

## WELCOME

The 2011 Walsh Fellowships Seminar at the RDS is primarily an event where Teagasc acknowledges and highlights the achievements of our postgraduate students as they near the completion of their studies. We also challenge them to identify the contribution their research makes to achieving the Food Harvest 2020 vision for the agri-food sector. The vision is summarised by three actions "Acting Smart, Thinking Green and Achieving Growth". Delivering this vision requires both new and existing knowledge to drive profitability and sustainability in the sector. It emphasises the importance for young researchers to be increasingly aware of their important role in supporting and delivering the innovation that is central to achieving the ambitious growth targets set for the sector.

The Walsh Fellowship Scheme provides opportunities for Teagasc to collaborate with national and international universities and colleges on projects of relevance to our stakeholders. Equally important is the shared responsibility of preparing professionally trained people who can contribute to addressing the global challenges facing the sector or take advantage of the great opportunities it offers. This year I have taken the initiative of re-establishing the internal Walsh Fellowship Development Committee. Their task is to identify and implement actions that will deliver a nationally recognized post graduate programme of excellence in providing new knowledge and highly trained professional individuals to support and deliver sustainable development in the agri-food sector and wider bio-economy. Critical to their success will be building on the existing collaborations with universities and other stakeholders but more importantly in terms of new initiatives to support and develop our new strategic alliances at home and abroad.

The continued support of the RDS for this event is appreciated and I would also like to thank the Institute of Food Science and Technology of Ireland (IFSTI) for their award to the best food science and technology presenter. The seminar as usual is held as part of Science Week Ireland. Finally, thank you to the Walsh Fellows for their contribution to our research programme and I wish them much success and fulfilment in their future careers.



**Professor Gerry Boyle,**  
Teagasc Director

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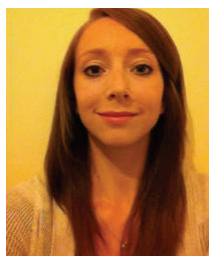
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## Irish seaweeds: Potential use as functional foods with antioxidant and heart-health benefits

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Seaweeds tolerate a harsh and constantly changing marine environment and thus have developed chemical defence strategies to cope with these conditions. These strategies include the production of compounds known as secondary metabolites, some of which have antioxidant properties. To date the potential of Irish seaweeds as a source of antioxidants has remained largely untapped. Therefore the aim of this research project was to investigate Irish seaweeds, namely *Fucus spiralis* (Spiral wrack), *Pelvetia canaliculata* (Channelled wrack), *Ulva intestinalis* (Gut weed) and *Ascophyllum nodosum* (Knotted wrack) as sources of antioxidant and heart-health ACE-I inhibitory compounds. Antioxidants have a multitude of food uses and are traditionally used in the meat, milk and marine industries to control oxidative processes such as lipid oxidation. They are also scavengers of damaging free radicals which may contribute to chronic diseases, such as cancer and high blood pressure. A bioassay-guided approach was employed in this work and food-friendly, antioxidant extracts were generated from brown seaweeds using accelerated solvent extraction<sup>®</sup> (ASE<sup>®</sup>) and enzymatic hydrolysis. High pressure liquid chromatography, MS and NMR spectrometry were employed to further purify and characterise the crude antioxidant extract, resulting in fractions with enhanced polyphenol and peptide content. Mining the untapped potential of sustainable Irish marine resources as sources of bioactive compounds is a key recommendation of both the Teagasc Vision programme and Food Harvest 2020. If achieved this could contribute to the ambitious target of increasing by 40% added value output in the food and beverage sector by 2020 as laid out in Food Harvest 2020 by providing bio-active ingredients for the lucrative functional food market.



## Biofilm formation in strains of *Salmonella enterica* Typhimurium DT104 isolated from the pork chain

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The aim of the project is to evaluate *Salmonella* DT104 and DT104b isolates for their ability to form biofilms, to determine the factors that contribute to biofilm formation at a transcriptomic and proteomic level and to investigate the mechanisms that allow highly virulent pathogenic bacteria to persist and survive in the food chain. Phenotypic characterisation involved examining colony morphology on Congo red agar, monitoring capability of surviving chlorine treatment, and observing their ability to attach to stainless steel and PCV plastic. PCR was utilised to detect the presence of *Salmonella* genomic island I (SGI1) which increases capacity for biofilm formation. In expression studies, a number of isolates were exposed to acidic conditions, and the regulation of biofilm formation genes was investigated. The results show that cellulose may not be critical in the formation of the mature biofilm. In addition, total biofilm formation appeared to be inhibited when nutrients are limiting. It was found that the majority of strains possess biofilm forming capabilities. Molecular and phenotypic comparisons of strong and weak biofilm forming strains indicate that biofilm development is not solely dependent on the acquirement of SGI1, but the regulation of individual genes may also come into play. Results from this study will allow for the implementation of novel control measures to enhance food safety from farm to fork whilst improving the health of animals and humans.

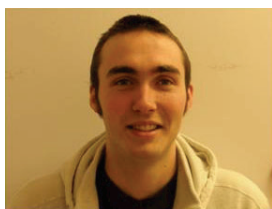


## Re-engineering infant formula manufacture

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This abstract describes the development of a more sustainable process for the manufacture of powdered infant formula. The rationale is to replace processing steps used during manufacture of infant formula, including evaporation, by changing formulation dynamics and the introduction of a steam shockwave device for heating/emulsification. The new process differs from conventional manufacturing in that raw materials are rehydrated to higher total solids content, i.e., 60 % w/w as opposed to typically 20-40 % w/w. Heat treatment is achieved by injecting steam directly into the 60% w/w formulation via a shockwave injector. A conventional manufacturing process (rehydration to 30 % w/w) incorporating evaporation to 55 % w/w prior to spray drying was used as a control. The physical stability of the control and experimental formulations were determined using particle size (fat globule size distribution) and viscosity measurements. After heat-treatment (using the shockwave injector), the 60% w/w formulations were diluted to approximately 55% w/w as a result of steam condensation. The fat globule size distribution was ~ 3.1 microns, as opposed to ~ 3.5 microns for control formulations post evaporation, and viscosity was 50% lower. No stability issues were observed while spray drying. In conclusion, the removal of the evaporation step using a high solids approach and alternative thermal treatment was successful in producing stable emulsions capable of being dried on conventional spray drying operations used in the infant formula industry. The process outlined above, if implemented on a commercial scale, will satisfy the "Smart, Green, Growth" criteria outlined in Harvest 2020. The energy saving results of the process could help maintain the economical and environmental sustainability of the infant formula industry in Ireland into the future.



## Effect of altering curd washing on the biochemistry, sensory and quality of Cheddar cheese

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Relatively little information is available on the effects of seasonal variations in milk lactose (4.0 to 4.8% (w/w) over lactation) on the quality of Cheddar cheese. The aim of this study was to investigate the effects of curd washing, as a means of varying the concentration of lactose plus lactic acid in cheese moisture (LLAM<sub>C</sub>), on the quality of Cheddar cheese. The level of curd washing in the cheese vat was varied from to give target levels (% w/w) of LLAM<sub>C</sub> in the cheese of 5.3, 4.5, 4.3 and 3.9; these values correspond to expected LLAM<sub>C</sub> levels in non-washed cheeses made from milks with lactose levels of 4.8, 4.6, 4.3 and 3.8 % (w/w), respectively. The cheeses were manufactured in triplicate from mid-lactation milk and analysed over a 270-day ripening period. Increasing curd washing significantly reduced mean levels of lactose, total lactate and LLAM<sub>C</sub> over the ripening period and the reduction in LLAM<sub>C</sub> coincided with a significant increase in pH by ~0.3-0.4 at times  $\geq 90$  days. Otherwise, alteration of curd washing generally did not affect gross composition and the populations of starter or non-starter lactic acid bacteria. Grading of the cheese indicated that curd washing resulted in cheeses that were less acid, sweeter and creamier than non-washed cheese. The results will help to overcome seasonal variations in the milk supply, hence improve the overall Cheddar cheese quality and achieve a higher value-added product in response to market, which meets the target of Food Harvest 2020.



## Emulsification and microencapsulation increase oxidative stability of omega-3 rich foods

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Lipid oxidation can cause off-flavours and odours, decrease shelf life and have nutritional implications, when omega-3 rich oils are incorporated into food systems, such as emulsions. Omega-3 rich oils (camelina and fish) were emulsified (oil-in-water (O/W) / oil-in-water-in-oil O/W/O emulsions) or microencapsulated (spray dried), with the aim to improve oxidative stability. High levels of lipid oxidation products (lipid hydroperoxide and p-Anisidine values, measuring primary and secondary lipid oxidation products, respectively) were detected immediately after emulsification, of caseinate stabilized (3%) omega-3 rich O/W emulsions, in comparison to their corresponding bulk oils. While hydroperoxides increased three-fold in the bulk oil after two days storage at 60°C, levels in the emulsions decreased by a factor of 6-46, depending on fat globule size. This behavior was highly temperature dependant ( $p < 0.05$ ), higher storage temperatures resulted in lower levels of detectable oxidation products. Table spreads were produced (75% fat) using double emulsion technology, where various omega-3 rich O/W emulsions were dispersed in a hardstock/rapeseed oil blend. Such spreads had better oxidative stability than their corresponding omega-3 bulk oils. Antioxidant addition (green tea extract) further improved the oxidative stability of spread systems. High fat powders (71.7-85.6%) were produced by spray drying sodium caseinate stabilised omega-3 rich O/W emulsions, containing dried glucose syrup as the carrier material. Initially, lipid hydroperoxide and p-Anisidine values of spray dried oils were similar to bulk oil values, but they became far more stable as storage time at 15°C increased. This study contributes to DAFM's Harvest 2020 goals for the agri-food sector, regarding production of smart ingredients using dehydration technology: functional value added ingredients have been produced, using emulsification and microencapsulation (spray drying) technology, and oxidative stability of omega-3 rich oils has been achieved as a result, allowing built-in functionality for food applications worldwide (e.g., spreads, mayonnaise, yoghurt, salad dressing, milk, orange juice).





## Composition of the intestinal microbiota of elderly Irish subjects

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The ELDERMET project aims to assess the intestinal microbiota of the elderly Irish community as an indicator of health and to exploit the data generated to develop functional foods or food ingredients for elderly consumers. Knowledge of the baseline composition of this intestinal microbiota is being acquired through a combination of traditional microbiology and more modern next generation sequencing technologies. Almost 500 elderly Irish people (male and female, >65 yrs) have taken part in this study, including individuals in long-term residential care and rehabilitation centres, people being treated with antibiotics, with *Clostridium difficile* infections and healthy individuals. Total levels of *Lactobacillus* spp., *Bifidobacterium* spp. and *Enterobacteriaceae* were determined through bacteriological culturing techniques, which compared favourably to data from culture independent sequencing methods. The study also demonstrated the impact of antibiotic administration and living environment on the composition of the intestinal microbiota. This work has also identified a diverse range of bacteriocinogenic strains (including unexpected species like *Lactococcus lactis*, *Streptococcus mutans*) of elderly human origin. Along with the ELDERMET Culture Collection (currently housing >6000 bacterial isolates comprising of both bifidobacteria and lactobacilli), these constitute a bank of potential probiotic bacteria for the future. The information on the composition of the intestinal microbiota and links to health and diet offer the food industry and medical community knowledge, that can be used for the development of functional foods aimed at health improvement in the older consumer.



## Potential biocide tolerance in verocytotoxigenic *e. coli* and its impact on gene and protein expression

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Biocides are deployed at all stages of the farm to fork chain to eliminate microorganisms. An increase in biocide tolerance would be of concern as it could contribute to increased persistence of foodborne pathogens in the food chain. The aim of this study was (i) to establish the ability of verocytotoxigenic *E. coli* to acquire tolerance to commercially available biocides and biocidal active agents (ii) to identify differences between an *E. coli* O157:H19 wildtype isolate and its triclosan tolerant mutant at a genomic and proteomic level. A stepwise broth method was used to isolate potential biocide tolerant mutants. Stable mutants were isolated displaying an increased tolerance to the biocidal active agents triclosan (n=3) and benzalkonium chloride (n=1). Gene and protein expression profiling were performed on the *E. coli* O157:H19 wildtype (triclosan MIC 6.25 µg/mL) and its triclosan tolerant mutant (MIC >8000 µg/mL) in the presence and absence of triclosan. The Affymetrix GeneChip® *E. coli* Genome 2.0 Array and two dimensional fluorescence difference gel electrophoresis (2-D DIGE) were used to investigate differences in gene and protein expression respectively. Wildtype gene expression was found to be significantly different to the triclosan tolerant mutant for a large number of genes, regardless of triclosan exposure. In accordance with these results, differentially expressed proteins were identified between the wildtype and mutant both in the presence and absence of triclosan. Data from this study indicates that the transcriptome and proteome of the wildtype *E. coli* O157:H19 was different to its triclosan tolerant mutant and differential expression of proteins and genes was not dependent on triclosan exposure. Assurance of food safety underpins the goals set out in Food Harvest 2020. The research undertaken helps support this by increasing our understanding of a critical food safety issue and enabling better guidance for the food industry on biocide usage.

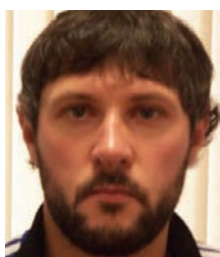


## Rapid pyramiding and multiplexing of disease resistance traits in potato breeding using marker-assisted selection

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Potatoes are the world's third most important food crop; consumption is particularly high per capita in Ireland and with a production of 455,000 tons of potato. Commercial variety development currently involves a conventional potato breeding scheme relying on a single round of crossing and over a decade of phenotypic selection and advanced trialling to identify improved varieties. The goal of any potato breeding programme is to obtain new varieties with improved characteristics such as high yield, quality traits and resistance to numerous pests and diseases. Significantly however, the implementation of such a breeding scheme makes production of varieties that combine all these traits extremely difficult to achieve. Food Harvest 2020 highlights the need "to urgently prepare for the impact of the new EU pesticide regulations to ensure the sustainability of crop production in Ireland". One of the most effective strategies to reduce pesticide use in agriculture is to develop disease resistant varieties. The arrival of molecular markers for a number of commercially important traits in potato raises the possibility of developing accelerated breeding programmes that can target the more rapid development of varieties combining traits relating to both quality and disease resistance. Therefore, a small scale experimental breeding programme was developed to investigate the potential for the routine deployment of marker-assisted selection (MAS) as part of the potato breeding programme at Oak Park. This accelerated breeding strategy has resulted in the "stacking" of multiple disease resistance traits (potato cyst nematode, late blight and potato virus Y) into potato genotypes over three successive annual rounds of crossing. Implementation of marker-assisted selection within the Oak Park potato breeding programme will enable the earlier identification of superior varieties to secure the future of sustainable potato production in Ireland.



## Towards the development of ethanol tolerant *Fusarium oxysporum* strains to facilitate the microbial-based production of bioethanol from wheat biomass.

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Microorganisms such as *Fusarium oxysporum* can convert lignocellulosic biomass into fuel ethanol providing an alternative source of renewable energy from waste agricultural materials such as wheat straw. During the fermentation process, these microbes must overcome several environmental challenges in order to produce ethanol efficiently and cost-effectively. One key obstacle is the ability of the producing organism to tolerate the toxicity of the ethanol produced. Consequently, understanding the genes and/or mechanisms involved in ethanol stress tolerance is critical to enhancing ethanol production of these microbes. In this study, a high throughput *Agrobacterium tumefaciens* transformation system was developed in parallel with a rapid bioassay for ethanol and butanol tolerance in order to simultaneously construct and screen a library of *F. oxysporum* gene disruption mutants ( $n=1,562$ ). Mutants demonstrating altered tolerance to ethanol ( $n=36$ ), butanol tolerance ( $n=96$ ) and tolerance to both alcohols ( $n=50$ ) were selected. After verifying the genetic modification of each transformant using PCR, each transformant underwent a more intensive screen for alcohol tolerance, relative to the non-transformed wild type *F. oxysporum*. One transformant demonstrating significantly lower ethanol tolerance ( $P<0.05$ ) compared to the wild type parent strain has been selected for further characterisation. A comparative global transcriptome study of this selected mutant under ethanol stress will help to understand and reveal components of the ethanol stress response in *F. oxysporum* in order to facilitate efficient bio-alcohol production. Output from this project will contribute to the Food Harvest 2020 vision of assisting the agricultural sector to achieve its sustainable energy requirements through novel approaches that maximise existing resources.



## Micropropagation, polyploid induction and $\gamma$ -irradiation of *Hebe* cultivars

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Many varieties of *Hebe* are important ornamental shrubs. Research aims were to develop micropropagation protocols, obtain disease-free mother plants and regenerate new variants. *In vitro* methods were used for their genetic improvement. Optimal propagation media were determined for the seven cultivars: different hormone combinations and/or concentrations were required according to variety. The micropropagation rate was determined for different explants (single nodes, double nodes, shoot apices and clusters of buds) and for at least one year. The most productive explants were single nodes and the micropropagation rate varied according to variety from 2.1 to 5.3. No systemic bacteria were found in the plant material *in vitro*. Polyploids were induced using antimitotic agents for varieties 'Pink Fantasy', 'Oratia Beauty' and *H. albicans* 'Cobb'. The confirmed tetraploid plants were characterized. Flow Cytometry showed the presence of: diploids (2x), mixoploids and tetraploids (4x) plants after treatments. Confirmed tetraploid plants had, thicker stems, larger guard cells, a lower density of stomatal guard cells and more chloroplasts per guard cell compared to diploid controls. An altered phyllotaxis in shoots immediately after treatment was also observed. Cuttings from tetraploid plants rooted at the same rate as diploids and their rooting rate was the same. Variants were produced using ionizing radiation ( $\gamma$ -rays) on five cultivars. The radiosensitivity of 'Oratia Beauty' and 'Wiri Mist' *in vitro* through two culture cycles was studied and the LD<sub>50</sub> values obtained were 37 Gy and 48 Gy respectively. Putative mutants were found among treated plants. They had leaf variegations, rounded leaves, glossy leaves, deformed leaves, red leaf midrib and stunted / prostrate growth. Further propagation and characterisation of this material will determine their commercial potential and new cultivar could be registered.



## Pathogen removal from agricultural wastewater treated in integrated constructed wetlands

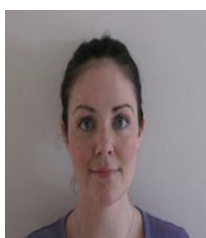
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Integrated constructed wetlands are biological wastewater treatment systems consisting of a series of linked ponds or cells, through which the wastewater flows sequentially. They have been shown to successfully reduce nutrients, such as nitrogen and phosphorus, in agricultural wastewater. The objective of this study was to investigate the removal of pathogenic (disease-causing) bacteria. Faecal indicator bacteria were enumerated in the wastewater influent as well as the effluent from the first, mid- and final cells of nine on-farm ICWs treating piggery (3) or dairy (6) wastewater. The presence/absence of *Salmonella* was also determined. Reductions in faecal indicator bacteria were observed across all nine ICW systems, with *Escherichia coli* and *Enterococcus* non-detectable in the final effluent. Mean removal rates were greatest for *E. coli* (81.5 %), with coliform and *Enterococcus* reduced by 55.9 % and 66.9 %, respectively. Antibiotic sensitive isolates of *Salmonella* Dublin, determined to be the same by molecular fingerprinting, were recovered from two dairy farm ICWs; from the influent to one and the cell 1 effluent of the other. However, *Salmonella* was non-detectable in the effluent from subsequent cells. On another farm, *Salmonella* Typhimurium was detected in the separated liquid fraction of anaerobically digested pig manure as well as in the effluent from the first and mid-cells of an ICW treating this material, but was absent in the final effluent. In conclusion, counts of faecal indicator bacteria were reduced in agricultural wastewater treated in on-farm ICWs, and *Salmonella*, when present, appears to have been removed. ICW's can therefore be considered a viable option for agricultural wastewater management that could overcome land-spreading restrictions. However, repeated sampling is required to investigate the effect of hydraulic retention time. These findings will help validate on-farm ICWs as "alternative waste usage and disposal options for the pig sector", as recommended in the 2020 Food Harvest Vision.



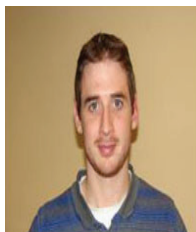
## Predictive segmentation using post-intentional variables

### A strategy to target the health segment in the Irish food market

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According to the Food Harvest 2020 report, the Irish food and drink industry must develop strategies to target mature markets, such as Ireland the US and the EU. These markets are characterised by consumers who increasingly seek out and pay a premium for foods with health attributes. Healthy eating is often regarded as self-directed health behaviour, given that individuals can take responsibility for their own wellbeing. To take responsibility, health intentions must be translated into behaviour. Hence, it is important to have a measure of post-intentional behaviours that facilitate healthy eating. Segmenting a population based on these post-intentional behaviours may prove beneficial for targeting consumers motivated to eat a healthier diet. This paper reports the study of a representative sample of Irish adults (n = 509) regarding consumption of confectionery foods and healthy dietary change. The target behaviour was selected in the context of industry trends which point to a continued focus on obesity and health along with initiatives to reduce sugar in products without affecting taste. The objective of the research was to compare predictive segmentation models to determine the most effective segmentation base for targeting consumers with healthier foods. Three predictive segmentation models were estimated using logistic regression analysis. The differences between the segments in each model were analysed using independent sample t-tests. The variables that influenced healthy dietary change differed significantly across the models. In the population model healthy dietary change was significantly associated with dietary control, planning behaviour, monitoring behaviour, and perceived need. The strength and significance of these variables varied across the demographic segments in model two and the behavioural segments in model three. Behavioural segmentation provided the most identifiable segments with discernible differences in age, Body Mass index (BMI) and sugar consumption. These findings suggest that a marketing strategy underpinned by initiatives to facilitate dietary planning, dietary monitoring and dietary control, may attain a price premium in different market segments.





## The role of the slurry microbial community in soil biomass dynamics

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Application of animal slurries have been associated with low efficiency of nitrogen (N) use, which increases risks of N-loss to the environment through nitrate leaching and gaseous emissions. Since most nitrogen transformations and pathways of nitrogen are mediated by microbiological processes, efforts to increase the N efficiency from slurry rely on the development of a deeper understanding of the quantity and dynamics of the soil microbial biomass. It has been well known that introduction of slurry to soil increases the soil microbial biomass, due partly to the additional nutrients in the slurry. However, heretofore there has been no information on the extent to which the microbial community within the slurry itself contributes to this growth, nor on the potential involvement of slurry microorganisms in N cycling. To elucidate and discriminate the roles of the indigenous soil microbial community and the exogenous slurry community, microcosms were established with mixtures of live and sterile soil and slurry and incubated at 15° for 42 days. Sterilisation of soil and dairy slurry were conducted by gamma irradiation. Soil nitrate and ammonium levels were measured before and after incubation. The increase in nitrate levels during incubation was significantly ( $P < 0.001$ ) higher in live soil treatments compared to sterile soil treatments, whereas no significant differences were found between live and sterile slurry additions. This experiment has demonstrated that the soil microbial community is the main driver in Nitrogen cycling in soil, and that the microbial community in slurry merely contributes to the total organic N content of the material. The results of this study contribute to our understanding of soil N dynamics; such understanding will aid the development of soil-specific N advice, which will result in higher production efficiencies, thus meeting the “Smart, Green Growth” objectives set out in Food Harvest 2020.





## Regulation of phosphorus cycling by the soil biota in grasslands systems

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There is increasing pressure to promote phosphorus (P) cycling and incorporation in grassland systems: particularly with respect to the goals set by Food Harvest 2020, whereby increases in productivity need to be aligned to sustainable management and improvements in water quality. Increasing the efficiency of P cycling in grassland systems will aid in sustaining plant yields whilst reducing fertiliser use. Despite increased awareness of the role that biota plays in nutrient cycling in the soil, there is little information on how soil biological communities may affect P cycling in grassland systems. Two experiments were carried out to investigate the role of soil biota in incorporating and cycling applied P into the soil. Experiment one characterised the soil biological communities of long-term field sites with different P fertilisation regimes (0, 15, 30 and 45kg P ha<sup>-1</sup> y<sup>-1</sup>). Experiment 2 studied the effects of different plant communities (perennial ryegrass or polyculture, plus no-plant control), fertiliser regimes and earthworms on P incorporation and cycling. Results from Experiment 1 showed that, surprisingly, different P fertilisation levels do not affect the P content of the soil microbial community. However, increasing P fertilisation significantly reduced the carbon content of the microbial community ( $p < 0.01$ ). Results from Experiment 2 showed that the presence of earthworms promoted a more even distribution of labile inorganic P within the top 10cm of the soil. Soil nematode community structure was affected by inorganic fertiliser additions to perennial ryegrass monocultures, indicating potential shifts in soil fertility. This study has demonstrated the potential of developing soil management strategies that promote soil organisms, specifically with a view to incorporating P into the soil matrix. Such optimisation of P cycling in agricultural systems will contribute to enhancing water quality and promoting sustainable grassland productivity and thus help achieve the goals of Food Harvest 2020.



## Measurements of ammonia and greenhouse gases following land spreading of cattle slurry

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Agriculture comprises 28% of national greenhouse gas and 98% of ammonia (NH<sub>3</sub>) emissions. Switching from splash-plate to trailing shoe application of cattle slurry, as well as applying slurry in spring rather than in summer, is effective at reducing NH<sub>3</sub> volatilisation. However the potential effects of such measures on greenhouse gases from soils is often ignored when formulating recommendations for farmers. This study addresses strategies for reducing both ammonia and greenhouse gases emissions associated with the land spreading of cattle slurry. Field plots were spread with four different slurries (maize-based or grass-based and of varying dry matter (DM) content). These slurries were splash-plate or trailing-shoe applied in April, July, August and September 2009 to provide a range of contrasting weather conditions. Continuous measurements of gas fluxes (NH<sub>3</sub>, N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub>) were taken after slurry application. Total nitrous oxide (N<sub>2</sub>O) emissions (including indirect emissions associated with NH<sub>3</sub> re-deposition onto the soil) were reduced by up to 35% when switching from summer to spring/autumn application, whilst lower dry matter reduced all gaseous emissions across all spreading dates. Switching from splash-plate to trailing-shoe application consistently reduced NH<sub>3</sub> volatilisation but the effect on direct N<sub>2</sub>O emissions was unclear and of a smaller magnitude than the estimated reduction of indirect N<sub>2</sub>O emissions. Overall, spring application of slurry, on a cold day, appeared to be more efficient in reducing the release of both ammonia and total greenhouse gases from grassland soils than a change in the application technique. The Food Harvest 2020 report envisages increased agricultural production, whilst EU climate and transboundary pollution directives simultaneously seek to limit gaseous emissions. Alterations in timing and dry matter content will not only reduce gaseous nitrogen losses, but increase slurry N fertiliser efficiency, reducing the mineral N fertiliser input costs and contributing to more sustainable farming without impacting on production.



## Impact of microbial communities associated with land use on pathogen survival

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Animal slurries are valuable resources that can enhance many soil properties and grassland productivity. However, these materials can contain pathogenic microorganisms to which humans and animals may be exposed. Thus it is crucial to identify factors that affect pathogen survival in soil, in order to gain maximum agronomic benefits whilst minimising the threats to health. An experiment was conducted to determine the impact of microbial community structure associated with different land uses upon pathogen survival in soil. A range of twelve contrasting soils were inoculated with known concentrations of model pathogenic microorganisms, including *Escherichia coli*, *Salmonella* and *Listeria* species. The nature of the microbial communities in each soil was determined by a technique which characterises the biochemical composition of the microbes and provides a form of 'fingerprint' of the community structure (phospholipid fatty acid analysis). Soils were also characterized for a range of physico-chemical properties such as texture, pH and organic matter. Pathogen survival in the soils was measured over 110 days, and the death rate calculated for each case. A multi-variate statistical technique (stepwise multiple regression) was used to determine the predominant factors related to pathogen-specific death rates. This showed that the microbial community structure was the most significant factor related to pathogen decay for all organisms tested ( $p < 0.01$ ), with the exception of an environmentally persistent *E. coli* isolate. This study demonstrates the importance of soil biological quality, specifically the configuration of the microbial community, in pathogen suppression. This work contributes to the 2020 Food Harvest Vision since it provides a framework to assess the inherent potential of soils to suppress pathogens, which will facilitate the development of management strategies to optimise this potential.



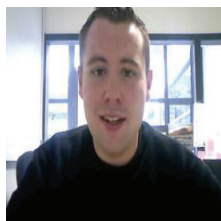
## Development of a probe based assay for the identification of nematodes infecting sheep

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A combined PCR and probe based reverse line blot assay (RLB) has been developed for the identification of gastrointestinal nematodes in sheep. Gastrointestinal nematodes are of economic importance worldwide causing significant profit losses in livestock production. Identification of nematode species causing infection allows for a better understanding of their epidemiology and biology. Traditional methods for the identification of nematodes from faecal samples are laborious and time consuming, taking up to fourteen days before the species composition is known. The internal transcribed spacer (ITS) region of the ribosomal DNA of nine common sheep nematode species from the super family's Trichostrongyloidea and Strongyloidea were sequenced and compared. A universal PCR primer set was developed to amplify all nine species. Species specific probes for use on the RLB were also developed based on the variable regions of the ITS2. The combined PCR/RLB assay has been shown to be species specific and being able to detect femtogram amounts of DNA template. The PCR/RLB assay has the potential to overcome the shortfalls of the traditional methods of identification facilitating epidemiological studies and increase our understanding of anthelmintic resistance. This will facilitate better parasite control which, in line with the objectives of food harvest 2020, will allow for increased meat production.



## Implementing quality parameters of ryegrasses into a national variety evaluation scheme

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DAFM Recommended List trials assess the seasonal and annual yield and persistence of perennial ryegrass varieties. Information on the ruminant feeding value of varieties was not provided as costs and workloads for chemical analysis were prohibitive. Near infrared reflectance spectroscopy is a rapid and cost effective scanning method of analysing nutritive quality and was studied to create calibration models for four quality traits; buffering capacity, crude protein, *in vitro* dry matter digestibility, water-soluble carbohydrate. A total of 2076 ryegrass samples from DAFM trials were scanned and accurate calibration models produced ( $R^2 = 0.86 - 0.98$ ). These models were subsequently applied to the scans of sub-samples from plots of replicated field trials in five harvest years. A REML statistical analysis was used to assess the seasonal and total annual responses of species, ploidy, maturity and age of sward. From within this database a weighted average across all seasonal periods and years was taken for each of the four quality characters. The ranking of 15 perennial ryegrass varieties was calculated for each trait to provide the definitive variety ranking. By performing a spearman rank correlation analysis on each individual seasonal period the variation in variety ranking across the season was measured. From this a preliminary testing protocol was produced to minimise sampling workloads for DAFM variety testers while still maintaining the statistical accuracy needed to confidently identify varieties with superior feeding quality. DAFM are now routinely providing digestibility and water-soluble carbohydrate information to Irish farmers through the annual recommended list publication. This will promote merchants and breeders to provide and local farmers to use higher quality varieties that can reduce dependency on high cost concentrates, help lower the carbon footprint and greenhouse gas emissions of ruminant production, while maximising the efficiency and productivity of Irish grassland enterprises.



## Effect of dietary fat supplementation on the reproductive performance of lactating dairy cattle

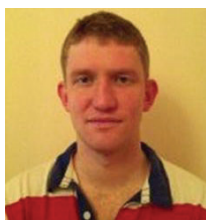
I.A. Hutchinson,<sup>1,3</sup> R.J. Dewhurst,<sup>2</sup> A.C.O. Evans,<sup>3</sup> P. Lonergan,<sup>3</sup> and S.T. Butler<sup>1</sup>

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A series of studies was carried out to determine the effects of fat supplementation on the fertility of lactating dairy cattle. Investigations were carried out to determine the effect of feeding supplemental lipid-encapsulated conjugated linoleic acid (LE-CLA) on bioenergetic status and reproductive performance. Preliminary results demonstrated reduced milk fat concentration and yield in cows receiving LE-CLA, resulting in improvements in energy balance, body condition score and indicators of reproductive performance. In a second study, with greater numbers of animals, milk fat concentration and yield were reduced, but milk yield was increased with LE-CLA supplementation, resulting in no difference in milk energy output or any measure of reproductive performance. These results suggest that in pasture-based systems of dairy production, where energy intake limits milk production, energy spared by LE-CLA induced milk-fat depression is partitioned primarily towards increasing milk yield rather than towards body reserves. The effects of four fat supplements containing different fatty acids on the mechanisms involved in reproductive performance were also investigated. Plasma progesterone concentration and corpus luteum volume were increased in cows supplemented with a saturated fat source or LE-CLA compared with both flaxseed and fish oil n-3 fat supplements. Finally, the effects of fat supplementation and herbage allowance on progesterone metabolism were examined. Measures of progesterone metabolism were not affected by either herbage allowance or fat supplementation, but the half-life of progesterone tended to be increased in fat-supplemented cows on a restricted grazing allowance. Reduced catabolism of progesterone can lead to increased plasma progesterone concentrations. Collectively the results of the studies suggest that feeding specific fat supplements may result in increased plasma progesterone concentrations and this may have beneficial effects on embryo survival and subsequent fertility. This research will contribute to the 2020 Food Harvest Vision by reducing the economic costs associated with poor reproductive performance on Irish dairy farms.



## The effect of genetic merit for fertility on reproductive efficiency

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To elucidate the underlying physiological basis of declining reproductive performance, the current study compared the phenotypic performance of Holstein cows with divergent genetic merit for fertility traits, but with similar genetic merit for milk production traits. Results showed cows with good genetic merit for fertility traits (Fert+) had improved reproductive performance during a 20 week breeding season, maintained greater body condition score and had greater circulating concentrations of insulin-like growth factor-I (IGF-I) and insulin during lactation compared to cows with poor genetic merit for fertility traits (Fert-). During the oestrous cycle, Fert+ cows developed a corpus luteum that was 16% larger and had 25% greater circulating progesterone concentrations compared with Fert- cows. Maximum preovulatory follicle diameter was larger in Fert+ than Fert- cows, and a greater proportion of Fert- cows ovulated following a silent heat compared with Fert+ cows. Fert+ cows exhibited greater hepatic expression of IGF-I and reduced hepatic expression of lower molecular mass binding proteins, explaining the elevated circulating concentrations of IGF-I in this genotype. There was minimal effect of genotype on morphological classification and transcript abundance of key genes in immature oocytes, and morphological classification of embryos, with subtle differences in blastocyst quality. Hence, this study emphasizes the importance of genetic selection for fertility as a vital strategy to alleviate reproductive wastage, and achieve the desired expansion of the national herd highlighted in the Food Harvest 2020 document.





## Novel nanovaccine development to prevent respiratory disease in cattle

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This project designed and investigated a novel nanovaccine against bovine parainfluenza type 3 virus (BPI3V), a pathogen associated with bovine respiratory disease (BRD). Multiple pathogens are associated with BRD and they cause substantial economic loss (€35m) to the beef industry annually in Ireland. Nanoparticles encapsulating (BPI3V) antigens were prepared and optimised for intranasal delivery. A series of studies were conducted in calves, 1), with and without maternal antibodies, and 2), with and without a 'booster' inoculation, and the immune response to the nanovaccine was compared with a commercial vaccine. The nanovaccine induced a stronger ( $P \leq 0.05$ ) immune response over the study period in the presence of maternally derived antibodies compared with the commercial vaccine. Booster immunisation with the nanovaccine elicited a controlled and gradual rise in protective mucosal immune response due to sustained release of antigen from the nanoparticles. The effect of weaning stress on the immune response to vaccination was also examined in beef calves immunised at 1 and 4 weeks pre-weaning. Calves immunised with the commercial vaccine and the nanovaccine at 1 week pre-weaning did not show an increase in serum antibody levels until 3 weeks post-weaning. Calves immunised at 4 weeks pre-weaning with a commercial vaccine showed a significant rise in serum antibody levels 14 days after immunisation. The greatest antibody response was present in weaned calves immunised with the nanovaccine at 4 weeks pre-weaning with calves having no clinical signs of BRD post-weaning. Results indicate that the nanovaccine, after a single intranasal dose, induced BPI3 antigen-specific secretory IgA in the upper respiratory tract of calves that lasted for 17 weeks which is indicative of the sustained release of the antigen. This project, applying novel nanotechnology for vaccine delivery, will drive state-of-the-art prevention strategies against BRD which will enhance animal health and welfare and greatly reduce economic losses.





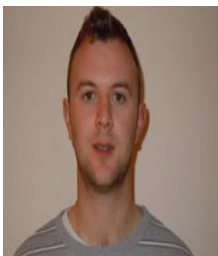
## Options to improve productivity from dairy pastures: Grazing management and perennial ryegrass cultivar

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The objective of this study was to examine options to improve the productivity of pasture based dairy systems; grazing management and perennial ryegrass cultivar were explored as possible options to achieve this. Maintaining varying levels of pre-grazing herbage mass (1200, 1500 and 2000kg DM ha<sup>-1</sup>) did not affect milk output, but pasture output was substantially reduced (-0.9t DM ha<sup>-1</sup>) by maintaining pre grazing herbage mass at 1200kg DM ha<sup>-1</sup>. Lower enteric methane emissions were recorded from cows grazing the lower herbage mass pastures, due to the improved organic matter digestibility of these pastures (+12g kg<sup>-1</sup>). Choice of perennial ryegrass cultivar was also selected as an option to improve productivity from dairy pastures. It was found that perennial ryegrass cultivar influences milk production performance due to cultivar differences in sward structure and chemical composition. In order to assist growers in selecting perennial ryegrass cultivars, many countries have independent official trials. It is imperative that these trials have a management in place that will identify the most suitable cultivars for the end user. This study reported that conservation based managements do not identify cultivars suitable for intensive grazing due to cutting frequency × genotype interactions. The work completed demonstrates the potential for the Irish dairy industry to achieve the targets set out in The Food Harvest 2020 Report. Implementing correct grazing management allowed the utilisation of 12.2t DM ha<sup>-1</sup> of pasture, producing 1176kg MS ha<sup>-1</sup>, approximately double what is achieved at farm level. Having an appropriate perennial ryegrass evaluation management and choosing the most suitable cultivars for one's system allows further production increases. However, expansion must be environmentally sustainable; improving pasture quality reduced methane emissions by 17% per kg milk solids, making it a suitable methane mitigation strategy.



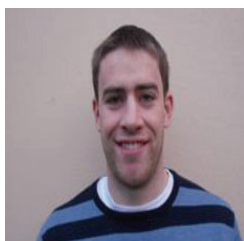
## Reducing greenhouse gas emissions from Irish dairy production

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Ireland's dairy sector emitted 10% of the nation's greenhouse gas (GHG) emissions in 2010. As a member of the EU, Ireland has committed to reduce GHG emissions from non-emission trading sectors, such as the agricultural sector, to a level 20% below those of 2005 by the year 2020. However, the Food Harvest report targets a 50% increase in milk production by 2020. Thus, the purpose of this study was to develop a model to assess strategies to reduce the carbon footprint (kg of GHG per unit of milk) of dairy production and to compare the principle methods for modelling GHG emissions; Life Cycle assessment (LCA) and the Intergovernmental Panel on Climate Change (IPCC) method. The model assessed the effect genetic merit of cows has on GHG emissions. The study showed selecting cows solely for milk production increased the carbon footprint of dairy production relative to cows selected on a combination of production, reproductive and health traits, because of an increase in emissions from non-productive animals. The model evaluated GHG modelling methodologies and found that the IPCC and LCA methods ranked the carbon footprint of dairy systems differently. For instance, the IPCC method produced a carbon number 14.5% lower for the confinement system when compared to a grass-based system, but the LCA approach calculated that the confinement system increased the carbon number by 18%, because of the inclusion of off-farm emissions e.g. concentrate and fertiliser production. The analysis shows that mitigation strategies should be calculated using approaches that quantify total GHG emissions, thus ensuring that the policy in place was not causing emissions displacement. This study will guide policy makers with information in relation to carbon footprint and national inventory calculations and will provide information in relation to the potential to achieve Food Harvest 2020 targets while complying with environmental regulations.



## An econometric estimation of risk aversion and risk exposure of Irish dairy farmers

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The Irish dairy sector has been exposed in recent years to an increase in price volatility. The Food Harvest 2020 report highlights the need for risk management tools such as forward contracts and insurance to play a key role in unlocking the potential of the Irish agriculture by enabling the necessary investment to take place. Their success depends largely on farmer's risk aversions and risk exposure. Risk aversion relates to the amount a farmer (or investor) would be willing to pay to avoid a risk. Thus a more risk adverse investor would expect a higher return or risk premium on a more risky investment. Risk exposure relates to the riskiness of the activity engaged, which from a farmer's perspective can relate primarily to input cost, output cost, yield and policy risk. In this study, we estimate a statistical model of these issues amongst dairy farmers using the National Farm Survey. When farmers choose a production system they also in effect choose the range of anticipated profits. For example, renting additional land indicates the anticipation of a reasonable return on the additional production from that land rather than the risk of losses or lower profits if future product prices fall. However, where a farmer takes a conservative production plan, the exposure to risk diminishes as well as expected profit. Farmers engage therefore in a trade-off between expect profit and risk. Hence, the input mix impact on expected profit and risk offers a good indication of the level of risk aversion. In order to get a sense of risk exposure, we assume that risk is the difference between farmers' expectation and what actually happens. However without collecting data on expectations, we use a proxy for risk. Consistent with the literature, our proxy is based upon the residuals of a regression where we control with a fixed effect estimator unobserved and constant heterogeneity. We found that on average, farmers would have been ready to pay 16% of their gross margin in 2009 to get rid of all sources of risk. This is their risk premium. Lastly, we show that risk exposure played an important role in the diversification of activity such as the uptake of REPS, suggesting that risk considerations are central to devise a smart way to achieve sustainable growth.



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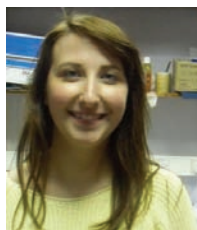
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## Enhancement of calcium bioavailability *via* milk bioactives

L. O'Brien<sup>1,2</sup>, H.Schellekens<sup>2</sup>, T.G. Dinan<sup>2</sup>, J.F. Cryan<sup>2</sup>, G.F. Fitzgerald<sup>2</sup> and C. Stanton<sup>1</sup>

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From a nutritional standpoint, human milk contains the best source of bioavailable calcium for developing infants. Calcium bioavailability is described in terms of percentage calcium solubility, with human milk containing approximately 65% soluble calcium, followed by cows' milk, which contains 45% soluble calcium, while infant formula typically contains 22% soluble calcium. The objective of this study was to develop and employ a rapid bioassay to identify and exploit bovine milk constituents (milk protein-derived peptides and dairy fats) for enhanced calcium uptake across the gut epithelium, with a view to their functional application in infant formula for the promotion of calcium bioavailability. The standard method for assessing calcium bioavailability *in vitro* involves quantification of radio-labelled calcium isotopes transported across a cell monolayer consisting of CaCo-2 colorectal adenocarcinoma cells. Drawbacks associated with this approach include the need to use radio-labelled materials, high expense, and the time-consuming nature of the assay. The rapid assay which was developed as part of this study employed the HT29 colorectal adenocarcinoma cell line in conjunction with fluorescence analysis using the FLIPR 4 Calcium Assay kit to quantify intracellular calcium uptake. Using this approach, the potential of milk-derived constituents for enhanced calcium bioavailability was demonstrated, and these bioactivities are currently being verified using radio-labelled Ca isotopes in conjunction with CaCo-2 monolayer's. This research will contribute to the 2020 Food Harvest vision with regard to product innovation and increased sustainability within the dairy sector, by identifying potential beneficial milk constituents for incorporation into infant formula, of which Ireland retains 12% of global productivity.



## The use of mixture design to formulate optimized nanoemulsions

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The objective of this study was to investigate the effect of mixture formulation on the stability of nanoemulsions. Mixture design software was used to formulate carbohydrate-protein systems comprising of lactose (0% to 20% w/w), trehalose (0% to 20% w/w), and  $\beta$ -casein (2.5%, 5%, 7.5%, and 10% w/w). They were analyzed for glass transition temperature in maximally freeze-concentrated state ( $T_g'$ ), onset of ice-melting temperature in maximally freeze-concentrated state ( $T_m'$ ) and viscosity ( $\mu$ ). A significant ( $P < 0.05$ ) decrease in  $T_g'$ , and significant increase in viscosity and  $T_m'$  were observed with increasing protein content. The optimization function was applied to these results to predict quantities of lactose and trehalose required, at fixed protein contents, that will minimize and maximize  $T_g'$  and viscosity. 13 optimized mixtures were selected for the continuous phase for nanoemulsions (10% w/w sunflower oil), microfluidized at 70 MPa. The nanoemulsions were analyzed for mean particle size, viscosity, stability, and  $T_g'$  &  $T_m'$ . Significant ( $P < 0.05$ ) increases were observed in viscosity (5 to 156 mPa.s), mean particle size (186 to 199 nm) and stability (40.9 to 4.2 m/d) with a significant decrease in  $T_g'$  (-45 to -50 °C) with increasing protein content (2.5% to 10% w/w). In this way, it was shown that a continuous phase suitable for nanoemulsion systems can be designed based on rheological and glass transition properties through the use of a statistical mixture design. This research will contribute to the 2020 Food Harvest Vision by showing how an innovative approach, such as statistical experimental design, can be used to formulate stable food emulsion systems.





## Establishment of an optimum harvest window and pre-harvest treatment of *Miscanthus Giganteus*

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Delaying the harvest date of *Miscanthus giganteus* from the conventional harvest time of autumn until spring has been found to improve the combustion quality of the fuel while also aiding the storability of the biomass material by reducing the moisture content (MC) of the material (Lewandowski, et al. 2003). The aim of this work is to determine the optimum time of harvest of *Miscanthus* between the months of January and March to achieve the lowest possible MC, using three cutting dates as treatments, Cut January, Cut February and Cut March, while also examining the drying rate under different harvest subtreatments, namely uncut (control), cut and left flat and cut and left in a swath. This trial took place in 2009 and was repeated in 2010. Biomass sampling took place on a weekly basis in which a sample of *Miscanthus* was taken from each of the subtreatments in order for the MC to be determined. A significant difference in MC was observed between treatments in both years of the trial. Cutting the crop in March was found to result in a more rapid loss of moisture than cutting the crop in January. No significant difference was observed however between subtreatments flat and swath while the control was found to behave significantly different to the flat and swath subtreatments, showing harvest method and cutting time influence the rate of moisture loss of the crop. In 2009 and 2010, an increase in MC on flat and swath subtreatments was observed when compared to that of the control in the first weeks of the trial. When looking at the Cut February treatment, apart from a little fluctuation in both 2009 and 2010, the overall trend was MC dropped below that of the control after weeks 3-4. The rate of moisture loss observed in the Cut March treatment varied significantly more between 2009 and 2010 than the previous treatments with significantly higher moisture loss occurring in 2009 than in 2010.

Contrary to this, the control treatment in 2010 saw a significantly higher loss in MC when compared to 2009 over the course of this trial (January to April). The results of the trial show that while the time of harvest can significantly affect the rate of moisture loss from the biomass material, no difference was observed between flat and swath subtreatments. However, a significant difference in MC and rate of moisture loss was observed between the aforementioned subtreatments and the control (uncut) material.



## Detection of adulterants in food using mass spectrometry techniques

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This paper reports the development of a new analytical method for routine screening of known and designer adulterants in food supplements. This Q-TOF based method enables screening of a large number of targets at the same time without loss of sensitivity. It also has a strong potential for unequivocal confirmation of the analyte identity due to the capability to combine both accurate mass measurement and tandem mass spectrometry. The unknown peaks can be identified based on accurate mass and isotopic profile evaluation. There is no need to use standards a priori and data can be reprocessed a posteriori for additional compounds, which had not been yet investigated. The screening procedure was complemented with UPLC-MS/MS method for quantitative analysis of known adulterants. The confirmatory analysis was validated according to Commission Decision 2002/657/EC criteria. The developed methods have been extensively evaluated through application for routine examination of authentic adulterated slimming food supplement samples available on the Irish market. Various undeclared drugs, being restricted in food supplements by regulatory agencies, were detected in tested samples. It confirms that slimming food supplements, regardless of the label claim, are often purposely adulterated with synthetic drugs to enhance desired action. The outcome of this research has been the improvement of the safety of food supplements through the application of new sensitive test methods. This research will contribute to the 2020 Food Harvest Vision by underpinning food safety, leading to improved consumer protection. The approach developed in this work has the potential to be applied in different fields of work including bioactive discovery and veterinary drug residue analysis.

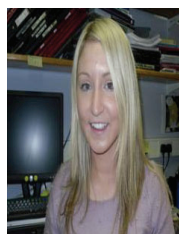


## Maximising the flavour potential of lactic acid bacteria for dairy applications

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Cheese requires lactic acid bacteria (LAB) to produce lactic acid from milk lactose and to develop flavour. In cheese, once lactose is depleted the next most available energy source; peptides and free amino acids are utilised. It is this nitrogenous metabolism that creates most of the characteristic aromatic compounds associated with cheese flavour. Numbers of LAB reduce rapidly over ripening due to competition for energy resources, high salt levels, and low water activity. It is now known that most of these bacteria enter a “non-culturable” state and continue nitrogenous metabolism, remaining the prime source of flavour development in cheese. “Non-culturable” LAB can be created by high pressure microfluidization and this study was designed to evaluate the metabolic flavour potential of “non-culturable” LAB in comparison to live intact culturable cells. A sterile model system was developed which consisted of pre-hydrolysed lactose free UHT milk containing a limited carbohydrate source and significant levels of peptides and free amino acids for metabolic activity. Six commercial single strain starter LAB were grown under optimal conditions and each split into two portions; with one portion passed through a microfluidizer to create “non-culturable” cells. Both cell types were evaluated under identical conditions and their flavour potential assessed by headspace-solid phase micro extraction gas chromatography mass spectrometry. In all cases the “non culturable” cells produced more flavour compounds and higher levels of flavour compounds than live intact culturable cells. These “non-culturable” cells were subsequently utilised in the production of enzyme-modified cheese and shown to enhance flavour development and have significant potential as flavour sources in a wide range of applications. These results also align with targets set within the 2020 Food Harvest Vision in relation to adoption of new technologies, been at the forefront of research and development, having a direct application to industry.



## The effects of aromatic compounds on taste transduction and gastrointestinal satiety signalling

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Enteroendocrine cells are found throughout the gastrointestinal tract and respond to ingested nutrients by producing satiety signals including glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK) and peptide YY (PYY). These cells also express taste receptors and previous studies have demonstrated that activation of the taste transduction pathway can lead to the release of satiety signals. This study aims to establish a molecular link between aroma compounds, taste and satiety by examining the impact of volatile dairy aroma compounds on taste transduction and satiety signalling. The murine enteroendocrine cell line, STC-1, produces CCK, GLP-1 and PYY, and expresses transmembrane receptors involved in taste transduction. STC-1 cells were exposed to aqueous solutions of the volatile compounds for 4 hours. RNA was isolated from cell lysates, quality assessed and reverse transcribed into cDNA for gene expression analysis of *proglucagon*, *PYY* and *CCK* genes using Lightcycler and SyBr Green technology. Results demonstrated that the compound diacetyl significantly down-regulated *proglucagon* and *CCK* mRNA levels over time. Specific antagonists of taste receptors and siRNA inhibition of the G-protein coupled receptor gustducin, which is involved in taste transduction, were used to investigate the activation of taste signalling pathways. Gene expression data indicated that diacetyl may inhibit  $\text{Ca}^{2+}$  channels and gustducin in order to mediate its effects on downstream satiety signals. Understanding the mechanisms by which food components affect gastrointestinal signalling is important in the development of functional foods with health benefits. This research targets the area of functional ingredients and will therefore contribute to the effective R&D strategy outlined in the 2020 Food Harvest Vision.



## Calcium induced aggregation of dephosphorylated bovine $\beta$ -casein

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Protein in human milk can be divided into two groups known as serum proteins (~ 60%) and caseins (~ 40%). The casein fraction can be subdivided into  $\beta$ -,  $\alpha$ -, and  $\kappa$ -casein, whereby  $\beta$ -casein is the main constituent. Infant formulae are manufactured to simulate, as closely as possible, the nutritional profile of human milk and proteins sourced from bovine milk are typically used as part of the formulation. However, bovine  $\beta$ -casein differs to human  $\beta$ -casein, mainly by the number of phosphate groups bound to the protein (5 in bovine; 0 to 5 in human). Consequently, human  $\beta$ -casein has different functionality, e.g., it is more readily hydrolysed at pH 4, resulting in a faster rate of gastric emptying. The aim of this study was to investigate the effect of enzymatically (potato acid phosphatase) removing phosphate groups from bovine  $\beta$ -casein (i.e., dephosphorylation) on functional characteristics such as calcium induced aggregation. The phosphorous content of the dephosphorylated  $\beta$ -casein was 1.38 mg g<sup>-1</sup> dry matter (DM) compared to 4.90 mg g<sup>-1</sup> DM for the non-dephosphorylated  $\beta$ -casein. Dephosphorylation resulted in reduced electrophoretic mobility, due to removal of phosphate charged groups and an increase in minimum solubility value from pH 5 to ~ pH 5.5. Addition of calcium (30 mM) had little effect on the viscosity of dephosphorylated  $\beta$ -casein measured at 37°C (~ 5.5 mPa.s); however, it did result in an increase in viscosity from 6 to 12 mPa.s in solutions of non-dephosphorylated  $\beta$ -casein. The increase in viscosity in the non-dephosphorylated  $\beta$ -casein was attributed to calcium induced aggregation as shown by increases in turbidity measured at 440 nm. The results demonstrate that, in the presence of calcium, it is possible to reduce aggregation of bovine  $\beta$ -casein by reducing the number of phosphate groups to levels similar to those of human milk. The work presented here plays a key role in the continuation of research and provision of knowledge to the infant formula sector which is a major user of dairy ingredients in Ireland, supporting growth and development as outlined in the Food Harvest 2020 report.



## Technologies and systems to control *Campylobacter* in the poultry industry

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*Campylobacter* is the most frequent cause of bacterial gastroenteritis in Ireland and the EU. In Ireland, over 98% of broilers are contaminated with *Campylobacter*. The objectives of this study were; (1) to investigate the effect of different chemicals, concentrations and application methods on *Campylobacter* levels on poultry carcasses; (2) to investigate the efficiency of cloacal treatments as a *Campylobacter* control technology and (3) to investigate the effect of modified atmospheric packaging (MAP) on *Campylobacter* levels and the shelf-life of chicken fillets. After dipping and spraying poultry carcasses, the two most effective chemicals were 14% (w/v) tri-sodium phosphate and 5% (w/v) citric acid, which achieved 2.49 and 1.44 log<sub>10</sub> CFU cm<sup>-2</sup> reductions respectively. The most effect cloacal treatment was 5% lactic acid (v/v), which achieved a 1 log<sub>10</sub> CFU cm<sup>-2</sup> reduction on carcasses in the slaughtering plant. Of the seven different gaseous combinations used in the MAP experiments, 40:30:30% CO<sub>2</sub>:N<sub>2</sub>:O<sub>2</sub>, resulted in a 1.17 log<sub>10</sub> CFU per gram *Campylobacter* reduction and most significantly trebled the shelf-life. The technologies developed and validated in this project provide a science based solution to the *Campylobacter* issue for Irish poultry processes and contribute to the Harvest 2020 object of sustainable growth in the Irish agri-food sector through the “delivery of high quality, safe and naturally based produce” while providing consumer safety.



## The generation of bioactive peptides from bovine myofibrillar proteins

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In human nutrition the role of red meat as a valuable source of protein and essential nutrients including iron, zinc and vitamin B12 within the diet is well documented. Furthermore, the potential of meat proteins to harbour bioactive peptides which are capable of exerting physiological effects in the body have received some attention. The aim of this study was to identify bioactive peptides released following the *in vitro* digestion of bovine myofibrillar proteins. Hypertension is one of the major risk factors associated with cardiovascular disease, Angiotensin 1-converting enzyme (ACE) plays an important physiological role in regulating blood pressure, inhibition of this enzyme can exert an anti-hypertension effect. Hydrolysates generated via proteolysis of myosin and actin with pepsin, trypsin and  $\alpha$ -chymotrypsin were screened for ACE inhibitory activity. Myosin hydrolysates generated via proteolysis with pepsin and trypsin exhibited ACE inhibitory activity. The hydrolysates were subsequently separated using RP-HPLC into individual fractions. The peptides responsible for the observed ACE inhibitory activity within individual fractions were identified using a combination of MALDI-TOF MS and the peptide amino acid sequence confirmed by Tandem MS, in total four ACE inhibitory peptides were identified using this approach. This study demonstrates the potential of myosin as a source of bioactive peptides in meat that may be released during the physiological digestion of beef. Bioactive peptides generated from proteins found in low value cuts of meat and waste streams could contribute to Food Harvest 2020 by adding value to the waste streams of the meat industry.





## Chemical amendment of dairy cattle slurry for the control of phosphorus in runoff

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Phosphorus (P) loss from grassland to a waterbody can adversely affect water quality. Land application of dairy cattle slurry can result in incidental P losses to runoff in addition to increased chronic P losses from soil as a result of a build-up in soil test P. This study comprised laboratory and field-scale experiments, which investigated the effectiveness and feasibility of chemical amendments in reducing solubility of P in dairy cattle slurry, taking into account for the first time their pollution swapping potential. Following a preliminary study, the most feasible amendments (alum, poly-aluminium chloride (PAC), ferric chloride ( $\text{FeCl}_2$ ) and lime) were mixed with slurry before it was applied to soil contained within runoff boxes and then subjected to simulated rainfall applied at an intensity of  $11.5 \text{ mm h}^{-1}$ . A laboratory chamber experiment was used to address the potential for 'pollution swapping' as a result of the effects of chemical amendments on emissions of ammonia, nitrous oxide, methane and carbon dioxide. The most feasible amendments were then examined at field plot-scale in Johnstown Castle, Co. Wexford. The results of this study show that chemical amendment of dairy cattle slurry with alum or PAC could be used to control P solubility and reduce incidental P losses from soils receiving dairy cattle slurry without adversely effecting metal and nitrogen losses. This research will contribute to the 2020 Food Harvest Vision by identifying a short-term P mitigation measure which may be used to reduce P solubility of slurry and allow farmers to better utilize other nutrients in slurry.





## Biodiversity and soil food web activity affected by p fertilisation in grasslands

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Grasslands are the main agricultural land use in Ireland, and are extensively fertilised to increase productivity. To understand more about the consequences of fertilisation, especially with P, we sampled the Cowlands long-term experiment at Johnstown Castle, where 0, 15 or 30 kg P ha<sup>-1</sup> yr<sup>-1</sup> has been applied for the last 50 years. Our study combined morphological and molecular techniques (Terminal restriction fragment length polymorphism) for analysing nematode communities with measures of the microbial community. Results showed that P fertilizer increasing nematode abundance, especially in the medium P treatment. P application also changed soil microbial properties. Soil microbial biomass C, N and P increased significantly by 27%, 38%, and 65%, respectively from no P to high P application Phospholipid Fatty Acid Profiles (PFLA), and bacterial qPCR indicated that P application increased soil bacterial and fungal biomass, but changes to the nematode community showed an increasing reliance on bacterial pathways as P increased. Our study determined the effect of grassland P fertilizer management intensity on soil microorganisms, enabling us to understand the grassland soil food web.



## Renewable energy production and greenhouse gas mitigation by biomass crops

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The EU has committed to a 20% target for renewable energy by 2020, with an additional national co-firing target of 30% in the peat-burning power stations to be achieved by 2015. Land-use change to biomass production has been identified as a means of mitigating greenhouse gas (GHG) emissions through the substitution of fossil fuels and the enhancement of carbon storage in vegetative and soil carbon reservoirs. However, the size of this potential terrestrial carbon sink is unknown. The aim of this research was to investigate the carbon balance implications of establishing biomass crops by quantifying soil stocks during conversion and measuring ecosystem-scale carbon fluxes in the newly-established plantations. Field-scale stands of *Miscanthus × giganteus* and Reed Canary Grass (RCG; *Phalaris arundinacea*) were established on land previously under permanent pasture in the south-east of Ireland. Ploughing resulted in a peak in CO<sub>2</sub> emissions, which decreased to background levels in a matter of hours. The total emissions suggested that if the fallow period could be minimised, ecosystem C losses could be limited to less than 1 t C ha<sup>-1</sup>. Measurements showed a larger sink of C in RCG (3.3 t C ha<sup>-1</sup>) compared to *Miscanthus* (0.5 t C ha<sup>-1</sup>) during the establishment phase. This was explained by greater leaf cover in the RCG canopy which increased light interception, leading to higher rates of photosynthesis. However, once the crop has established, *Miscanthus* may achieve comparatively higher rates of C sequestration (circa. 7 t C ha<sup>-1</sup>). This research will contribute to the vision outlined in Food Harvest 2020 as biomass systems will reduce the carbon intensity of agricultural activities by enhancing carbon sinks. In addition, biomass crop production has strong potential as an innovative and sustainable solution to meeting energy requirements and to achieving renewable energy targets.



## Genetically enhancing *Fusarium Oxysporum* for added value products

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The plant pathogenic fungus *Fusarium oxysporum* has the potential to convert agricultural waste products into value added phenolics. *F. oxysporum* releases ferulic acid from cell walls of higher plants, such as wheat and converts it into poly-phenolics such as vanillic acid, protocatechuic acid, ethyl vanillate and ethylguaiacol which are antioxidants, anti cancerous, food and flavouring agents of significant economic importance which exceeds \$3.4bn globally. The goal of this research was to enhance the capacity of *F. oxysporum* to generate added value phenolics by the genetic disruption of genes involved in the ferulic acid production pathway. To this end, *Agrobacterium* mediated transformation was employed to generate a collection of random gene disruption transformants of *F. oxysporum*. From an initial library of 1500 transformants, a subset of 170 transformants were screened using high-throughput HPLC analysis for ferulic acid bioconversion efficiency and by-product synthesis, relative to the wild type *F. oxysporum* profile. Four transformants (T302, T319, T321, and T334) exhibited elevated bioconversion efficiency, which equated to a 30-fold increase in the ability to convert ferulic acid to vanillic acid. In addition, ethylguaiacol production was increased 8 fold in these transformants. In parallel, those *F. oxysporum* transformants with significantly different ferulic acid bioconversion efficiencies compared to the wild type strain were screened for hyper-tolerance to the targeted phenolics, which can become fungi-toxic at high concentrations. Radial growth and biomass production were also examined as they are basic features required for large scale fermentation. These 30 transformants showed increased and decreased tolerance to ferulic acid, vanillic acid and ethylguaiacol. With this study we have successfully identified four transformants which have a hyper ability to convert ferulic acid to vanillic acid and ethylguaiacol compared to wild type *F. oxysporum*. The second phase of the work programme is to investigate the genetic mechanisms underpinning these novel phenotypes.



## The implications of sexual recombination in *phytophthora infestans* for potato blight control in Ireland

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Potato growers in Ireland can suffer crop yield losses of up to 10t/ha arising from blight disease caused by *Phytophthora infestans*. The situation is complicated by the recent occurrence of hyper aggressive genotypes of *P. infestans* in Irish potato fields. These include the 13\_A2 ('Blue 13') and 6\_A1 ('pink 6') genotypes. The emergence of these strains, which are opposite mating types has dramatically increased the risk of sexual recombination and the subsequent generation of complex genotypes. As the implications for blight control strategies are as yet unknown, an investigation was carried out to determine whether recombinant progeny from these genotypes exhibit fungicide resistance and whether they have the ability to overcome established cultivar resistance. An F1 population was raised from *in vitro* crossings between Blue 13 x Pink 6 and Blue 13 x 8\_A1 (traditional Irish genotype) isolates of *P. infestans*. The Blue 13 parental isolate is resistant to metalaxyl, which is a primary fungicide used to control potato blight disease. This is in contrast to Pink 6 and 8\_A1 which are metalaxyl sensitive. Twenty four and 40 F1 progeny were recovered from the Blue 13 x Pink 6 and Blue 13 x 8\_A1 crosses respectively. Metalaxyl sensitivity of the F1 progeny was assessed using the floating leaf disc technique. The metalaxyl profiles of the F1 population segregated into 3:16:3 and 6:25:5 (sensitive:intermediate:resistant) for 13\_A2 x 6\_A1 and 13\_A2 x 8\_A1 pairings. This study has demonstrated that *P. infestans* isolates with a Blue 13 genotype will mate with opposite mating types of Pink 6 and 8\_A1 genotypes producing a segregating F1 population with a low frequency of progeny with sensitivity and resistance, and a high frequency of those with an intermediate phenotype. The F1 populations are currently being tested for their aggressiveness on potato. This research is highly relevant to the recommendations of Harvest 2020 as it generates genomics-based knowledge to support existing crop management regimes.

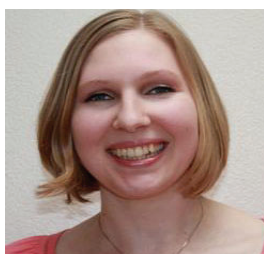


## N<sub>2</sub>O emissions from land use change into bioenergy crops

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Biomass crops have the potential to contribute to EU 2020 climate and energy targets. Land use change (LUC) for bioenergy production can displace fossil fuel emissions and reduce agricultural greenhouse gas emissions since biomass crop nutrient requirements are lower compared to arable or grassland systems. Our research investigated the impact on nitrous oxide emissions of LUC from pasture to two energy grasses (*Miscanthus x. giganteus* and *Phalaris arundaceae*) on two soil types of contrasting drainage. A field study and two lysimeter trials investigated the effects of ploughing technique, plant establishment, soil type and crop type on N<sub>2</sub>O and leached nitrate and on N cycling within *Miscanthus*, *Phalaris* and pasture systems. The emissions in the first two years post-LUC were associated with agricultural practices (tillage, plant establishment), with the early stages of land conversion, in particular, generating large N<sub>2</sub>O fluxes due to enhanced N mineralisation. Ploughing losses were affected by soil type and cultivation technique, with higher N<sub>2</sub>O emissions observed for heavier soils and higher leached N losses for well-drained soils. Ploughing technique shifted the balance between gaseous and leached losses but did not affect the magnitude of losses. Post-establishment, field-scale N<sub>2</sub>O emissions from the biomass crops were reduced by 50% compared to managed grassland systems. The lowest emissions were observed for *Miscanthus*, due to higher plant N utilisation. In conclusion, the conversion of a small proportion of grassland to biomass production substantially reduces field N emissions, with losses associated with cultivation offset via improved N utilisation and reduced fertiliser requirement. These reductions, allied to fossil fuel substitution, will contribute to both sustainable energy requirements and the reduction in agricultural carbon intensity as envisaged under the Think Green vision in Food Harvest 2020.



## Pesticide leaching to groundwater from diffuse agricultural sources in an agricultural catchment

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A groundwater monitoring network was installed in seven locations across Ireland to review the occurrence of pesticides in contrasting settings under the influence of diffuse agricultural sources. This paper deals with two sites located in one arable catchment in Co. Wexford. Both sites are intensively managed with spring barley and associated pesticide applications. The catchment has Clonroche acid brown earth soils underlain by highly weathered till which spans a depth between 7-9m. Beneath this is a fractured aquifer of Ordovician metasediments. Monthly samples were collected from three multilevel monitoring wells to screen the subsoil (8 m below ground level (b.g.l)), interface (16 m b.g.l) and bedrock (36 m b.g.l). Each month 18 samples were collected. The most frequently detected pesticides were both transformation products (TP): firstly 2,6-dichlorobenzoic acid, a TP of dichlobenil (Casoran) which was banned in March 2010 and of 3,5-dichlorobenzamide. Second most frequently detected was phenoxyacetic acid which arises from the degradation of 2,4-D (BanDock). Mecoprop (ALNETTEL), mecoprop-p (Foundation), MCPA (Larke) and bentazone (Basagran) were also detected but not in quantities exceeding the drinking water standard of 0.1µg/L as the TP's did. This project aims to provide data to help the sustainable use of plant protection products in Ireland.

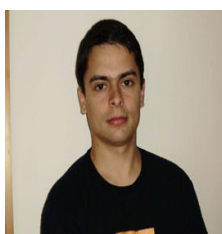


## Effect of feeding genetically modified mon810 maize to pigs from 12 days post-weaning for 110 days on serum and urine biochemistry

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To assess the effects of genetically modified Bt maize (GMm) on pig health, 40 male pigs (~40 days old; ~10.7 kg) were assigned to diets (d0); T1- non-GM maize (nGMm) diet to d110; T2- GMm diet to d110; T3- nGMm diet for 30d followed by GMm to d110; T4- GMm diet for 30d followed by nGMm to d110. Liver [alanine and aspartate aminotransferase (AST),  $\gamma$ -glutamyl transferase, alkaline phosphatase] and kidney [total protein (TP), urea (SU) and creatinine (SC)] health indicators were analysed in serum on d0, 30, 60, 100 and 110. Urinary creatinine and protein were measured on d110 to investigate kidney health. On d30, SU was lower for T3 than T1, T2 and T4. On d110, SC was higher in pigs fed T3 and T4 than T1 and T2. Serum TP was lower on d110 in pigs fed T4 than T1, T2 and T3. On d110, serum AST tended to be lower in pigs fed T2 than T1. All values were within the normal range for pigs of this age. The changes observed were not indicative of organ dysfunction therefore indicating that Bt maize is well tolerated by pigs. This study shows no adverse effects of feeding Bt maize to pigs on clinical biochemistry and can offer assurance to producers, regulators and consumers as to the safety of this GM crop. As GM feed ingredients are less expensive than their non-GM counterparts, these results provide confidence in using GM feed ingredients thereby reducing feed cost per kg carcass and improving the competitiveness of Irish pig production.



## Effects of sow back fat and gestation feeding level on metabolic stress levels in sows and growth indicators in the serum of progeny

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Maternal stress and undernutrition can influence foetal development and reduce birth weight in offspring. This metabolic programming may involve hyperinsulinaemia, hyperleptinaemia and anomalies in the insulin-like growth factor (IGF) axis. Using a pig model, we investigated interactions between body condition (thin or fat) and gestation feeding level (restricted, control or high) on maternal cortisol levels during pregnancy as a biomarker for stress, and IGF-1 levels in offspring as a biomarker for growth. We also evaluated litter size and offspring birth and weaning weight. Saliva was collected from the gilts during pregnancy and cortisol levels quantified using an EIA kit according to manufacturers' instructions. At farrowing, piglets were weighed. At weaning, weight and blood samples were obtained and IGF-1 levels quantified. During pregnancy restricted gilts had higher cortisol levels in the morning than gilts on the high feed and control feed level. Piglets born to fat sows had the highest average daily gain and weaning weights. Sows on the high feed level gave birth to the heaviest piglets. Light and heavy birth weight offspring did not differ in their serum IGF-1 levels at weaning. In conclusion, the results showed that excessive feed restriction during pregnancy raised maternal cortisol levels. As cortisol is a biomarker for stress, these results may indicate that stress caused by undernutrition during pregnancy is a contributory factor in foetal programming. Food Harvest 2020 recommends increasing sow productivity through the adoption of new technology and best practice and this research underpins that goal.





## Manipulating the ensilage of maize conserved as whole-crop, cob or stover

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Consistently satisfactory yields of maize of high nutritive value followed by minimal conservation losses are required in climatically marginal areas such as Ireland in order to sustain the economic viability of this crop. The objective of this project was to examine methods to reduce losses in yield and nutritive value associated with maize silage production. The DM yield, nutritive value and ensilage characteristics of whole-crop, cob and stover from contrasting cultivars of maize (early vs. late maturing), grown under two application rates of nitrogen (N) fertiliser (low = 33 kg N/ha, high = 168 kg N/ha) and harvested on different dates (early, conventional and late) were investigated. Higher N application did not confer an advantage in yield or nutritive value for whole-crop, cob or stover silage, whereas delaying harvest date or growing early-maturing cultivars resulted in reduced dry matter (DM) losses during the ensiling period, primarily due to the increased DM concentration of these herbage at ensilage. A subsequent study indicated that lactic acid bacteria additives, which differed through their promotion of either a homofermentative or heterofermentative lactic acid fermentation, did not improve the conservation or aerobic stability of cob or stover silages. The potential for improvements in the nutritive value of maize stover components (leaf, upper stem, lower stem) through their digestion by white-rot fungi (*Pleurotus* or *Trametes*) was examined. High DM losses and the lack of an improvement in the nutritive value of leaf, or upper or lower stem, indicated that herbage digested with white rot fungi did not confer an advantage over conventionally ensiled stover. These results contribute to the 2020 Food Harvest Report by highlighting the potential to improve maize silage yields and nutritive value through reduced ensiling losses, which may increase the efficiency of the forage's production system.

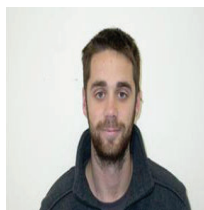


## *In vitro* rumen methane output of grassland treatments determined using a batch culture technique

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In Ireland, agriculture accounts for 0.29 of greenhouse gas emissions, approximately half of which is enteric methane (CH<sub>4</sub>) from ruminants. Since grassland is the dominant (*circa* 0.9) use of agricultural land in Ireland, any enteric CH<sub>4</sub> mitigation strategies must be effective within the predominantly grass-based ruminant production systems used. The objectives were to determine the effects of (1) perennial ryegrass herbage mass (HM) and sward allowance (SA), (2) perennial ryegrass variety (PRGV) and perennial grass species (PGS), (3) grassland forb species, and (4) perennial ryegrass sample drying method (thermal drying at 40°C for 48 hours *versus* freeze-drying at -55°C for 72 hours) on herbage chemical composition, and *in vitro* rumen fermentation variables and CH<sub>4</sub> output using a batch culture technique. All herbage samples were dried, milled and incubated *in vitro* with buffered rumen fluid at 39°C for 24 hours. The perennial ryegrass HM had a small-scale effect, while SA had no effect, on *in vitro* rumen CH<sub>4</sub> output. Furthermore, no differences in CH<sub>4</sub> output were found between the PRGV or PGS examined. These findings reflected the general absence of effects on herbage chemical composition and *in vitro* rumen fermentation variables. Some grassland forb species had reduced *in vitro* rumen CH<sub>4</sub> outputs, but these were achieved mainly through a reduction in the extent of *in vitro* rumen fermentation. Thermal drying of grass samples had a similar effect to freeze drying on CH<sub>4</sub> output. Overall, the results suggest that the HM, SA PRGV, PGS and forb treatments assessed would not reduce enteric CH<sub>4</sub> unless they directly impacted on animal productivity. Thus they may not assist in achieving the climate change objectives in the Food Harvest 2020 report by the Department of Agriculture, Food and the Marine.



## Technical and scale efficiency of Irish dairy farms

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The Irish dairy Industry will for the first time since the introduction of milk quota have the opportunity to expand unhindered post 2015. Key factors affecting increased profitability, efficiency and productivity must be exploited if the Irish dairy industry is to become a key exporter in the international dairy markets. The objectives of this study were to estimate the levels of efficiency, both technical and scale efficiency for Irish dairy producers, to determine the optimum scale and identify factors associated with technical and scale efficiency. The analysis was carried out using Data Envelopment Analysis with National Farm Survey Data for 2008. The results show on average a potential to increase technical and scale efficiency at farm level. On average technical efficiency could increase by 24.3% under constant returns to scale and 21.1% under variable returns to scale with scale efficiency 4.9% below optimum. Based on the sample selected the optimum scale on Irish dairy farms was found to be 80 cows farming 41 hectares of land. Twelve per cent of the sample was operating at optimum scale, 56% at sub optimum scale and 32% were operating above optimum scale with significant differences in terms of land, quota quantity and hired labour. Increased farm size, intensification and dairy specialisation were associated with both increased technical and scale efficiency at farm level. The implications of this research highlight a potential to enhance efficiency through increasing scale on Irish dairy farms. As Food Harvest 2020 has set goals to increase milk output by 50% in the Irish dairy industry, this study has shown that there is substantial scope to increase efficiency on dairy farms and that the expected increased scale can be associated with increases in efficiency at farm level.



## The effect of dicyandiamide on herbage production when applied at different times and rates

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The nitrification inhibitor dicyandiamide (DCD) has been shown to reduce nitrate leaching and nitrous oxide emissions from grassland by slowing the conversion of ammonium in urine patches to nitrate. In New Zealand DCD has increased herbage production when applied to urine patches. Food Harvest 2020 targets an increase in milk production of 50%; therefore increased herbage production will play a role in realising this target. Increasing nitrogen (N) available for plant uptake will potentially result in increased herbage production. The objective of this work was to establish if DCD applied in autumn and winter would increase spring and annual herbage production. Four simulated grazing plot experiments were undertaken at two sites, a free-draining brown earth (Moorepark) and a heavy brown earth (Ballydague). No N fertiliser was applied in experiment one (Moorepark) and two (Ballydague), while 350kg N fertiliser ha<sup>-1</sup> yr<sup>-1</sup> was applied in Experiment three (Moorepark) and four (Ballydague). Dicyandiamide was applied at rates of 0, 5 or 10kg ha<sup>-1</sup> in September, October or November to plots receiving artificial urine or zero urine. Spring and annual herbage production, spring herbage crude protein (CP) content and herbage N uptake were subsequently measured. Dicyandiamide application did not increase spring herbage production. Applying 5 or 10kg DCD ha<sup>-1</sup> to urine patches in October and November increased annual herbage production by 10 and 20%, respectively, and 13 and 18%, respectively, in experiment one. Dicyandiamide increased herbage N uptake and spring herbage CP content in experiment one. Urine application increased spring and annual herbage production in experiment one by 47 and 27%, respectively, and in experiment two by 17 and 15%, respectively. The effect of DCD on herbage production was variable across experiments; therefore the use of DCD to increase herbage production and achieve Food Harvest 2020 targets cannot be considered reliable.



## The ensilage of grass as a biomass feedstock and the subsequent utilisation of the fibrous fraction as a biobased material

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In the initial stages of a 'Green Biorefinery' process, plant biomass is separated into a fibre-rich press-cake and a nutrient-rich press-juice, and these separated fractions are further refined to recover or produce industrial products such as proteins and particleboard, etc. As with traditional farm enterprises, grass grown for industrial purposes would have to be ensiled to ensure year round availability. This project investigated the effects of two inorganic nitrogen fertiliser inputs (low = 0 kg N ha<sup>-1</sup>, high = 125 kg N ha<sup>-1</sup>) and five harvest dates (fortnightly from 12 May to 7 July) on the dry matter (DM) yield, chemical composition, ensilage characteristics, physical fibrous architecture and engineering properties of five common grass species (perennial ryegrass (PRG), Italian ryegrass (IRG), cocksfoot, timothy and tall fescue). The effect of hydrothermal conditioning and mechanical pressing on the fractionation of grass silage was also investigated. Herbage grown under the high N treatment and harvested at a later date had a higher DM yield while herbage harvested later had a higher concentration of fibre and had a lower digestibility. Timothy produced the highest herbage digestibility (as well as PRG), despite having the highest total fibre concentration compared to other species. The IRG had the highest WSC concentration and lowest buffering capacity making it the most suitable species for ensiling. Differences in physical fibrous architecture characteristics (i.e. fibre length and width) and engineering properties (i.e. tensile strength) were observed both between grass species and harvest dates. The isolation of a plant fibre can be improved by optimising fractionation treatments (i.e. mechanical pressing). This research characterises a potential biomass feedstock for industrial purposes and thereby directly contributes to stated research priority in the Harvest 2020.



## The effect of stocking rate and calving date on the productivity of spring-calving milk production systems in a no quota scenario

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The objective of this study was 1) to evaluate the impact of SR on the productivity of grass-based milk production systems from the available literature using meta-analytical techniques and 2) to investigate the biological characteristics of Irish milk production systems differing in SR and CD post EU milk quotas. The results of the meta-analysis indicates that although production per cow is reduced, a strong positive relationship exists between SR and milk production per ha. A farm systems experiment was established to investigate the effect of SR and CD on animal performance. Two groups of Holstein-Friesian (HF) dairy cows: early calving (mean CD February 12) and late calving (mean CD February 25) were established and animals within each CD were randomly allocated to one of three SR treatments, low (2.51 cows/ha), medium (2.92 cows/ha) and high (3.28 cows/ha). A total of 138 spring-calving dairy cows, comprised of two strains of HF (North American HF and New Zealand HF), were used during 2009 and 2010, respectively. The SR tested in this study exceed those commercially used in production systems in Ireland and provide an insight to the potential productivity of such systems post EU milk quota removal. Although reducing per animal productivity, body weight and body condition score during lactation, increased SR results in increased milk, fat and protein production per ha. Increased SR increased grazed grass utilisation but had no effect on herbage production while winter feed production was reduced as SR increased. The results also show that earlier calving and increased SR has no effect on overall pregnancy rates. The results illustrate the potential for increased productivity per ha with increased SR, but suggest that increased grass production and utilisation is required to fully harness the benefits of increased SR.



## Grazing management of white clover in grassland

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White clover facilitates atmospheric N fixation and thereby reduces fertiliser requirements for grassland. However, cognisance of correct grazing management is essential for its success. This is particularly relevant for the wet soil conditions that affect grazing management on most Irish farms. This project investigated various aspects of autumn, winter and spring grazing management on the productivity of grass-clover swards under wet soil conditions at Solohead Research Farm, Tipperary. The highest clover content, N fixation and clover stolon mass were all achieved with a six-week grazing interval in autumn, extended winter grazing (until mid-January) and tight (3-4cm) post-grazing height. Applying 100 kg per ha of fertilizer N in spring significantly increased annual herbage production while reducing clover content and N fixation. However, grazing during the winter promoted a high clover content during the following year, higher N fixation and herbage yields similar to where 100 kg per ha of fertilizer N was applied. Trampling damage on wet soils significantly reduced herbage yield by up to 58% and was greatest in early spring. ANCOVA revealed that this was due to the shorter grazing intervals in spring when compared to autumn/winter grazing. Despite the relatively wet soil conditions at Solohead, the clover-based systems produced 65% higher milk solids per ha than the national average with 32% lower input of fertiliser N. The appropriate management of white clover on Irish farms will contribute to the harvest 2020 targets of a 50% increase in milk output, a 40% increase in the value of beef output and a reduction in fertiliser N inputs.





## The prediction of grass dry matter intake for grazing Irish dairy cows

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Grazed grass is the cheapest source of feed available to Irish dairy farmers. Maximising the intake of grass in the dairy cow diet leads to reduced costs and increased profitability. In order to increase the proportion of grass in the diet, farmers must be able to adjust their grazing management daily or weekly. At present Irish dairy farmers do not have accurate daily grass dry matter intake (GDMI) estimates. Thus, farmers make sward and grazing management decisions with no information on GDMI which is a critical factor influencing milk production. A database containing information on GDMI and associated animal, sward and management variables from studies conducted in Moorepark from 1988-2009 was constructed. The database was used to develop simple multiple regression equations for the prediction of GDMI during the grazing season. These equations highlighted the extent to which variables in the database had an influence on GDMI. The prediction accuracy of a more complex model to predict GDMI was then investigated using the database. The model used is a French model called Grazeln. Grazeln predicted the GDMI of Irish dairy cows well using input variables available to farmers, such as herbage allowance, grass quality, animal body condition score and milk production. However, prediction accuracy of Grazeln could be improved specifically for high quality grass in the autumn and also in general by adapting the persistency of the lactation curve to reflect the cow type predominant in Irish grass-based production systems. Adaptation of the Grazeln model will produce a more accurate prediction of GDMI for Irish dairy cows. The adapted model will ultimately help farmers to make more informed decisions on grazing management and supplementation, and as a result will help dairy farmers to achieve the 50% increase in milk production target set out in the Harvest 2020 report.





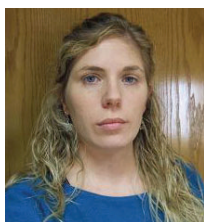
## Analysis of trends in climate and grass growth and evaluation of grass growth models

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Meteorological conditions, as well as management factors, influence grass growth. As a result grass growth is variable, making grass budgeting at farm level challenging. Grass budgeting will have an increasingly important role on farms as milk production is targeted to increase by 50% in the Food Harvest 2020 Report. The objectives of this study were to (1) determine climatic factors having the greatest influence on grass growth; (2) examine trends in meteorological data at Moorepark from 1982 to 2010; and (3) evaluate three grass growth models (Johnson and Thornley (1983), Jouven *et al.* (2006) and Brereton *et al.* (1996)). Relationships between meteorological factors and grass growth were examined using regression analysis; trends in meteorological data were examined using moving averages and regression analysis; and the accuracy of prediction of the models was examined using root mean square error and mean square prediction error. Main results are that (1) meteorological factors having the greatest influence on grass growth between January and March are evapotranspiration and soil temperature at 100 mm; from April to mid-June is soil temperature at 50 mm; from mid-June to August are maximum and minimum daily temperature, evapotranspiration, and sunshine hours; and from September to December are evapotranspiration, minimum and mean daily temperature; (2) soil temperature at 100 mm increased significantly over the period, twice faster than air temperature; and (3) the Johnson and Thornley model over predicted grass growth in all years, with a high primary grass growth peak, while the Jouven and Brereton models predicted grass growth closest to that measured. The models with the greatest potential for grass growth prediction in Ireland, albeit with some modifications, are the Jouven and Brereton models. This research will contribute to the Food Harvest 2020 targets providing information for grass growth prediction allowing more accurate feed budgeting on farm.



## An examination of the genomic control of fertility in cattle

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A significant decline in cow fertility has been reported worldwide. It is well documented that conception rate in cattle is heritable. Furthermore, 70-80% of reproductive wastage is attributed to early embryo loss within the first two weeks of pregnancy. The objectives of this study were: (i) to identify genes and pathways contributing to early embryo survival by comparing gene expression profiles of endometrial tissue on days 7 and 14 of the oestrous cycle between heifers of high or low fertility (HF; LF); (ii) to identify transcriptional mechanisms regulating these differences using *in silico* analysis of the microarray datasets and; (iii) to identify DNA variations affecting fertility via sequencing of 10 genes highlighted as important from the transcriptional profiling results. Microarray analyses revealed 419 and 430 differentially expressed genes (DEG) between HF and LF animals on days 7 and 14, respectively. These DEG were members of biological pathways including cell growth and proliferation, tissue morphology and lipid metabolism. *In silico* analyses of the regulatory mechanisms governing these transcriptional profiles revealed significant enrichment for the forkhead box (FOX) family of transcription factors, known regulators of tissue development. Based on these analyses, DNA sequences of 10 genes with putative roles in fertility—*ALB*, *BMP2*, *COL4A3*, *COL4A4*, *CYP4F2*, *DAP*, *FST*, *GALNT6*, *PCCB* and *SFRP1*—were selected. Candidate sequencing using DNA from 150 Holstein-Friesian bulls (75 displaying high genetic merit for calving interval [HCIV] and 75 with low genetic merit [LCIV]) uncovered 366 DNA sequence variations. In addition, more than 50% of the gene coding variants identified could potentially alter protein structure and function. In summary, this study has identified genes, pathways, transcriptional mechanisms and potential DNA variations regulating bovine fertility that may serve as genetic markers for breeding programmes designed to increase reproductive efficiency.



## Effect of stage of cycle and progesterone on the bovine uterine proteome and differences from plasma

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The majority of bovine embryonic mortality occurs within the first 16 days after fertilisation and is due to a variety of factors. For most of this time the embryo resides free-floating in uterine fluid on which it depends for normal growth and development. Characterisation of the uterine proteome and how it changes during this critical period will broaden our understanding of early embryo loss and in addition improve strategies designed to reduce this loss. The objectives of this study were to (i) develop a protocol for the immunodepletion of high abundance proteins from bovine plasma, (ii) identify uterine-predominant proteins i.e., those present at a higher concentration in the uterus than in blood plasma and (iii) determine the effect of stage of cycle and concentration of systemic progesterone on uterine protein composition. An efficient and reproducible protocol for immunodepletion was developed and was effective in the depletion of >97% albumin and >92% of IgG from bovine plasma. Analysis of immunodepleted blood plasma (n=4) and uterine flushings (n=6) on day 7 of the oestrous cycle identified 35 proteins which were up 11.3-fold higher in uterine flushings than plasma. A comparison of the uterine proteome on days 7 (n=10) and 15 (n=10) of the cycle resulted in the identification of 29 proteins which had up to 6.1-fold difference in expression. No effect of systemic progesterone or side of uterus was found. This study confirms that the uterine proteome is dynamic, differs from plasma and is markedly affected by stage of cycle consistent with the changing demands of the developing embryo. Overall, it is hoped that the results of this study, will help elucidate the biological mechanisms surrounding early embryo loss, optimise efficiency and enable the application of new and emerging genomic technologies that will contribute to a more profitable dairy and beef sector.



## Effect of compensatory growth potential on the transcriptome profile of *m. longissimus dorsi* in steers

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Compensatory growth is the ability of an animal to undergo accelerated growth after a period of restricted feeding. This study aimed to examine the transcriptional regulation of key genes and pathways controlling muscle growth during feed restriction and compensatory growth in Aberdeen Angus × Holstein-Friesian (n = 12) steers. During a differential feeding period, steers were offered either a high energy control diet (n = 6) or an energy restricted diet (n = 6). After 99 days, all animals were then offered the high energy diet. During the realimentation period, live weight gains were 1.74 kg/day vs 1.26 kg/day for previously restricted and unrestricted steers, respectively. Additionally, 32 days into the realimentation period, there was no difference in ultrasonically scanned *M. longissimus dorsi* depths between treatments. Biopsies of this tissue were harvested at the end of the differential feeding period and again 32 days into the realimentation period. RNA-seq, a highly sensitive approach to transcriptome sequencing, was used to conduct the transcriptional sequencing analysis. During the differential feeding period, gene pathways relating to lipid metabolism were significantly different between the two treatments and consistent with plasma leptin concentrations and ultrasonically scanned fat depth data. During the realimentation period, when previously restricted steers were experiencing compensatory growth, the TGF- $\beta$ 1 gene involved in the TGF- $\beta$  signalling pathway, a negative regulator of growth, was down-regulated in expression. The results obtained from this study offer a new and exciting insight into key regulatory genes and pathways controlling compensatory growth in skeletal muscle of cattle which following appropriate validation may be incorporated into genomically assisted selection strategies for beef cattle.



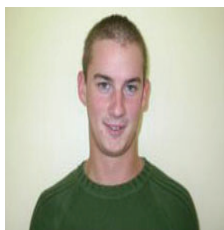
## Examination of the physiological and molecular response of the bovine leukocyte to weaning stress in beef calves

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Weaning has been documented to cause a physiological stress response in beef calves, altering blood metabolites, leukocyte cellular distribution patterns, acute phase proteins and hormones of the neuroendocrine system. Weaning is a multifaceted stressor and may involve numerous husbandry practices, including the abrupt separation of the calf from its dam, a nutritional adjustment to a non-milk diet and social reorganisation and, additionally, is often associated with other husbandry practices, such as housing. There is limited data concerning the molecular response to weaning stress in beef calves. Therefore, the aim of this PhD project was to fully elucidate the bovine leukocyte response to weaning, allowing an accurate assessment of the immune state during the stress response. This was accomplished by carrying out three studies. In the first, the physiological response to either housing or weaning at housing was examined, the results of which indicated that cattle response to a stressor with an inflammatory response mediated by cytokines. The second study examined the difference between calves weaned next to the dam and those weaned away from the dam on gene expression of a select panel of molecular biomarkers. In the final study, calves were either housed or weaned at housing and RNA-seq analysis was carried out to assess the leukocyte response on a transcriptome-wide basis. The combined results of these three studies demonstrate that weaning results in a somewhat inflammatory response that may initially enhance the immune response to pathogens. This work has aided in our understanding of the effect of the stress response to weaning on the immune system and may aid in future studies aimed at reducing the prevalence of bovine respiratory disease challenge in weaned beef calves.



## Analysis of total methanogens in rumen fluid using Qpcr and Gc/Ms

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Archaeol is a lipid ubiquitous in the membranes of Archaea, including methanogens, and is a potential methanogen biomarker. The analysis of archaeol is performed by gas chromatography-mass spectrometry (GC-MS) after the extraction, fractionation and derivatisation of the total lipid extract. Twelve rumen fluid samples were analysed for archaeol, with the same samples analysed for total methanogen populations and *Methanobrevibacter ruminantium* using quantitative real-time PCR (qPCR). Archaeol and qPCR results were compared using regression analysis. There were significant relationships between archaeol concentration and total methanogen numbers estimated using the qMet ( $P = 0.011$ ) and *mcrA* primers ( $P = 0.073$ ), and between archaeol and *M. ruminantium* ( $P = 0.002$ ). Variations in the relationships were present for total methanogens, which may be due to difficulties in the analyses, variation in the archaeol concentration of Archaea, gene and genome copy numbers and the presence of non-methanogenic Archaea. The highly significant relationship between archaeol and *M. ruminantium* may be due to its dominance within the rumen microbiome. This research will contribute to the 2020 Food Harvest Vision by providing an alternative method for the study of methanogens, and therefore contributing to the study of methane mitigation in agriculture.



## Evaluation of protocols to synchronise oestrus and ovulation in seasonal calving dairy production systems

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In seasonal calving dairy production systems, maximising the proportion of the herd that successfully establish pregnancy in the first six weeks after mating start date (MSD) is a prerequisite for a compact calving period in the following spring. Our objective was to examine the effect of whole-herd intervention with protocols to synchronise oestrus or ovulation on herd reproductive performance in seasonal-calving dairy cows. In addition, understanding the physiological mechanisms responsible for differences in fertility performance between different synchronisation protocols was a key objective. Ovulation synchronisation protocols, which facilitated timed artificial insemination (TAI), were associated with increased likelihood of earlier conception after MSD due to higher submission rates, shorter intervals from MSD to conception, and a higher proportion of animals successfully establishing pregnancy during the first 42 days after MSD. Circulating concentrations of progesterone (P4) remained elevated for the entire period of ovulatory follicle growth for animals treated with CIDR-based protocols. Ovulation induction using GnRH in TAI protocols caused a rapid decline in circulating concentrations of oestradiol (E2), resulting in a narrow range in time to ovulation. Use of a conventional CIDR-based oestrous synchronisation protocol resulted in a longer period of ovulatory follicle growth, increased final ovulatory follicle size, greater circulating concentrations of E2, and greater variation in the time of ovulation. No differences in postovulatory concentrations of P4 or luteal volume were observed. Presence of a corpus luteum, higher body condition score, and greater days in milk at the onset of synchronisation were associated with increased likelihood of synchronisation, submission for insemination and conception at first service. The Food Harvest 2020 report has targeted increased productivity and efficiency within the Irish Dairy Industry. These findings will contribute to that Vision by providing new technologies to dairy farmers to concentrate herd calving pattern, thus enhancing productivity and economic efficiency on Irish dairy farms.





## Health of farmers in Ireland

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This paper reports on the occupational health problems among Irish farmers with a particular focus on musculoskeletal disorders. A questionnaire survey, using quota sampling, to achieve 100 farmers from each of the six main National Farm Survey enterprise systems (n=600) was conducted. Of the 600 farmers, 61% (n=363) had experienced a significant health problem in the previous year. The most common health problems reported were musculoskeletal disorders 54% (n=325), respiratory problems 4% (n=26), digestive problems 3% (n=20), diseases of the ear 3% (n=18), and mental health problems 3% (n=16). Musculoskeletal disorders (n=325) which included back (37%), neck/shoulder (25%), hand/wrist/elbow (10%), knee (9%), ankle/foot (9%), and hip (8%) disorders were by far the most frequent health problem reported. Lifetime, annual and point prevalence of low back pain were 46% (n=227), 24% (n=146) and 13% (n=78), respectively. Of those with a lifetime history of low back pain, 35% attributed their low back pain to a specific farm injury and 36% to repeated activities, with over 50% of farmers attributing onset to lifting. Of the low back pain respondents, 36% reported needing help to carry out some of their farm tasks, while 54% reported having to change work habits due to their low back pain. A majority (80%) of farmers considered their health to be 'very good' or 'good' where excellent, fair and poor were 17%, 2% and 1%, respectively. However, in the previous year 73% (n=427) of farmers had visited a health professional, most commonly a medical doctor. The findings of this research should facilitate the development of targeted health promotion strategies for farmers. Such investment in human capital is vital in realising the Food Harvest 2020 Vision, as good farmer health is essential in ensuring farmers' competitiveness in the international market place.





## Agricultural pests and climate change: a modelling approach for Ireland

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While a number of studies have attempted to assess the impact of climate change on crop production both nationally and internationally, the relationship between climate and agricultural pests is an area that has largely been neglected, due primarily to the complexity of the relationship. Our ability to predict future dynamics of agricultural pests under climate change is dependent on an understanding of the underlying mechanisms which regulate the population dynamics of pest species, as well as data availability to support any modelling attempts. Temperature has been demonstrated to be one of the main driving variables of insect pest development and despite the growing concern regarding the potential impacts of future temperature increases on agricultural production, the area of pest impacts on crop production remains largely unknown, particularly in the Irish context. In terms of area, spring barley is the most widely grown cereal in Ireland and yield losses due to pest or disease activity can directly impact gross margins at harvesting. *Sitobion avenae*, the aphid vector of Barley Yellow Dwarf Virus (BYDV), has been reported as the most abundant aphid in March and April sown barley trials in Ireland and yield losses as high as 1.56 t/ha have been recorded (0.86 t/ha attributed to direct aphid feeding and 0.73 t/ha to BYDV). This research utilises a mechanistic modelling approach using available suction trap data to make preliminary assessments of the potential changes in aphid population dynamics and subsequent BYDV levels under future climate change scenarios for Ireland. The research will directly contribute to the 2020 Food Harvest vision by highlighting adaptation options in the crop production sector in response to new EU pesticide regulations.



## Spatial modelling of agricultural greenhouse gas emissions and effects of the 2020 Food Harvest

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Ireland's targets for the reduction of Greenhouse Gas Emissions (GHGs) reflect the current level of agreement on climate change policy at a national, European and global level. However, current policy typically centres on the achievement of aggregate national targets with little focus on the potentially disparate spatial effects. The consequence of this is to miss opportunities to design more tailored mitigation strategies taking into account regional variations. The model draws on a socio-economic assessment spatial micro-simulation model developed by the Rural Economy Development Programme (Teagasc) to create a farm level baseline model of GHG emissions. Results for a spatially disaggregated model of Irish agricultural greenhouse gas emissions disaggregated to Electoral District level are reported. The potential spatial effects of the 2020 Food Harvest targets on 2020 agricultural emissions are reported drawing on the agricultural output predictions from the FAPRI-Ireland model. The development of a baseline spatial model is necessary in order to assess the likely effectiveness of future mitigation and adaptation strategies. This will involve the investigation of options and their likely adoption given the adaptive capacity of current regimes and the significance of the presence of barriers to change/entry, be they economic, information driven or institutional. With a substantial share of national emissions currently attributed to the agri-sector, the design and assessment of agri-mitigation and adaptation options will be required in order to ensure that emissions targets and the 2020 Food Harvest targets are complementary.



## Upgrading performance of Irish dairy product exports

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In 2010, Irish dairy product exports were valued at €2.3bn, accounting for 29% of all agri-food exports (Department of Agriculture Fisheries and Food, 2011). Irish exports go to over 200 destinations worldwide. Presently, the market outlook for the sector is positive. A focus on innovation in dairy products is key to delivering on this opportunity. However, engaging in innovation may not be enough to increase or sustain market position if rate of innovation is lower than competitors. This process of how fast a sector is innovating compared to competitors is referred to as upgrading. In 2010, the strongest performing export dairy categories in terms of value were butter, cheese and food preparations for infant use. Their combined value accounted for 78% of total dairy produce exports. The methodology used to assess product and process upgrading and downgrading is based on the framework developed in Kaplinsky and Readman (2005). Using trade data, changes in import unit price and market share are analysed to reveal product upgrading performance between 2000 and 2010. The analysis looks at changes over time and uses the highest level of disaggregation available in global trade statistics. Relative innovation performance of each product type is identified by means of four possible outcomes - failed product upgrading, product upgrading, product and process downgrading and process competition. The results conclude that there are good and bad innovation performers across categories of Irish dairy products. Ireland's most valuable dairy export, food preparations for infant use has experienced failed product upgrading in two of its largest markets, Asia and Europe between 2000 and 2010. Various butter and cheese product categories record different upgrading performance. For example, cheddar (not grated, powdered or for processing), Ireland's most valuable cheese export experienced process competitiveness. The comparative upgrading performance of dairy products in global markets is valuable for stakeholders across dairy product value chains. This work provides the opportunity to highlight the importance of a focus on relative innovation to enhance income growth and achieve targets set out in Food Harvest 2020.

