reproductive characteristics will be inseminated, scanned for pregnancy at Day 28 and re-programmed to return to oestrus and be re-inseminated on a further 3 occasions. With the aim of creating a 'high' (n~15) and 'low' fertility (n~15) group. All heifers had their oestrous cycles synchronised using two inter-muscular injections of 500µg of the prostaglandin $F_{2\acute{\alpha}}$ analogue, cloprostenol (Estrumate®, Schering-Plough Limited. Heat detection was carried out five times daily (8am, 11am, 2pm, 6 pm and 10pm). Estrotect™ patches were used as an aid to detection. Inseminated heifers are pregnancy tested on day 28 after insemination.

Each heifer will then be ascribed an embryo survival score based on their cumulative embryo survival rate over the 4 rounds. Uterine fluid and blood plasma will be collected from 15 "low" and 15 " high" fertility dairy heifers based on the outcome of 4 rounds of inseminations. Blood plasma are collected by jugular veni puncture. Uterine lavage will be harvested on Days 7 and 13 of successive oestrus cycles, by catheterisation of each uterine horn and flushing with saline. Flushings will be subjected to proteomic analysis.

Tissue samples of importance such as the endometrial tissue, ovaries and liver will be collected from heifers from each of the "high" and "low" fertility groups immediately following slaughter. Total RNA will be isolated from the tissue and processed for array hybridisation. To-date four rounds of inseminations have almost been competed on all one hundred and twenty heifers. The conception rates are shown in Table 22 below.

Table 22: Conception rates to date

Round number	Conception rate %
Round 1	74%
Round 2	70%
Round 3	70%
Round 4 (to date)	76%

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