001

Alternative feed ingredients – Overview from an end-users perspective with specific reference to bioethanol co-products

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Embracing new alternative feed ingredients in feed formulation is an essential component of future sustainable intensification of feed production. Furthermore identification of alternative raw materials is a means of increasing resilience in the supply chain in the face of increased risk due to the pending unreliability of climate. Appreciating the gap between the identification of an alternative raw material and the approval of this material in a commercial raw material data matrix is imperative to a successful adoption of the new product. In this presentation the presumption is made that any new raw material is listed in a national list of permitted raw materials. It is not the intention to review regulatory issues. Unfortunately however it does not follow that a material listed for use in feed in a national list is then used routinely in feed formulation. How a raw material is used can be viewed in terms of either animal factors and/or logistic and commercial factors. The presence of anti-nutritional factors can have a significant impact on how a new raw material is utilised. Independently it can influence the species of animal, the age and stage of growth of an animal and the quantity of material used in the formulation. Furthermore traditional nutrient components such as phytate and fibre are now recognised as anti-nutritional factors in for example poultry and aqua nutrition respectively. Means of eliminating or negating the impact of anti-nutritional factors is an important aspect of developing an alternative raw material. Least cost ration formulation techniques have been developed to design diets that meet specific requirements from readily available ingredients at the lowest cost. Invariably a new ingredient will only be used in a diet formulation as long as it will reduce the cost of the diet unless the ingredient can provide additional benefits not taken into account in the least cost formulation. This would be the case when a product delivers health benefits in addition to its nutrient specification. A new raw material may satisfy all major nutrient requirements but in addition a number of logistic issues require to be fulfilled. The product must be produced in a quantity to support a supply chain and to be incorporated in one or a number of feed formulations to merit a feed mill allocating space in the mill for storage. Finally the benefit of using the new alternative feed material must create sufficient value to be attractive to the producer of the product, all the active members in the supply chain and finally the livestock producer who will use the product. Biorefineries processing large quantities of grain for biofuels are prime examples of processes capable of producing novel raw materials. Currently the biorefinery process tends to produce a single medium protein, high fibre co-product, distillers dried grains and solubles (DDGS). It is questionable whether this process is viable when large quantities of energy are required to dry the product for feeding to ruminants, the segment of livestock production with the poorest feed conversion efficiency. With the expansion of the biofuels industry research has been undertaken to create new feed co-products which when used in feed formulation are more valuable than DDGS. Essentially the research has focused on separating the components of DDGS to produce separate, yeast, oil, protein and fibre fractions. The individual components have value in monogastric and aqua nutrition where the lower feed conversion ratio of the monogastric is a more efficient use of the energy used for drying the product. The scale of the biorefinery process is such that the new bioprocessing co-products are able to satisfy the majority of the constraints listed above and therefore to embody the features of new feed raw materials produced in a sustainably intensive manner.

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002

Insect protein for animal feed?

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The European Parliament has recently adopted an 'own initiative' resolution with respect to the EU's protein deficit. This resolution tabled measures that seek to end Europe's dependence on imported protein crops for animal feed and improve on our currently less than 30% self-sufficiency in protein supply. A significant proportion of this protein is imported soya (whether as beans or meal), a crop that has historically been implicated in sustainability issues in Latin America. The UK currently imports approximately 2.5 million tonnes of soya per year, the majority of which is destined for animal feed, principally that for pigs and poultry. The EU resolution indicates that urgent action is needed to replace much of the imported protein crops with alternative European sources.

Insects grown specifically with the intention of being fed to domestic animals/fish, has been the subject of evaluations for several decades (e.g. Bondary and Sheppard, 1987; Newton *et al*., 2005; Hem *et al*., 2008) but has never reached a stage that has led to any significant replacement of traditional plant/fish-based protein used for livestock production with insect based protein. This is largely due to systems being explored and developed on a local, isolated level with no integration or co-ordinated development of know-how to enable adoption at the national and international levels. Importantly, much of the work to date has made little or no attempt to process the produced insect material or to assess safety, social and acceptability issues. Land availability issues and the rising costs of plant and fish derived protein provides a critical platform for the development of a co-ordinated approach to fully evaluate insects as an alternative source of protein for animal feed. The global adoption of insect production systems would reduce reliance upon non-sustainable protein sources and hence increase protein availability for humans without requiring a reduction in meat consumption. Whilst it is widely acknowledged that reduced meat eating is highly desirable, in the absence of social and political will throughout much of the world, alternate strategies designed to ameliorate the detrimental impacts of livestock are required. As a result, the work presented here aims to place the primary research emphasis on evaluating the potential for the development of large-scale insect production methodologies for the production of material for the **safe** incorporation into animal feeds.

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003

Evaluation of novel feed ingredients for aquaculture to meet fish production, health and sustainability in the 21st Century

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Introduction

Aquaculture is recognised as the fastest growing sector of the agri-business industry amounting to a total world production exceeding 70 Million metric tonnes ($120 billion) per annum and contributing to over 47% of annual sea food production (FAO, 2012). Fish (66 million tonnes) has now overtaken beef (63 million tonnes) in the global consumption of meat products (Earth Policy Institute, 2013).

Expansion is particularly concentrated in SE Asia with China being the largest producer of farmed fish. Total UK farmed fish tonnage amounted to 169,800 tonnes in 2012 with 90% contributed by farmed Scottish salmon.
The major constraint towards the production of fish and crustacean is the source of high quality protein and energy in the diet and aquafeeds have been reliant on expensive marine proteins derived from pelagic fish species in the form of fish meal and fish oils. This has been the most criticised aspect associated with the aquaculture industry from both an ethical and environmental view with conflicting accounts of whether this can remain sustainable or viable for the production of carnivorous fish such as salmon.

This quest has prompted considerable progress in the inclusion of various categories of protein concentrates and oil sources. These have ranged from mainly plant by products e.g. soya bean meals, rapeseed meal, lupins, corn gluten and wheat meals, as well as pulses, cereal and grain based proteins. The use of processed animal proteins (PAPS) has now been approved for inclusion in aqua feed formulations within Europe since June 2013 after a period of restriction over the last 20 years after the incidence of BSE.

More recently attention has focused on single cell proteins (SCP's) that range from microbial derived proteins to yeasts and algae that can provide a significant replacement for fishmeal as well as being feed additives and supplements with various functional properties that can enhance the health and production of farmed fish

Alternative protein sources already provide from one- to two-thirds of the dietary protein in commercial feed that is supplied for the cultivation of fish. Soy-based protein can provide up to 40 percent of dietary protein in fish feed without significantly affecting the feed conversion ratio, the protein efficiency ratio, or the net protein utilization—in essence, without impacting the health or nutritional value of the fish. In the laboratory, 100 percent replacement of fishmeal protein in feed has been achieved, but it is not yet considered cost effective for commercial-scale production. The inclusion of novel proteins can make a significant contribution to the partial replacement of fish meal in compound diets for farmed fish.

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004

Emerging and re-emerging diseases of livestock in Europe

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In the last 15 years, several severe outbreak scenarios of virus diseases of livestock could be observed in Europe. Well known transboundary pathogens re-emerged in the last years, e.g. foot-and-mouth disease virus (FMDV) in UK in 2001 and classical swine fever virus (CSFV) in Germany in 2006, and also arthropod born (ARBO) viruses like Bluetongue virus serotype 8 (BTV-8) and Schmallenberg virus (SBV) emerged in the centre of Europe and spread to large parts of Europe including the United Kingdom. Furthermore, the zoonotic highly pathogenic avian influenza A virus (HPAIV) of subtype H5N1 was transmitted to Europe by migratory wild birds and induced several outbreaks in poultry which could be fortunately controlled in an early phase.

Nevertheless, the different scenarios demonstrate that further outbreaks have to be expected, and very recently African swine fever virus (ASFV) reached the European Union with outbreaks in wild boar in both Lithuania and Poland. The discussed factors for the occurrence of those very different emerging and re-emerging diseases in the last years, and with some focus on ARBO viruses and involvement of wild life animals, are multifarious. Globalization and global trade, travelling and tourism, but also changes in environmental factors including the climate are some of the suspected key players.
For all scenarios, awareness and early detection is a key issue of utmost importance for the implementation of control measures. Modern molecular routine diagnostics, especially real-time PCR, allows today the very fast detection and identification of the known emerging and re-emerging viruses. Furthermore, novel diagnostic technologies like metagenomics using next generation sequencing allow even the detection and full-length genome analysis of before unknown pathogens. However, the right samples have to be analysed as soon as possible and differential diagnostic approaches should be used to exclude in as many cases as possible those notifiable transboundary diseases.
Here, different introduction scenarios, diagnostic procedures as well as control possibilities for selected examples will be presented and discussed. The Schmallenberg virus emergence will be used as an example for the detection of a novel pathogen including the possible consequences and the “lessons learned”.

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005

Emerging and re-emerging arboviral diseases in Europe: The emergence and control of Bluetongue in Northern Europe

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Implications The movement and incidence of arboviral diseases of livestock and humans have recently increased, both in Europe and in other parts of the world. These events, which have been linked to increased trade, travel, and climate change, have demonstrated the importance of rapid, sensitive and specific diagnostic systems, for the development, implementation and targeting of effective control strategies that have included restriction of animal movements and vaccination campaigns.

Introduction Outbreaks of disease in European ruminant populations have been caused by multiple strains/serotypes of bluetongue virus (BTV) and more recently by Schmallenberg virus (SBV). Several other important arboviruses, including African Swine fever virus, African horse sickness virus and Epizootic haemorrhagic disease virus have extended their range, with increased activity near the borders of Europe. Zoonotic and human arboviral diseases have also occurred in the Mediterranean region (e.g. caused by West Nile virus, Nairobi sheep disease virus, Chikungunya virus and Toscana virus) demonstrating an increasing risk posed by these important viral pathogens, to both human and animal health.

On a global scale Bluetongue (BT) is one of the economically most important diseases of ruminants. It is transmitted by certain species of biting midge (*Culicoides spp*.) and can cause high levels of morbidity and mortality particularly in naive populations of sheep and certain species of deer. The bluetongue virus has a genome composed of ten segments of linear dsRNA, packaged within a three layered icosahedral protein capsid. The genome encodes 11 distinct proteins, seven of which are structural components of the capsid. Multiple strains of the 26 different serotypes of BTV were obtained from the reference collection (http://www.reoviridae.org/dsRNA_virus_proteins/ReoID/virus-nos-by-country.htm) which is held at the Pirbright Institute. Sequencing studies of genes encoding the outer capsid proteins of these viruses, confirmed that virus-serotype is controlled primarily by outer-capsid protein VP2 (encoded by genome segment 2 (Seg-2)). This has allowed development of conventional and real-time RT-PCR assays to detect and type the virus within hours of sample receipt, representing a massive improvement over more conventional serological assays that could take weeks to complete. Virus serotype is important as the protection generated by BTV vaccines is type-specific.

The first outbreak of bluetongue ever recorded in northern Europe started during 2006, in the Maastricht region of the Netherlands and Belgium. The outbreak affected only a small number of animals that year but successfully over-wintered, re-emerging with massively increased severity in 2007, killing many thousands of animals and spreading across most of Europe. Work by the Met Office indicated that infected midges were blown across the Channel to the UK on the 4th and 5th of August 2007, leading to detection of the first cases of bluetongue in the UK a month later.

The virus was identified as BTV serotype 8, using the novel RT-PCR based diagnostic assays and sequencing of Seg-2. A new inactivated vaccine was rapidly developed by the major pharmaceutical companies (Intervet (MSD), Pfizer (Zoetis) and Merial) and deployed in the UK early in 2008, prior to re-emergence of the adult midge vectors. The Joint Action against Bluetongue (JAB) vaccination-campaign (led by Defra) was very successful, achieving over 80% coverage in affected regions and as a result the UK was the only country in Europe to fully control the disease that year. In contrast massive further spread of the disease and losses occurred in France and Germany. Subsequent vaccination campaigns across Europe in 2008-9 eradicated BTV-8 from the entire region, representing a major success for veterinary medicine.

The results of sequencing and PCR based molecular epidemiology studies, indicating the origins and movements of different BTV strains in Europe, will be presented and briefly discussed. As well as different serotypes, BTV also exists as multiple geographic variants (topotypes) around the world, allowing the origins of individual strains and even individual genome-segments to be identified. Using full-genome sequencing and phylogenetic analyses it becomes clear that that there is a high frequency of BTV genome segment exchange (reassortment) in the field, leading to the emergence and of novel virus strains each year. Some of the reassortant strains that have appeared in Europe, have altered virulence characteristics and represent further possible threats to the health of ruminant populations across the region.

The BTV-8 strain that arrived in Northern Europe in 2006 is most closely related to an isolate from Nigeria in 1982. Schmallenberg virus also appears likely to have a sub-Saharan origin. However, it is not clear how these viruses arrived in northern Europe, although these events indicate there is a continuing risk that other important arboviral diseases will arrive and spread to affect both human and animal health. This process may be further influenced by continuing climate change, as well as changes in travel and trade. There is clearly a need for further enhancement of surveillance technologies, as well as better vaccines and antiviral agents to combat these diseases.

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006

Modelling climate-driven changes in the seasonality of *Fasciola hepatica*

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Implications Over the past 10 years, disease related to infections with the parasite *Fasciola hepatica* appears to have made a step change with farmers up and down the UK recording heavy fasciolosis-associated losses. A validated mathematical model of *Fasciola* abundance will be able to identify risk periods, thereby guiding the timing of the application of worm drenches, as well as facilitate explorations of effective future control options.

Introduction *Fasciola hepatica* is a zoonotic trematode parasite mainly of clinical and economic importance in farmed ruminants. In recent years, the UK-recorded number of outbreaks of acute and chronic fasciolosis in cattle and sheep has risen at an alarming rate (van Dijk *et al* 2010). Apart from increases in overall abundance, there are early indications that patterns in the seasonal abundance of the parasite are changing. Moreover, the liver fluke, traditionally mainly associated with the wetter western parts of the UK, now appears to also fare well in dryer eastern parts, such as East Anglia and South East Scotland. Climate change is likely to play a role in these changes but it is currently not quantified to which extent. We also do not know what future changes to expect and how to adapt current fluke control strategies, which are already under strain from emerging anthelmintic resistance. The Ollerenshaw Index, still used to as a UK fluke forecasting system, was developed in the Fifties (Ollerenshaw et al., 1959) Up to now, it has worked very well but, as it takes into account climatic conditions of certain months of the year only, its accuracy is likely to diminish as our climate changes. For the same reasons, the Index is unlikely to be applicable to other parts of Europe. Moreover, while predicting the within-year severity of disease, it provides no guidance on when pastures become dangerous exactly. We develop a mathematical model of the ecology of the free-living stages of *Fasciola hepatica*, aiming to predict periods of risk under current and future climate scenarios and to explore control strategy adaptations.

Material and methods Our differential equation model of the effects of temperature on the development and mortality rates of the free-living stages of *F. hepatica* and its snail intermediate host, *Galba truncatula,* was populated from our own experimental work and data in the published literature. Experimental work conducted to populate the model included explorations of the over-winter survival of *Fasciola hepatica* eggs at pasture. Unembryonated eggs were kept at freezing temperatures between -8 and 4 ˚C for up to 14 days and survival was tested by placing the eggs at 26˚C for 2 weeks and assessing subsequent hatching.

Results No discernible mortality was detected in batches of Fasciola hepatica eggs kept at temperatures down to -2˚C. Mortality rates went up rapidly below -4˚C, with 100% mortality observed in batches of eggs exposed to -8˚C for 48 hours. Eggs frozen after embryonation had started all died. *Fasciola* eggs deposited at pasture in late autumn and winter are predicted to survive all but extremely cold winters. However, eggs which start to develop during late summer/ early autumn will perish. The model produced realistic patterns of metacercarial emergence and survival, which are being validated using historic field data. Initial sensitivity analyses indicate that the effects of climatic conditions on snail abundance at pasture are the most important determinants on overall metacercarial abundance.

Conclusion The complex life cycle of the liver fluke clearly poses a challenge to ecologists and modellers, but it appears that simple temperature-driven model of the free-living stages captures the parasite's seasonality well. The relative importance of the effect of temperature on fluke abundance may be underestimated and will be investigated after incorporation of rainfall-related parameters in the model. A better understanding of the effects of temperature and rainfall on vital rates of the *Galba* lifecycle should be prioritised for future work.

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007

Comparative proteome analysis of nasal secretion from healthy, malignant catarrhal fever (MCF) challenged and vaccinated cattle

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Implications Malignant catarrhal fever (MCF) caused by alcephaline herpesvirus-1 (AlHV-1) is a fatal disease of cattle that is very important to African small-holders. Nasal secretion offers a novel method of quantifying responsiveness to new, developmental vaccines.

Introduction Nasal secretion from animals has potential as a source of biomarkers for bovine disease. In previous work, we have shown that bovine nasal secretion has higher alkaline phosphatase (AP) activity than serum and that AP is synthesized and secreted in the nasal tissue (Ghazali, *et al*., 2012). In the present study, we undertook a comparative proteomic analysis of nasal secretion from naive and vaccinated, MCF challenged cattle. The vaccine under investigation was a passaged, attenuated strain (C500) of A1HV-1. The aim of the study was to compare the level of AP activity and to use Difference Gel Electrophoresis (DiGE) in nasal secretion from naive and vaccinated, challenged animals.

Material and methods Samples of bovine nasal secretion were obtained from MCF immunisation studies carried out at the Moredun Research Institute (Haig, *et al*., 2008). Disease-free and OvHV-2 seronegative male Friesian-Holstein-cross calves between 3 and 5 months of age were randomly assigned to 6 groups according to the following routes of administration of primary:booster:challenge: adjuvant only with intravenous (IV) challenge (n=4); phosphate buffered saline (PBS) only with intranasal (IN) challenge (n=3); intramuscular (IM)/IM/IV (n=3); IM/IM/IN (n=3); IM/IN/IV (n=5); IM/IN/IN (n=4). Vaccination was at d-0 and boosted at d-30, using attenuated C500 strain of AlHV-1 and challenge was at d-70, using virulent C500 strain of AlHV-1. Nasal secretions were collected (Haig *et al*., 2008) from the animals at d-56 and at d-84. The AP activity in nasal secretion pre and post challenge was measured using para-nitrophenyl phosphate (pNPP) enzymatic reaction. The ratio of AP measured at d-84 to d-56 was used as the outcome variable (AP response). AP response data were not normally distributed, so the non-parametric Kruskall-Wallis test was used for univariate analyses and data were also log10-transformed for ANOVA. The protein concentration of nasal secretion in selected group was determined using Bradford assay (Sigma-Chem Co, Poole, UK). DiGE separation was carried out on IPGphor (GE Healthcare) in the first dimension (IEF electrophoresis, pI 4 -7) and using a DALT 12 vertical gel electrophoresis system (GE Healthcare) in the second dimension. Gels were scanned using Typhoon™ 9400 Imager (GE Healthcare) and the protein spots analysed with DeCyder 2-D Differential Analysis Software v7.2 (GE Healthcare). One-way ANOVA was used to select protein spots, which were differentially expressed with P value < 0.05. Selected protein spots were excised and subjected to tryptic in-gel digestion. Mass spectrometric (MS) analysis was performed using a continuous duty cycle of survey MS scan followed by up to ten MS/MS analyses of the most abundant peptides, choosing the most intense multiply charged ions with dynamic exclusion for 120s. MS data was processed using Data Analysis software (Bruker) and the automated Matrix Science Mascot Daemon server (v2.1.06). Analysis of the MS and MS/MS spectral data was performed using SwissProt database version 57.15.

Results Only animals from the IM/IM and IM/IN group survived a lethal challenge with AIHV-1 given by the IN route. The challenge route did not have a significant effect on AP response (P=0.43), so data were combined within groups of IM/IM and IM/IN. Primary and booster immunisation treatments had significantly different effects on AP activity in nasal secretion, being highest in the IM/IM treatments ($P<0.05$). Median responses (ratio of AP at d-70:d-56) were 0.69, 0.72, 0.77 and 2.4 for PBS, Adjuvant, IM/IN, IM/IM treatments respectively. Following trypsin digest and peptide mass fingerprinting, 13 major protein spots were identified. 23 protein spots were significantly differentially expressed as a result of IN challenge and IM vaccination route. Most of the plasma protein such as albumin, apolipoprotein A1 and fibrinogen beta chain were decreased in challenged animals. Immunoglobulins, odorant binding protein and serpin were increased in both disease and protected group. Proteins involve in antimicrobial and innate immunity increased in protected group but decreased in disease group.

Conclusion AP response to vaccination and challenge differed significantly with treatment, being strongest after two IM treatments, suggesting an adaptive immune component in the regulation of nasal AP secretion. We also demonstrated for the first time a comparative proteome analysis of nasal secretion from healthy, disease and vaccinated cattle and have identified change in protein expression in response to MCF and following vaccine protection where 23 protein spots were differentially expressed as a result of IN challenge and IM vaccination route.

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008

Retrospective profiling of the 2012 Schmallenberg virus incursion into Ireland

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Implications Identifying the route and timing of Schmallenberg virus (SBV) entry is critical to making predictions, national planning and other preparations for future emerging vector-borne diseases.

Introduction Schmallenberg virus (SBV) is a recently emerged pathogen of ruminants, new to Europe in 2011 (Hoffman *et al*, 2012). Like Bluetongue, it is a vector-borne disease, thought to be transmitted by biting insects such as midges and mosquitoes. It causes a transient disease (diarrhoea, milk drop) in adult animals but may lead to abortion and birth defects in pregnant females. Ireland lost its freedom from SBV in October 2012, one of the last EU countries to do so. This study described here uses archived material from an eradication programme to best identify retrospectively the point-of-entry and time-of-entry of SBV into Ireland. Retrospective sourcing of samples requires some creativity to match material availability with expected usefulness and relevance.

Material and methods The Irish BVD eradication programme commenced in January 2012, and generates blood samples for the laboratory confirmation of BVD virus status. All samples are archived and provide an excellent resource in terms of geographical and temporal coverage. All available (570) archived samples from six counties – Carlow, Cork, Kilkenny, Waterford, Wexford and Wicklow - were used in this study. These counties were selected as being the first to have herds which showed SBV clinical disease in late 2012/early 2013. In Ireland, digitized data are available for all farms, in terms of the Irish National Grid map co-ordinates for the largest fragment of land within that farm. This data is managed by the Department of Agriculture, Fisheries and Food and permits the precise mapping of the location of all herds.
All samples had been collected between 1st March 2012 and 31st December 2012 and comprised of 287 calves and 279 dams (and four other adult animals).Samples were tested using ID Screen Schmallenberg virus Competition Multi-species ELISA, according to the manufacturer's instructions without modification. Absorbances were read in a Tecan Sunrise™ microplate reader (TECAN Austria GmBH, Salzburg, Austria). All results reported as sample/negative control (S/N%), with samples reporting S/N% <=40 deemed positive.

Results One hundred and twenty-six (22.1%) samples were found to be SBV antibody positive. Overall, the proportion of SBV seropositive calves and dams is strikingly similar: 22.6% and 21.8%, respectively. The first sample detected as SBV antibody positive was on 5th September 2012 (Week 36) in an adult female from Co. Kilkenny. In total, counties: Carlow, Cork, Kilkenny, Waterford, Wexford and Wicklow recorded 39, 3, 9, 44, 31and 8 seropositive animals, respectively over the period tested. This data is presented in a set of sequential maps illustrating the virus's geographical progression during the period tested.

Conclusions All indications suggest that SBV first circulated in the south-eastern portion of Ireland, in an area about 300km long and within 50km of the coast. This result pattern is consistent with trans-marine transfer of infected vector species.
Based on this studies results and the accepted 14 day post-infection seroconversion period, the most likely latest incursion date for SBV into Ireland was Week 34 (week beginning August 20th 2012). Using this information, it should be possible to cross-reference contemporary wind direction and speed data from around that period and conclude if the risk of introduction could have been predicted.
For this dataset, there is no detectable age-related difference in seroprevalence pattern among cattle which likely reflects the biting patterns of the vector involved.

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009

Improving feed efficiency in dairy production systems – challenges and possibilities

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Implications The best means to assess and improve feed efficiency among dairy cattle remains an active area of scientific investigation, particularly for lactating cows. This review summarizes the latest research on the topic.

Introduction Improving production efficiency has always been a goal of animal agriculture to ensure an abundant food and fibre supply, and to maintain producer profitability. In recent decades, the concept of sustainable agriculture emerged, which includes the additional goals of safeguarding natural resources, promoting a clean environment, and improving both producer and animal well-being. Within the dairy cattle sector, enormous gains have been made in farm operations, herd management, and animal nutrition, health, and genetics since the commercialization of milk production in the late 19th century to increase efficiency of production. Despite this progress, advances in dairy science must continue to meet the food demands of a growing world population, to promote environmental stewardship, and to sustain producer profitability. One of the greatest threats to producer profitability is the cost of feeding animals, which accounts for 40 to 60 percent of total production costs (Makkar and Beever, 2013;USDA-ERS, 2013). Opportunities exist to reduce feed costs and environmental impacts associated with dairy production by identifying and maintaining animals exhibiting superior feed efficiency within the herd that do not exhibit associated declines in milk production, fertility, or health. However, tools are needed to assist producers in identifying these animals. Although means to improve feed efficiency among poultry, swine, and beef cattle are well investigated, research focusing specifically on feed efficiency of dairy cattle is less prevalent and only recently has appeared consistently in the scientific literature (Berry, 2009). As a result, much debate persists on how best to evaluate feed efficiency in lactating cows, and how to make genetic progress in efficiency-related traits among dairy cattle populations without negatively affecting other traits, such as energy balance (Pryce *et al*., 2014). Therefore, this review focuses on the opportunities and challenges for improving feed efficiency in dairy herds, including the most common approaches for its estimation and some of their limitations, potential implications of genetic selection for greater efficiency, and means to evaluate efficiency in commercial dairy operations. Some suggestions for future research directions, particularly related to understanding the physiological basis for variation in feed efficiency among animals, are discussed.

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010

Improvement of feed efficiency in pigs and poultry: current knowledge and future challenges

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Feed efficiency (FE) in livestock represents the efficiency of the production at the animal level. It is usually computed as the ratio of outputs (body weight gain in growing animals) on inputs (feed intake), or its inverse, feed conversion ratio (FCR). It is an integrative trait quantifying gross FE. Combined management, nutrition and genetic strategies have improved FE in the past. Monogastric livestock are usually bred indoors and fed from composite feed: breeding environment (temperature and ventilation, pathogen loads) and energy and nutrient supply have been adjusted to maximise animal performances. Genetic selection essentially has improved FE by increasing lean growth rate in growing animals. In prolific monogastric species, feed efficiency in breeding adults has been essentially studied in laying hens (eg, Bordas and Merat, 1981), as FE of these adults has a lower economic importance compared to FE of growing animals, and furthermore, is difficult to evaluate.

Decreasing FCR for improving FE is generally associated with increased growth rate and leanness (Aggrey *et al*., 2010) and reduced excretion (Saintilan *et al*., 2013). Correlations with reproductive traits are generally low, but excessive leanness and reduced FI impairs female reproduction (Knap, 2009). Residual feed intake (RFI) has been proposed to estimate net FE, i.e. FE independent from production levels. It is usually computed as the difference between recorded FI and FI predicted from production and maintenance requirements of the animal. It represents from 60 to 85% of the variation of FI in growing animals and is moderately to highly correlated with FCR (Saintilan *et al*., 2013). Among other traits, RFI covers intake for basal requirements, activity, protein turn over and digestibility. The correlation between RFI and production performance is null when calculated at the phenotypic level, and is also generally reported to be low at the genetic level. It has been shown to have similar unfavourable correlations with meat quality traits as FCR (Gilbert *et al*, 2007). The RFI trait is understood as a buffer compartment for the animals to cope with stress and diseases, so low RFI has been expected to be associated with reduced ability to cope with stress (Knap, 2009). Adverse genetic correlations can be accounted for by including the corresponding traits in the breeding objectives.

Improving FE reduces maintenance requirements and activity and generates a change in energy metabolism and protein turn over. It modifies growth and feed intake dynamics over the growing period, together with energy and nutrient requirements. Digestive efficiency is only marginally improved when selecting for improved FE under high quality feed where energy is not limiting and digestion not challenged. However, the access to high quality feed resources is expected to diminish and studies are needed to improve the animals' use of lower quality feed, thus challenging their digestive capacity (Kyriazakis, 2011). This requires development of phenotyping strategies for digestive efficiency, and defining feeding challenges to reveal the inter-individual variability. Similarly, the efficiency of use of specific nutrients (protein, N, P) is now questioned.

One of the major limitations to improving FE remains availability of suitable phenotypes. Among components of FE, individual FI records are complex and expensive: animals are either raised in individual cages where FI is recorded manually, or, using electronic identification, animals in groups are measured for FI with one or multiple single-place electronic feeders. As competition is a major driver of FI and breeding in individual cages is a welfare issue, recording FI in groups should now be the standard. Complementary, indicator traits or biomarkers of FE and FI are necessary to record data large cohorts in diverse environments. Some blood markers associated with FE or its components, together with genomic markers, have been proposed, but use in selection needs validation. Alternatively, genomic selection, in which a reference population would be phenotyped for FE, could be used. However the economical advantage of genomic selection in monogastric is not demonstrated for all production systems. Finding good predictors might be a more interesting strategy.

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011

Improving feed efficiency of beef cattle: Current state of the art and future challenges

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Implications Feed efficiency is a key trait governing the economic and environmental sustainability of beef cattle production systems, worldwide. Despite this, there is a dearth of information on the biological control of the trait and this has resulted in little genetic progress. This review aims to discuss current knowledge on the biology of feed efficiency in beef cattle, as well as future opportunities for progress.

Introduction Feed is the single greatest variable cost in beef cattle systems, typically accounting for 70 to 80% of total costs of production. Additionally, up to 70% of dietary energy is used for body maintenance while in the region of 75% of ingested nitrogen is excreted and thus, unavailable for productive proposes. Consequently, there is significant worldwide interest in feed efficiency (FE) as a means of improving both the economic but also the environmental sustainability of beef production systems. Despite this, however, when compared with monogastric species, little direct improvement in FE has been achieved to-date (Bottje *et al*., 2009). The concept of residual feed intake (RFI), rather than feed conversion ratio, is becoming the preferred measure of FE across many livestock production enterprises, and in particular for beef cattle (Arthur and Herd, 2012). Animals with low RFI (efficient) eat less than expected based on their weight and growth. We and others have also demonstrated significant genetic variance in the trait and that, genetically, it is not antagonistically associated with desirable growth or carcass traits in growing beef cattle (Crowley *et al*., 2011). The challenge is, therefore to reliably and cost-effectively identify these feed efficient animals and proliferate their genetics through structured animal breeding programmes. However, the main impediment to genetic progress and adoption of selection strategies based on FE is the difficulty and enormous expense of measuring individual animal body weight and feed intake over a period of up to 90 d. Consequently, developing predictive biological markers specifically for improved FE is an attractive alternative to direct measurement on large numbers of animals (Moore *et al*., 2009). Our own and other work to date has also clearly shown that FE is a complex multifaceted trait, under the control of many biological processes (Moore *et al*., 2009; Kelly *et al*., 2010; Lawrence *et al*., 2012). These include inter-animal variation in feeding behaviour, digestion, absorption, metabolism, nutrient partitioning, cellular energetics as well as, potentially, susceptibility to stress. Studies have also shown that feed efficient cattle are likely to have different ruminal microbial profiles (Carberry *et al*., 2014) and emit less methane (Fitzsimons *et al*., 2013) than their inefficient contemporaries. Thus, a multi-facted integrated research approach is required to fundamentally understand the biochemical mechanisms regulating this trait at a cellular level (Bottje and Kong, 2013) and this will not be disentangled using solely genome wide association studies (Moore *et al*., 2009). Additionally, our own data (Kelly *et al*., 2010) and others clearly shows that, although relatively repeatable, ranking of beef cattle for FE offered the same diet is not consistent over time, and this is further exacerbated when diets differing in energy density are fed successively (Duranna *et al*., 2011), as per commercial practice. This strongly indicates the presence of a genotype x environment interaction for the trait. However, the existence of such a phenomenon has not been adequately tested to-date. Despite this, worldwide, breeding values for FE are typically derived from progeny performance based on *ad libitum* access to energy dense diets whereas, in many countries including Ireland and UK, the lifetime gain of most commercial beef cattle is achieved from diets consisting, to a significant extent, of lower energy density feeds such as grazed grass and/or ensiled forages. Thus, any future effort to develop predictive tools for selection of beef cattle with improved FE potential must be reflective of possible confounding contributory factors including stage of development and dietary management regime.

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012

Ewe nutrition – what are the known 'unknowns'

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Introduction Efficient use of feed resources is a key economic driver for sheep enterprises. The right balance between nutritional inputs and output is critical to profitability and is influenced by a myriad of factors. Historically it has been difficult to measure this, and the outcomes of changes in nutrition have been reported using short term factors e.g. lamb birth weights, colostrum quality, scanning result etc. With the advent of EID systems, which enable the rapid, accurate capture of individual data over long periods of time, it is possible to evaluate the impact of improved nutrition on profitability over production cycles and even the lifetime of individual animals. This paper aims to highlight some 'unknowns' in the context not only of gaps in our knowledge, but in the ability we now have to fully evaluate impacts in terms of profit, and this may mean re-prioritising our recommendations. To provide a framework these issues are broken down into short, medium and long term factors.

Short Term

Protein Requirements – ARC standards (1994) are the published levels of MP recommended for sheep. However, a number of papers have suggested that these are too low to allow for maximum udder development and colostrum production. Higher levels are also reported to reduce the impact of the Peri-parturient relaxation of immunity and the subsequent rise in faecal egg output around lambing (Coop and Kyriasakis, 1999) Conversely, (Robinson *et al* 2002) reminds us that MP utilisation increases by 15 % in ewes in late pregnancy.

Undegradable protein sources are expensive and often from less sustainable sources (eg soya bean meal), so what is the cost effective answer? How do we effectively harness advances in available forage varieties?

Trace elements – this remains an area shrouded in mystery and farmers spend £Ms per annum with questionable benefits. Recent work undertaken as part of an Eblex funded project (currently unpublished) confirms that ranges used for deficiency v. productivity are far from clear and the efficacy of methods of supplementation also require more work. Omega 3 and fish oils also require further evaluation in terms of cost effectiveness.

Medium Term

Body Condition (BCS) – the Eblex funded KPI work aims to evaluate the effect of BCS on productivity and profitability not simply at specific points in the production cycle, the effects of which have been well documented, but as a medium/long term process for the individual ewe. For example, we know that follicle maturation is a process that takes 6 months and while improved nutrition at mating (flushing) can reduce follicle attrition it cannot fully negate earlier effects. The mechanism is not fully understood and neither are the implications for flock management in terms of ewe BCS changes the impact of earlier weaning, preference over lambs for grass dry matter post-weaning etc. or indeed the effects over more than one production cycle. Examples from elsewhere of this approach include the 'Lifetime wool' project in Australia where flock management is based entirely on and driven by BCS targets.

Micro-nutrient programming in utero on lamb performance- for example cobalt deficiency in very early pregnancy has been associated with reduced lamb vigour.

Genotype interactions – production systems in the UK are moving towards more forage based systems and there are many unknowns with respect to the suitability of our genetics to meet this challenge. The influx of NZ genetics in recent years is testament to this and may require us to re-evaluate our selection criteria, taking more account of the sources of nutrition and the ewe's ability to perform without expensive supplements, except where the cost effectiveness is assured.

Long Term

It is known that nutrition from conception has an important influence on productivity of the adult ewe (Gunn *et al* 1995) but the impact this has on profitability in our flock systems has not been fully quantified. This is vital if we are to convince sheep farmers that rearing phase targets are essential for female replacements. We need to know which measurable benchmarks result in the most profitable outcome. It is not acceptable for us to push farmers towards smaller ewes simply because they have a lower maintenance requirement. Some farmers see the answer to this as a deliberate strategy to 'stunt' the growth of ewe lambs, a clear example of how a message is misconstrued. Rams that sire breeding females also require investigation in terms of the genotype x environment interactions and the implications for productivity.

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013

Maximising winter grass utilisation on sheep farms: all grass wintering

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Implications All Grass Wintering (AGW) has potential to reduce winter feed costs on UK sheep farms.

Introduction AGW is a rotational grazing system where a wedge of grass is built up in the autumn and fed back to the ewes through the winter using one- to four- day shifts. The theory is that the wedge plus regrowth should provide enough grass for the ewe flock, which avoids the need for supplementary concentrates. EBLEX and SAC Consulting have been working with a group of demonstration farmers to determine the benefits and practicalities of this system in the UK.

Material and methods Following a pilot in Cornwall with 950 New Zealand Romney in-lamb ewes for winter 2011-12, the system was implemented on five farms from Plymouth to Gloucester during winter 2012-13 (~110days from mid-November/early-December). The farmers began to shut up the trial fields from September and the grass cover in all fields was measured with a rising plate meter. A feed budget was calculated for two average winter grass growth scenarios: 5 and 10 kg dry matter (DM)/ha/day for each demonstration farm. Feed demand was estimated at 1.5% of bodyweight pre-scanning and 2% of bodyweight post-scanning. Daily demand is multiplied up to the flock level and divided by the area, this is deducted from the grass cover to estimate the cover at the end of the month. The aim is to have over 1500 kg DM/ha average grass cover at the end of the rotation, those calculated below this figure would not be feasible without reducing stock or feeding conserved forage.

The residual targets were 900 kg DM/ha pre-scanning and 1,200 kg DM/ha post scanning. The field size was calculated based on daily feed demand and plate meter measurements, and seven paddocks were established each week using three strand temporary electric fencing.

Results

Table 1 All Grass Wintering demonstration farm details

AGW flock size (ewes)	Breeds	AGW area (ha)	Initial grass cover (kg DM/ha)	February grass cover (kg DM/ha)	Lambing grass cover (kg DM/ha)	Scan results (%)	Supplements fed (tonnes)		Time fencing (hours/ week)	Time shifting (minutes/ day)
							Forage	Concentrates		
450	Mules, Texel Mules, Dartmoor	39	2658	1300	1200	146	0	0	3.5	10
160	NZ Romney	37	2302	1905	1713	193	3	0	10	20
160	NZ Romneys, Welsh Mules & Suffolk Mules	40	3018	1648	1850	160	8	0	10	20
840	Kent Romneys	140	2239	1464	1000	160	30	17	10	20

Lambing grass cover was short on two of the farms due to the late spring. Feed saving estimated at £15-17/ewe.

Conclusion AGW is feasible on South West England farms with free draining soils but amendments are required to prevent grass shortfalls at lambing. It is key that the system is flexible to cope with extreme winter weather. The next stage of this research will be to determine whether AGW can be successful in areas of low winter grass growth, how it stands up over multiple winters and how labour can be reduced with four day shifts.

Acknowledgements Thanks to all demonstration farmers that implemented all grass wintering on their system.

014

Maximising forage and grassland utilisation through outwintering in-lamb ewes on swedes

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Introduction For several years farmers in New Zealand have been utilising forage swedes to out winter in-lamb ewes. This policy has allowed them to reduce fixed costs as there is no requirement for winter housing, but more importantly it has allowed them to maintain a high stocking rate in the winter period without using concentrates.

New Zealand farmers have used the swede crop to rest their grass pastures in the early spring before putting in- lamb ewes out on to this pasture 3-4 weeks pre-lambing where grass covers are around 1,500kg/ha and grass growth is good.

This system fully utilises two of the cheapest feeds available to sheep farmers at a time of year when feed costs are normally high, with swedes costing about £62/tonne and grass/clover costing £72/tonne utilised dry matter (Forage Choice Costs & Rotation Report, Kingshay; April 2010).

Although there has been much research carried out in New Zealand, very little has occurred in recent years on how our climate might affect the success of the system in the UK. There has also been very little research done on the nutritional requirements of sheep as they move off the swedes and onto grass in the spring period. Many farms in the UK are now using brassica crops and swedes as a way to maximise returns from their sheep enterprise while maintaining a low labour, outside lambing system. However recommendations are very vague for such enterprises and very few costings have been made in comparison to standard housed sheep systems.

Therefore the project aims to give nutritional and financial recommendations to sheep farmers to run a successful outwintering system on swedes with no supplementary feeding.

Material and methods Ewe performance will be monitored on a commercial farm where the current system revolves around an outside April lambing system with the ewes outwintered on swedes from Christmas until early March. A proportion (at least half) of the flock of 400 Lleyn ewes, both single and twin bearing ewes, will be monitored and compared to a conventional inside lambing flock of 140 ewes on the same farm.

Monitoring will include:

- Regular body condition scoring and weighing of the ewes throughout the trial together with birth weight, 8 week weight, 21 week weight and slaughter weights of all lambs on the two different systems.
- Metabolic profiles of ewes on the swede system to include energy (betahydroxybutyrate - BHB) and protein (urea and albumin) status will be taken at 4, and 2 weeks prior to lambing.
- General health of the flock to be recorded daily including mortality, lameness and prolapse.
- Analysis of the swedes crop will include pre-grazing dry matter yield (by weighing swedes in 5 x 1 metre quadrants). Swedes will be analysed on three occasions during the grazing period (mid-December, early February and mid- March) for dry matter, crude protein and sugar content.
- Weekly grass growth measurements by plate metre and calculation of covers from 1st March until the end of the growing season will take place and will be complemented by nutritional analysis of grass on a fortnightly basis (dry matter, crude protein, sugars and predicted ME) until weaning.
- Physical and financial performance of ewes on the swede-based system will be compared with the conventionally managed flock.

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015

The effect of duration of removal from grazing on body weight in sheep measured with an automated weighing system

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Implications Body weight of sheep reduces as duration of removal from grazing increases over the day. Duration of feed and water withdrawal should be standardised, or corrected for, if weight data is to be used in systems research and in farm practice.

Introduction Short-term body weight (BW) loss or gut-fill loss is a well-known issue in animal experimentation, which is usually controlled by consistent methodology between sets of weighings within experimental environments (Scott 2011; Whiteman *et al*., 1954). However, a review of literature provides few examples where the methodology states the length of time of feed and water restriction. Electronic identification (EID) and automated weighing crates enable weight data to have greater practical potential with the use of precision livestock farming techniques. Sheep handling, including weighing to relatively high precision, typically involves a number of hours of removal from food and water. There is little information on weight loss following removal from grazing over a short time period, but gut-fill can account for 12-23% of total BW (Hughes, 1976). The aim of this study was to quantify and characterise short term weight loss when sheep are removed from grazing.

Material and methods Scottish Blackface dry ewes (n=100) were collected from grazing just before the first of three weighing sessions, which started at 9am, 12pm and 3pm. During each weighing session, ewes were weighed 3 times, each composite session lasted 40 minutes. A Prattley Auto Draft (Prattley Industries, Temuka, NZ), with Tru-Test™ MP600 load bars and XR3000 weigh head (Tru-Test Group, Auckland, NZ) was used to record weights automatically to 0.1kg. Sheep EID was collected via an Allflex® radio frequency identification portal reader (Allflex Australia, Queensland, Australia). Between sessions, sheep were kept inside without access to food or water. Session data for three sheep were discarded as the range between the three weights fell outside three standard deviations from the mean. This resulted in 891 weight observations for analysis. Means for the three weights per session were used in analysis. Nine body condition scores (BCS) per sheep were collected over the day for use in another study, the mean of which was used in this analysis. Generalized linear regression (GenStat, 15th edition) was used to test effect of ewe age, mean BCS and initial (9am) mean BW on weight loss.

Results Sheep lost significant weight during the day (P<0.001), with an average of -1.8kg or -3.5% BW change after a 3hr fasting period and -2.9kg or -5.6% BW change after a 6hr fasting period (Table 1). There was little difference between measures of variation and very high correlations of BW between sessions. Initial weight had a significant effect (P<0.001) on BW change at 12pm and 3pm, with heavier sheep losing more weight. After adjusting for weight, effect of age was not significant but BCS was a significant factor for BW change between 9am and 3pm (P=0.01), with lower BCS losing more weight. Age had a significant effect on proportion of weight loss at 12pm (P<0.05 for 1.5yrs old < 2.5yrs old) and at 3pm (P=0.01; 1.5yrs old < all others). No significant effects of BCS or initial BW were found on proportion of BW change.

Table 1 Effect of restricting sheep from grazing during a day on body weight (BW) and BW change

Weigh session	Mean BW (kg)	Standard deviation	Coefficient of variation	Correlations between sessions (r^2)		BW change from 9am (kg)	BW change from 9am (%)
				9am	12pm		
9am	51.2[a]	5.67	11.09	1	-	-	-
12pm	49.3[b]	5.37	10.89	0.996	1	-1.80	-3.51
3pm	48.3[b]	5.25	10.88	0.991	0.997	-2.87	-5.60

Data with different superscripts are significantly different (P<0.05)

Conclusion Sheep lose weight over the day when removed from grazing. Scale of likely weight loss in practice is much greater than the precision of the method. BW loss is consistent and predictable, therefore should allow for simple adjustments of weights to be made. Heavier sheep lose more weight than lighter sheep, and after adjusting for weight, thinner sheep lose more than fatter sheep. If unknown or uncorrected weight losses, due to removal from grazing, are not considered, potential anomalies in BW data are likely to be compounded when weights are conventionally compared within or across days. Therefore, adjustments should be made for weights collected from sheep after different durations of feed and water restriction.

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016

Automatic classification of foraging behaviour in sheep using Radio Frequency ID, movement sensor and video technology

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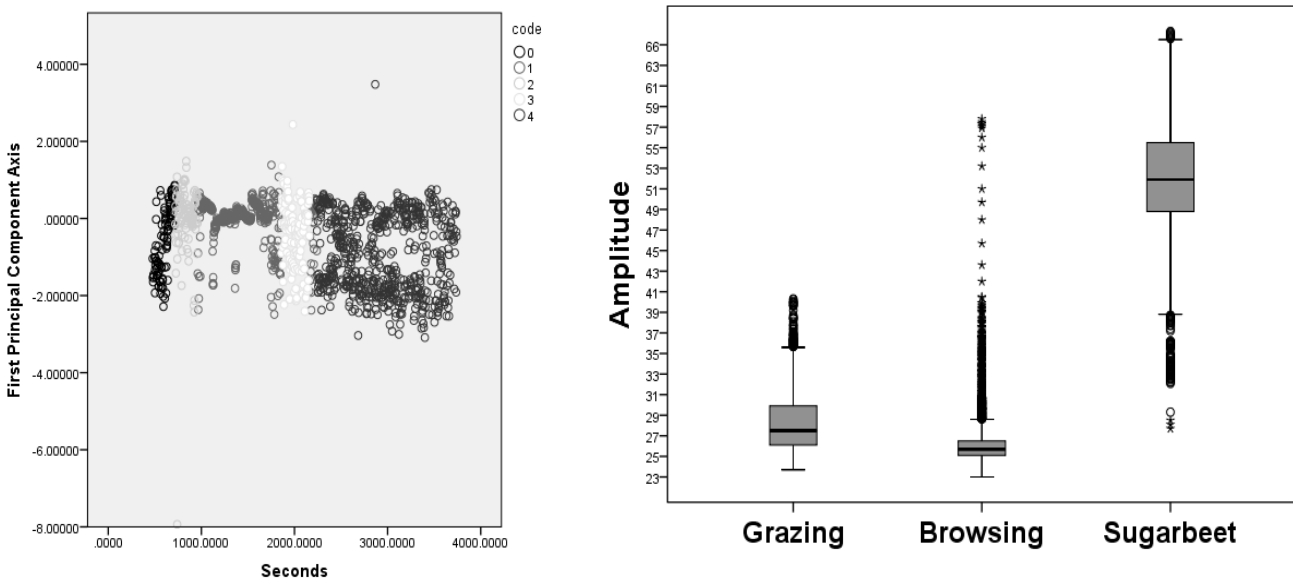
Implications A pilot Wireless Sensor Network (WSN) based Radio Frequency ID system is described that is designed to monitor, qualify and quantify real time foraging behaviour in free ranging sheep. Through the use of acoustic and video data for training and calibration, this system will be of future value for sheep farmers and land managers.

Introduction Little is known about foraging behaviour of free ranging sheep in bio-diverse pastures. There is a suggestion that grazing intensity is significantly influenced by fructan levels in grass in winter months ($p<0.02$) and that grazing bouts are longer at dawn and dusk, over seasonal timescales (Sneddon, unpublished data) . Clover is preferred over rye grass in early morning grazing bouts (Rutter, 2010). We have used a selection of technologies to describe foraging behaviour in 4 sheep grazing in two small bio-diverse paddocks (20 x 60 metres) in Cheshire.

Material and methods Four mature ewes were supplied at OS location 333781,371970 Shotwick, Cheshire UK. Foraging behaviour was examined during June and July 2013. The WSN system constructed for this work is based upon the Texas Instruments CC2431 system-on-chip microprocessor and developed custom software incorporating an accelerometer, and was small enough to mount on a sheep without any noticeable change in animal behaviour. Video cameras were attached to a halter (Kamer ®) harness near the mouth, & video recordings were made while grazing and browsing throughout June and July 2013. At least 4 recordings were made per sheep over several hours. Audio files were extracted from these videos using Format Factory ® and quantitative values of the sounds made by the sheep were produced using the software Sound Analysis Pro 2011®. These data were then analysed using Mann Whitney tests. The parameters compared were amplitude, pitch, mean frequency and Wiener entropy. In this abstract we will be presenting typical data for amplitude (n=4 sheep).

Results Four foraging-related behaviours were described with overall >80% accuracy using discriminate function analysis (SPSS v 21). A typical example for a Hebridean sheep is in the multicoloured Figure below. Video technology produced visual and acoustic recordings that could be used to distinguish ($p<0.05$) between foraging behaviours. Data for 4 sheep are also summarised below.



Conclusion A prototype WSN/video system has been developed and has yielded promising initial data which shows remote real-time recording of foraging behaviour in free range sheep. In particular we have shown significant variation in the output of an accelerometer sensor when an animal is grazing versus when it is simply standing. Ongoing work is linking this information to positional data in order that animal managers can assess in real time what animals are choosing to eat in a bio-diverse pasture.

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Home-grown alternatives for by-pass soya bean meal

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Implications Protected home-grown protein sources may reduce our reliance on by-pass soya bean meal for ewe diets.

Introduction The use of terminal sires and genetic selection for productivity are resulting in heavier and faster growing lambs, which increase ewe metabolizable protein (MP) requirements. Digestive-physiological constraints make it unlikely that microbial protein synthesis from effective rumen degradable protein (ERDP) alone can satisfy such increased MP requirements (AFRC, 1993). Therefore, complementary digestible undegradable protein (DUP) supply is increasingly needed to maximise ewe production potential.

By-pass soya bean meal Soya bean meal (SBM) is attractive as a protein supplement due to its high crude protein (CP) content, favourable amino acid (AA) profile, digestibility, palatability and availability. Feeding more SBM can increase DUP supply, though care should be taken to minimise excess of ERDP. Browning (heat treatment in presence of xylose) or formaldehyde treatments can almost double its DUP level (Hazzledine, 2008; Table 1) and reduce ERDP accordingly. As replacing SBM with browned SBM can increase ewe performance (Obeidat *et al*., 2010; Houdijk, 2014), by-pass SBM may be preferred over unprotected SBM to satisfy increasing MP requirements, provided that ERDP is not limiting.

Alternative by-pass protein sources Rapeseed meal (RSM) and pulses have smaller DUP levels than SBM (Hazzledine, 2008; Table 1). Earlier studies showed that RSM could replace SBM without affecting ewe productivity (Vincent *et al*., 1988). Replacing SBM for peas in dairy sheep producing ~1 kg milk per day (Renna *et al*., 2012) and for faba beans in cows producing ~27 kg milk per day (Tufarelli *et al*., 2012) also did not reduce productivity, but ewe milk production reduced when faba beans replaced browned SBM at similar calculated MP supply (Sakkas *et al*., 2012). This may be due to a smaller DUP to MP ratio, but may also arise from a reduced DUP quality (AA profile), as observed when high DUP maize gluten meal replaced SBM in ewe diets (Liamadis and Milis, 2007). Browning and pressure toasting can more than double DUP levels in RSM (http://www.lignotechfeed.com/Bypass-Protein/RaPass) and pulses (Goelema *et al*., 1998), respectively (Table 1). Cows gave more milk when browned RSM partly replaced a SBM-based concentrate (de Sousa Lamy *et al*., 2001), and when heat-treated RSM replaced untreated RSM (Bertilsson *et al*., 1994). First results are showing similar production responses to browned RSM and browned SBM over untreated SBM in hay- or straw-fed ewes (Wilkinson *et al*., 2014). Whether benefits of browned RSM feeding during pregnancy carry over into lactation, and whether toasted faba beans and browned RSM can replace browned SBM in silage-fed ewes is currently under investigation.

Table 1 Crude protein (CP) and digestible undegradable protein (DUP) levels in untreated and treated protein sources.

Feedstuff	CP (% in DM)	DUP (% of CP)	Treatment	DUP after treatment (% of CP)
Soya bean meal	51	33	Browning, formaldehyde	62
Rapeseed meal	35	17	Browning	70
Lupins	37	24	Pressure toasting	55
Faba beans	28	30	Pressure toasting	63
Peas	24	27	Pressure toasting	57
Maize gluten meal	62	55	-	-

Conclusions These desk study outcomes support the view that protected home-grown protein sources, subject to availability and palatability, can potentially reduce reliance on by-pass SBM to satisfy periparturient ewe MP requirements.

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018

Effect of forage type and level and source of DUP supply on ewe and lamb performance

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Implications Twin-bearing ewes may respond to additional MP supply from DUP above AFRC (1993) requirements.

Introduction The metabolisable protein (MP) requirements of ewes increase during late pregnancy and early lactation (AFRC, 1993). Failure to meet these requirements may reduce ewe and lamb performance. Over the last few years genetic selection for higher productivity has resulted in increased lamb birth weights and levels of milk production, with resultant increases in MP requirements. In addition, evidence suggests that ewes may require additional MP to maintain immunity to parasitic infection (Houdijk *et al*., 2006), and that current feeding standards may underestimate requirements (Robinson, 2001). The objective of the study was to investigate the effect of increasing DUP supply on ewe and lamb performance.

Material and methods 48 twin-bearing (Suffolk x Mule) ewes (86 kg) were housed individually from 6 weeks *pre-partum* to 4 weeks *post-partum* and allocated by parity, live-weight (LW) and condition score (CS) to one of 6 dietary treatments. Ewes were offered either hay (H) or straw (S) and one of 3 concentrates formulated to supply either a low (L) or high (H) level of protein, using either soya bean meal (S) or rapeseed meal (R) as the main protein source. All concentrates had a similar ingredient composition and were formulated to supply the same levels of ME, FME and ERDP, but different levels of CP and DUP. Concentrate SL supplied 188 and 26 g/kg DM CP and DUP, whereas concentrates SH and RH supplied 226 and 57 g/kg DM CP and DUP using xylose treated soya-bean meal or xylose treated rapeseed meal. Forages were chopped and offered at 0.5 and 0.8 kg/day DM during pregnancy and lactation. Concentrates were fed to satisfy the ME requirements of twin-bearing ewes producing 2.0 litres of milk (AFRC, 1993) with diets HSL and SSL supplying 1.0 and 0.8 of MP and diets HSH, HRH, SSH and RSH supplying 1.25 and 1.0 of MP requirements during pregnancy and lactation respectively. Ewe LW and CS was recorded weekly and colostrum and milk yield was estimated at 16 hours and 21 days. Lambs were separated from the ewes, which were then injected with 1 ml oxytocin and machine milked until the udder was empty. The procedure was repeated 4 hours later and milk secretion rate calculated. Litter weight and litter growth rate were recorded. The experiment was analysed by ANOVA as a 2 x 3 factorial design. Week -6 values were used as co-variates.

Results There was no significant effect of forage source on ewe performance *pre-partum*. However, ewes offered concentrates H gained more LW ($P<0.05$) and tended to lose less CS than those offered concentrates L. *Post-partum*, there was no significant effect of protein source on ewe LW or CS change, but ewes offered concentrates H had a higher colostrum yield ($P<0.05$). In addition ewes offered straw lost more CS ($P<0.05$) and tended to produce more milk than those offered hay. There were no significant effects of treatment on litter weight of litter gain.

Table 1 Effect of forage source and level and type of protein supply on ewe and lamb performance

	Hay			Straw			SED	Probability		
	SL	SH	RH	SL	SH	RH		For	Prot	Int
Pre-partum										
LW change (kg)	4.12	7.82	7.65	6.70	7.57	7.13	1.777	NS	0.027	NS
CS change	-0.63	-0.61	-0.44	-0.60	-0.40	-0.46	0.102	NS	0.086	NS
Post-partum										
LW change (kg)	-9.31	-10.45	-11.37	-11.18	-9.73	-10.92	2.135	NS	NS	NS
CS change	-0.92	-1.08	-1.40	-1.38	-1.42	-1.33	0.159	0.013	NS	0.053
Colostrum (l/d)	1.94	3.41	2.10	2.28	2.58	2.87	0.474	NS	0.042	0.061
Milk (l/d)	2.38	2.53	2.90	3.22	2.77	3.00	0.343	0.055	NS	NS
Litter weight (kg)	10.24	10.32	9.89	11.25	10.55	10.35	0.682	NS	NS	NS
Litter gain (g/day)	559	591	601	627	599	545	41.7	NS	NS	NS

Conclusion When fed diets designed to supply similar levels of ME and optimise microbial protein synthesis, enhancing DUP supply increased pre-partum ewe performance and colostrum yield, but had no effect of lamb performance.

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019

The influence of ewe nutrition pre and post lambing on the incidence of mastitis

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Implications It is well established that good nutrition of the ewe and management of body condition is fundamental to good flock performance. If it can be shown that ewe feeding has a direct impact on the incidence of mastitis then there could be very positive benefits of improving ewe nutrition on lamb performance, ewe longevity and culling rates and overall flock productivity.

Introduction Mastitis is common on many sheep farms and leads to poor milk yield, poor growth rate in lambs, higher culling rates and higher ewe and lamb mortality. In some flocks as many as 25% of certain ewe groups can be affected. Acute infections are usually seen from 10 days to about three weeks after lambing. This suggests that infection could be related to the lambing environment or to factors affecting milk yield (Watkins & Jones, 2005). Chronic mastitis is usually discovered later in the season at weaning or pre-tupping. In these cases the udder can feel hard or contain a number of hard lumps. Lambs from these ewes will often suffer ill thrift and poor growth due to low milk supply.

A major research project looking into factors leading to mastitis is underway at Warwick University. This project is an adjunct to that work and is looking specifically at pre- and post lambing nutrition of ewes and correlations between diet and the incidence of mastitis in a number of flocks with a known history of mastitis. Nutrition plays a key role in establishing and maintaining a successful lactation and trace elements like selenium have a role in the development of immunity so there is thought to be a direct relationship between nutrition and susceptibility to infection. Hungry lambs can inflict significant physical damage on the udder, which could be one contributory factor when milk yield is inadequate to sustain optimal growth rates. Maintaining the ewe in good body condition and providing adequate amounts of energy and protein is vital.

Material and methods A proforma questionnaire was designed in collaboration with researchers at Warwick university to capture full details of the rations offered to ewes before and after lambing on eight case study farms. This included analysis of forage and concentrate samples and details of all supplements offered – e.g. feed blocks and mineral supplements. Detailed information from the case study farms has been provided by research workers at Warwick university for lambing 2013 and will be provided for lambing 2014. The nutrients supplied by the diets offered have been compared to ARC (1983) recommendations by processing the information through the ADAS Sheepfeed rationing program for silage and hay diets and by Excel spreadsheet using standard equations for grass based diets. Some flocks were provided with diets that closely met recommended allowances for energy and protein but others showed significant variation from the optimum. This information has been reported to researchers at Warwick for further analysis and inclusion with other key data collected from the case study farms.

Results Only preliminary results are available for the 2013 lambing season and correlations with mastitis incidence on farm have not yet been completed. Initial assessments of the diets suggest some inadequacies in supply of nutrients to ewes on some farms pre- and post lambing.

Acknowledgements The financial support of EBLEX (division of Agriculture and Horticulture Development Board) is gratefully acknowledged.

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020

Effect of oral biotin supplementation on white line lesions in a lowland sheep flock

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Implications This study showed that oral supplementation with a bolus releasing 82 mg/day available zinc only or 5mg/day biotin and 82 mg/day zinc, compared to untreated controls had no significant effect on the severity of white line (WL) lesion scores. However biotin supplementation had a significant effect on lamb daily live-weight gain to 10 weeks of age.

Introduction White line separation and impaction is a common finding during routine ovine foot examinations but little is currently known about the pathogenesis of the condition. In dairy cattle oral biotin supplementation has been shown to reduce the prevalence of WL lesions (Hedges *et al.*, 2001). The aim of this study was to evaluate the effect of oral biotin supplementation on the percentage and severity of white line lesions in a lowland sheep flock and to monitor subsequent lamb performance.

Material and methods A single randomised split flock trial was conducted in a commercial North Country Mule flock with a history of WL. During a one-year longitudinal study, the feet of 302 ewe lambs were repeatedly scored using a categorical scoring scale (score 0, lesion free/healed lesion; score 1-3 minor, moderate and major separation of the white line respectively, score 4 discrete lesions without separation and score 5 active infection of the white line). Animals with WL in one or more feet (n=260) and those without WL (n=42) were randomly allocated to one of three groups; **C**ontrol – no supplementation, **Z**inc – zinc-based rumen bolus (releasing 82 mg bioavailable zinc/head/day) and **B**iotin - a biotin and zinc bolus (releasing 5 mg biotin and 82 mg zinc/head/day). At four-monthly intervals, sheep were re-bolused and individual feet scored by the same assessor who was blinded to the treatments. All study animals were managed as a single group. Birth weight and subsequent live-weight of lambs born to the trial ewes were recorded to 10 weeks of age.

Results Most sheep at study commencement (86%) and throughout the trial period were observed with WL although few were recorded with severe lesion scores at any assessment visit (Figure 1). Logistic regression analysis identified no significant difference ($p>0.05$) between the proportion or severity of WL scores recorded in Control, Zinc or Biotin groups at any of the four assessment visits. Figure 2 illustrates that in feet observed with WL in January 2012 (n=763) there was some reduction in the percentage of WL across all three treatment groups, although significant treatment differences were not observed. Lambs born to biotin supplemented ewes were significantly heavier (p=0.002) than Z or C lambs (23.7, 22.3 and 21.8 kg respectively) at 10 weeks of age.

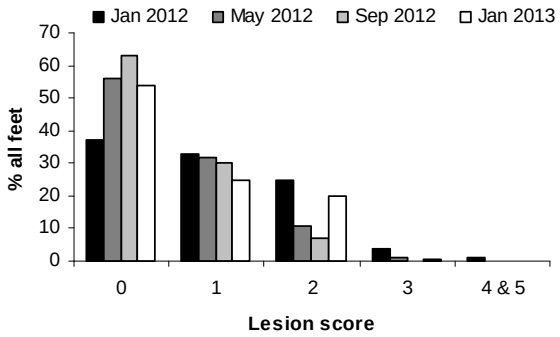

Figure 1 Distribution of WL scores at each assessment visit

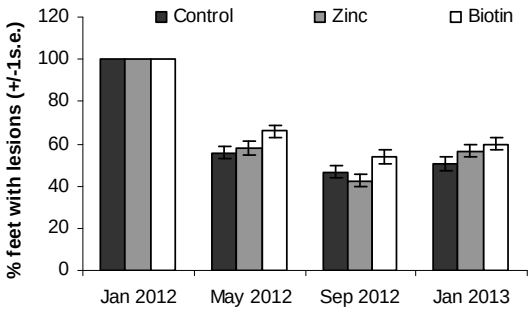

Figure 2 Feet with WL (scores 1-5) at the first assessment and the percentage of these feet recorded with WL at subsequent assessments

Conclusion Compared to control animals, oral supplementation with a bolus releasing 82 mg/day available zinc only or 5mg/day biotin and 82 mg/day zinc did not have a significant effect on the severity of WL scores suggesting there were no protective or healing effects of zinc or biotin during this study. As the study was conducted in a single lowland flock of sheep with relatively mild lesions it may be useful to evaluate the effect of biotin in a larger population with more severe lesions over a longer study period. The effect of environmental and climatic conditions and the role of genetics in WL of sheep also warrant further research. Biotin appeared to have a positive effect on growth rate of lambs born to supplemented ewes to 10 weeks of age.

Acknowledgements This project was funded by EBLEX (division of Agriculture and Horticulture Development Board). The support of the host farmer, DSM Nutritional Products UK Ltd and Agrimin Ltd is gratefully acknowledged

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021

To refine and confirm the level of selenium and iodine supplementation for breeding ewes

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Implications On a selenium deficient farm, supplementing ewes before mating with a slow release intra-ruminal bolus providing 0.1mg selenium per kg dry matter intake was effective in maintaining GSH-Px levels throughout pregnancy, with no additional benefit in either GSH-Px concentrations or production by supplementing with 0.2 or 0.3mg/kgDM. On two farms where iodine deficiency had previously been diagnosed, sustained release intra-ruminal boluses providing 0.5, 1.0 or 2.0mg iodine per kg DM, produced an initial response in PII levels and no differences in production between the three treatment groups. However, the response in PII was neither as great as expected nor of as long duration.

Introduction Current recommended dietary allowances for selenium and iodine for sheep are based on the 1983 ARC recommendations. For selenium the recommended dietary allowance in sheep is 0.1 mg/kgDM but anecdotal evidence suggests that higher levels could be beneficial in terms of fertility, lamb viability and immunity. For iodine, the situation is more complex. Recommended dietary allowances vary according to the season (summer or winter) and whether the diet contains goitrogens. Furthermore, the diagnostic tests are poorer and more difficult to interpret. The aim of the present study was to determine whether supplementing ewes with levels of selenium and iodine above those recommended by ARC demonstrated any production benefits.

Material and methods Four farms were recruited, two with previously diagnosed selenium deficiency and two where iodine deficiency diseases had been confirmed. On each farm, ewes were randomly allocated to one of three treatment groups, with 30 to 50 ewes in each group. As the farms were selected on the basis of known deficiencies, it was decided not to have control groups of untreated sheep, as the lack of supplementation may have been adverse to the animals' welfare. A slow release intra-ruminal bolus was administered to all the ewes on the trial. The boluses were manufactured by Agrimin Ltd and were based on commercially available boluses providing selenium, cobalt and iodine. Boluses used on the selenium farms were manufactured to provide 0.1, 0.2 or 0.3mg selenium per kg of dry matter intake, whilst those used on the iodine farms were predicted to supply 0.5, 1.0 or 2.0mg iodine per kg dry matter intake. The boluses were predicted to provide continual supplementation from 3 weeks prior to mating until lambing. Ewes were weighed, condition scored and blood sampled at randomisation, during pregnancy and post lambing. Lambing assessments were carried out by the host farmers. Adverse weather conditions in spring 2013 caused considerable difficulties at lambing time and one of the selenium farmers withdrew from the trial completely. The other farmers were able to identify lambs according to treatment groups but only to make an assessment of average birth weights and lamb vigour. The project design included examination of the thyroid gland of any stillbirths or neonatal deaths on the iodine farms, but this was not achieved. Lambs were weighed at least twice on the three farms still in the trial; once at 5-8 weeks of age and again at weaning. Blood samples from the ewes were analysed for GSH-Px concentrations to indicate selenium status and for PII concentrations as a measure of iodine intake.

Results On all four trial farms, no differences in ewe body condition score, ewe weight and pregnancy scanning were observed between trial groups. Lamb daily live weight gain was measured on 3 farms, and again there was no difference between treatment groups. No clinical signs suggestive of selenium or iodine deficiency were observed on any of the farms. On the selenium farms, all the boluses (0.1, 0.2 and 0.3mg selenium) resulted in a response in GSH-Px, with concentrations remaining well above the reference range for more than 120 days. On the iodine farms, there was also a response in PII concentrations with all the boluses (0.5, 1.0 and 2.0mg iodine), but the response was of a shorter duration than predicted from this type of bolus. Additionally, the PII concentrations recorded on one of the farms were not as high as expected.

Conclusion In this study, pre-tupping administration of a sustained release intra-ruminal bolus providing 0.1 mg selenium per kg DM was found to provide adequate levels of selenium on a known selenium deficient farm, with no observed benefits in supplementing with 0.2 or 0.3 mg selenium per kg DM. Supplementing ewes with iodine from a sustained release intra-ruminal bolus did not appear to provide an adequate iodine intake for the predicted life of the bolus. Also, PII concentrations on one of the farms did not rise as high as expected, raising the question whether the current interpretation of PII concentrations in sheep proposed by AHVLA (2013) is suitable, or whether iodine intake is adequate at lower PII concentrations than suggested.

Acknowledgements This project was funded by EBLEX (division of Agriculture and Horticulture Development Board). The project team would like to thank the host farmers and their vets. They also gratefully acknowledge Agrimin Ltd for supplying and administering the trial boluses.

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022

Effect of genotype and weight at mating on performance of ewes joined to lamb at 1 year of age

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Implications When lambed as one year olds ewe genotype and weight at joining have significant effects on the weight of lamb weaned per ewe joined. Target live weight at joining depends on ewe genotype. Belclare ewe lambs can wean a similar number of lambs as that achieved from the national adult lowland flock.

Introduction Ewe life-time performance, as measured by lamb carcass production, is influenced by age at first lambing and genotype. The proportion of ewe lambs that attain puberty before the end of the breeding season is influenced by genotype and weight at joining. The aim of the current study was to evaluate the effects of ewe genotype and weight at joining on the performance of ewe lambs joined to lamb at 1 year and the performance of their lambs.

Material and methods A total of 109 ewe lambs [38 Belclare (B), 38 B×Suffolk (S), 35 >75% S [mean initial (at joining) live weight 45.9, 46.4 and 48.9 kg; weight range 40.0 to 55.0, 37.8 to55.0 and 41.9 to 58.4 kg, respectively] were joined with Charollais rams. The >75%S ewe lambs were purchased from commercial farms in July and run with the B and B×S ewe lambs. Fourteen days prior to joining (23 October) the ewe lambs were exposed to vasectomised rams for 48 h to advance first oestrus via the "ram effect". The joining period lasted for 42 days. The lambs were shorn at housing in mid December and offered high feed-value grass silage *ad libitum.* All lambs received 200 g and 250 g concentrate daily from 14 to 27 January and from 28 January to 6 weeks prior to lambing, respectively. During the last 6 weeks prior to lambing a total of 18, 26 and 33 kg of concentrate was offered to ewes carrying (based on scan data) singles, twins and triplets , respectively. Mean lambing date was 31 March. Ewes rearing singles received no concentrate supplementation post lambing while those rearing twins received 0.5 kg concentrate for 5 weeks post lambing while their lambs had access to up to 300 g concentrate per head per day. From weeks 5 to 14 (weaning) all lambs had access to concentrate (up to maximum of 300 g/head daily). The data were analysed using Proc GLM and Proc MIXED of SAS, as appropriate.

Results The grass silage had DM and metabolizable energy concentrations of 210 g/kg and 11.7 MJ/kg DM, respectively. The effects of ewe genotype on animal performance are presented in Table 1. Belclare ewes had larger litters, reared more lambs than the >75% S ewes while B×S ewes were intermediate. Ewe genotype had no effect on lamb mortality, 64% of which occurred within 48 h of birth. The effect of ewe lamb weight at joining on the probability of rearing at least one live lamb is presented in Figure 1. The values in Figure 1 reflect the effects of ewe fertility, litter size, ewe mortality and lamb mortality. Increasing ewe live weight at joining increases the probability of rearing a live lamb, but is linked to ewe genotype. Ewes that failed to rear a lamb were 7.6 kg lighter at lambing, on average. There was a significant interaction between ewe lamb genotype and weight at joining for the probability of rearing at least one lamb.

Table 1 Effects of ewe genotype on ewe and lamb performance

		Breed[1]			s.e.	Sig
		B	B X S	>75% S		
Ewe wt (kg)	- lambing	52.9	54.6	56.4	1.13	NS
Litter size		1.81^{b}	1.39^{b}	1.27^{a}	0.096	***
Mortality (%)		27.3	27.3	26.3		
Lamb wt (kg)	- birth	4.0	4.3	4.4	0.14	NS
	- weaning	29.4^{a}	31.6^{ab}	32.0^{b}	0.82	*
Lamb gain	(g/d)	256^{a}	279^{b}	280^{b}	8.3	*
Efficiency[2]		39.4	32.5	29.8		
No. reared/ewe lambing		1.34^{b}	1.03^{b}	0.93^{a}	0.111	*

[1] B = Belclare, S = Suffolk, [2] Weight (kg) of lamb weaned per ewe lambing

The relationships between weight at joining and the probability of rearing at least one live lamb for the B, B× S and >75% S lambs are as follows:

Belclare: $y = 4.441\times10^{-5}e^{0.258x}/(1 + 4.441\times10^{-5}e^{0.258x})$
Belclare-X: $y = 1.162\times10^{-3}e^{0.160x}/(1 + 1.162\times10^{-3}e^{0.160x})$
Suffolk: $y = 1.376\times10^{-4}e^{0.200x}/(1 + 1.376\times10^{-4}e^{0.200x})$
where y = the probability of rearing at least one lamb
x = live weight at joining

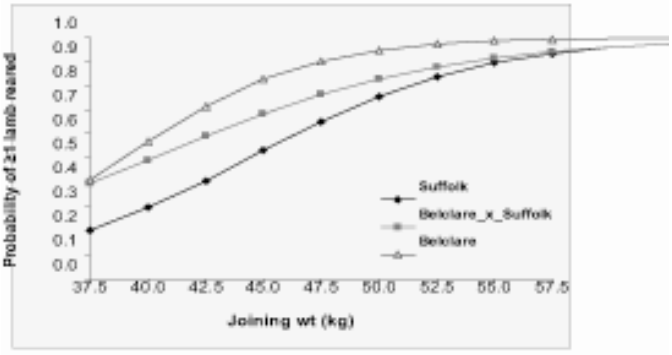

Figure 1 Effect of joining weight of ewe lambs from three genotypes on the probability of rearing ≥ 1 lamb

Conclusion Ewe genotype and weight at joining influenced the weight of lamb weaned. Belclare ewe lambs weaned 1.16 lambs per ewe lamb joined which is close to that recorded for lowland flocks in Ireland. To have a 90% chance of rearing at least 1 live lamb Belclare and >75% Suffolk ewe lambs need to be 62 and 66% of expected mature weight, respectively.

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023

First-cross ewes from Scottish Blackface dams: an evaluation of progeny sired by three lowland breeds

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Implications First-cross ewes by Charollais rams are essentially as productive as Belclare-X ewes but the performance of Chamoise-X is substantially inferior and the Chamoise does not merit consideration for use as a crossing breed.

Introduction The Scottish Blackface breed is the basis for most extensive hill flocks in Ireland. This sector is a source of crossbred replacements for lowland flocks but this role is poorly developed *vis-à-vis* Britain. While the performance of first-cross ewes from S Blackface dams, by a range of lowland breeds (Galway, Texel Suffolk, Border Leicester, Bluefaced Leicester and Belclare), has been evaluated (Hanrahan 2001) there is scant data on the use of Charollais rams and no comparative data on use of the Chamoise (a hardy hill breed from France with good carcass conformation) in this context. The present report concerns data from an evaluation of Charollais and Chamoise rams for crossing on S Blackface ewes under hill conditions and the performance of their first-cross ewes in a lowland grass-based system.

Material and methods The S Blackface flock at the Teagasc Hill Farm was used to produce all animals. Charollais-X (76) and Chamoise-X (115) ewes were born 1998 to 2000 and 2004 to 2006, respectively. Belclare rams were used on the flock every year; this provided a common reference for evaluations. The Belclare-X contemporaries of the Charollais-X and Chamoise-X ewes numbered 131 and 117, respectively. The Charollais and Chamoise breeds were each represented by 6 rams; 10 and 7 Belclare rams sired the Belclare-X contemporaries of Charollais-X and Chamoise-X, respectively. All crossbred ewe lambs were drafted from the hill (late August) to the Athenry Research Centre where they were managed as replacements for the midseason flock that is used for grazing and other management studies (care was taken to avoid confounding breed with experimental treatment). Ewes were first joined at 18 months and were (usually) only retained for 3 joinings. Mating at Athenry involved syndicate joining with purebred Suffolk rams. Core performance recording procedures were common between the hill and lowland flocks and included mating records, ewe live weight pre and post joining, lambing data (lamb birth weight; live or dead at tagging), lamb growth to weaning (~14 weeks) and mortality. Litter size at birth, number of lambs weaned per ewe, average ewe weight at mating, lamb growth to 5 weeks and weaning, and lamb weight at 14 weeks were used. Ewe survival to third joining was established. Lamb and ewe performance data were analysed using mixed models (Proc MIXED of SAS). The linear model for lamb traits had fixed effects for Year × Breed, rearing type (birth type in the case of birth weight), sex and dam age, with dam as random. In the case of ewe performance traits the model had Year × Breed × ewe age as fixed effect with ewe random. Linear contrasts, based on breed cohorts, were used to estimate breed effects with Belclare-X contemporaries as reference against which Charollais-X and Chamoise-X breeds were compared. Survival data were analysed using Proc GENMOD (SAS) with a logit link function

Table 1 Breed effects on live weight at mating and productivity

Estimate	Mating weight (kg)	Litter size	Lambs reared per ewe	
			Lambed	Joined
Belclare mean	67.7±0.30	2.03± 0.019	1.88±0.020	1.80±0.023
Belclare - Charollais	-3.2±0.99	0.02±0.050	0.04±0.055	0.04±0.062
Belclare - Chamoise	5.8±0.80	0.36±0.048	0.41±0.058	0.43±0.058

Results The birth weight of lambs sired by Charollais rams did not differ from those by Belclare rams but Charollais-X lambs had a higher growth rate and were 1.2 kg heavier at 14 weeks of age ($P<0.01$). Chamoise-X lambs were significantly lighter (0.5, s.e. 0.07, kg) than Belclare-X at birth and grew more slowly ($P<0.01$) being 2.8 (s.e. 0.35) kg lighter at 14 weeks. There was no evidence for any breed effects on perinatal lamb mortality or total mortality to weaning. Breed effects on ewe traits are given in Table 1. Charollais-X ewes were heavier than Belclare-X but while litter size and number reared were lower the differences were not statistically significant. Belclare-X ewes were significantly heavier than Chamoise-X as was litter size and the other reproductive traits ($P<0.001$). These estimated differences are all less than was predicted from previous evidence (Hanrahan 2001). There were no significant breed effects on fertility (i.e., proportion lambed) or annual mortality. The proportion of Belclare-X and Charollais-X ewes present for the third joining did not differ but the percentage for Chamoise-X exceeded that for Belclare-X and the difference approached significance (57% v 46%; $P= 0.053$).

Conclusion The use of Chamoise rams significantly reduced both growth rate of first-cross lambs and the prolificacy of first-cross ewes compared with the Belclare, or Charollais. Charollais-X and Belclare-X ewes did not differ significantly for litter size or lambs reared per ewe but lambs sired by Charollais had a significantly higher growth rate.

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Effects of forage and breed types on the efficiency of utilisation of energy in hill replacement ewes

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Implications Grass nuts have lower energy digetibility and metabolisability than fresh grass or grass silage. The accurate measurement of the energetic efficiency is essential for improving the production efficiency of sheep production systems.

Introduction Manipulation of dietary forage type can alter the rumen fermentation pattern and consequently influence nutrient degradability and energetic efficiency of the host animals. The aims of this experiment were to investigate how different forage and breed types affected the efficiency of utilisation of energy and to use these data to develop relationships between energy intake and CH_4 energy output in hill replacement ewes.

Material and methods Thirty six hill replacement ewes (18 pure Scottish Blackface and 18 Swaledale x Scottish Blackface (75:25)) aged approximately 12 months and weighing 42 ± 4.0 kg were allocated to 3 treatments balanced for breed and live weight. Each breed was offered 3 forages *ad libitum*: grass nuts, fresh grass and grass silage (CP = 152, 114 and 100 g/kg DM, respectively). Grass nuts were sourced from a commercial supplier (Drygrass South Western Ltd, Burrington, UK). Fresh grass was harvested daily from the primary regrowth of perennial ryegrass, while the grass silage was made from the 2nd harvest of perennial ryegrass ensiled with Ecocyl as an additive. The animals were individually housed and fed experimental diets for at least 14 days before being transferred to individual calorimeter chambers for a further 4 days with energy intake and output and CH_4 emissions measured for the final 3 days. Feed intake, live weight and CH_4 emissions were reported by Zhao *et al*. (2013). Data were analysed using two-way ANOVA for effects of forage type, breed and their interaction, with a probability level of P = 0.05 for significance between treatments, using the Genstat statistical package.

Results The effects of forage and breed types on the efficiency of utilisation of energy are presented in Table 1. There were no significant interactions between breed and forage types on any variable of energy intake or output or energetic efficiency. Sheep offered grass nuts had higher GE intake and faecal energy output (P<0.001) but lower DE/GE and ME/GE (P<0.001) compared to those given fresh grass and grass silage. The grass silage produced a significantly higher CH_4-E/GE than grass nuts (P<0.001). However, urinary energy output was not affected by diets but was produced more in Scottish Blackface than Swaledale x Blackface (P<0.05). There were no significant differences between the 2 breeds in any other variable. The statistical analysis using all data found that there was a significant relationship (P<0.001) between CH_4 energy output (MJ/d, y) and GE intake (MJ/d, x), $y = 0.03x + 0.43$, $R^2 = 0.70$.

Table 1 Effects of forage and breed types on the efficiency of utilisation of energy

	Swaledale x Blackface			Scottish Blackface			s.e.	Probability		
	Grass Nuts	Fresh Grass	Grass Silage	Grass Nuts	Fresh Grass	Grass Silage		Breed	Forage type	Interaction
GE intake (MJ/d)	30.6^{b}	17.6^{a}	14.2^{a}	30.0^{b}	17.3^{a}	15.9^{a}	2.65	0.824	< 0.001	0.815
Faecal energy (MJ/d)	13.1^{b}	2.8^{a}	3.0^{a}	13.1^{b}	3.7^{a}	3.7^{a}	0.95	0.261	< 0.001	0.768
Urinary energy (MJ/d)	0.30^{a}	0.47^{ab}	0.37^{ab}	0.47^{b}	0.50^{b}	0.47^{b}	0.080	0.049	0.281	0.530
CH_4 energy (MJ/d)	1.4^{b}	1.1^{ab}	0.9^{a}	1.3^{b}	0.9^{a}	1.0^{ab}	0.14	0.621	0.002	0.284
DE/GE	0.57^{a}	0.83^{b}	0.78^{b}	0.57^{a}	0.78^{b}	0.76^{b}	0.028	0.073	< 0.001	0.519
ME/GE	0.52^{a}	0.74^{b}	0.68^{b}	0.51^{a}	0.70^{b}	0.66^{b}	0.030	0.119	< 0.001	0.762
ME/DE	0.91^{b}	0.89^{ab}	0.88^{a}	0.90^{ab}	0.89^{ab}	0.87^{a}	0.012	0.628	0.032	0.779
CH_4-E/GE	0.044^{a}	0.063^{b}	0.067^{b}	0.043^{a}	0.052^{a}	0.066^{b}	0.0055	0.202	< 0.001	0.355

^{a,b,c} means within rows with same superscripts are not significantly different (P>0.05)

Conclusion Sheep offered grass nuts had higher energy intake but lower energy digetibility and metabolisability than those given fresh grass or grass silage. There was no difference in the efficiency of utilisation of energy between Scottish Blackface and Swaledale x Scottish Blackface. CH_4 energy output in hill replacement ewes can be predicted from GE intake.

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Effects of breed and forage types on methane emission factors for lowland replacement ewes aged between 8 and 19 months

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Implications Feeding grass nuts, rather than fresh grass or grass silage can reduce enteric CH_4 emissions as a proportion of feed intake in sheep. Methane emission factors for replacement ewes offered fresh grass were on average 7.3 and 6.9% for 8 and 19 months old ewes respectively, which is higher than the value currently used for adult ewes (6.5%, IPCC 2006).

Introduction Within the UK sheep industry, breeding of replacement ewes is normally delayed until the year after birth. This 'gap' year can represent a significant cost in terms of greenhouse gas (GHG) emissions from sheep systems. Within the Tier 1 methodology of IPCC (2006), methane (CH_4) emission factors of adult ewes are also applied to replacement ewes, despite their smaller body size, which may overestimate total GHG emissions. The aims were to establish CH_4 emission factors specific to growing lowland ewes and investigate the effects of different breeds and forage types.

Material and methods Animal and gas data were measured over three periods, using animals at approximately 8, 12 and 19 months of age respectively. For each period, 31 animals (8 months old) or 27 animals (12 and 19 months old) were allocated to two diet groups balanced for breed and live weight (LW). Three breed types were studied using crossbred ewes, defined according to the sire breed as: Belclare cross (B×), Highlander cross (H×) and Texel cross (T×). Animals were offered *ad libitum* either grass nuts (GN) or a diet representative of standard practice, i.e. fresh grass (G) (summer diet for 8 and 19 months olds) or grass silage (winter diet for 12 months olds). Grass nuts were sourced commercially (Drygrass South Western Ltd, Burrington, UK). Fresh grass and grass silage were obtained from a predominantly perennial ryegrass sward. The animals were individually housed and fed experimental diets for at least 14 days before being transferred to individual calorimetric chambers for a further 4 days, with feed intake and CH_4 emissions measured for the final 3 days. Live weight was measured at the beginning of the study and after leaving the chamber. Data were analysed for each period using a REML variance components analysis with breed, diet and their interaction as fixed effects, using Genstat (14th edition).

Results There were no significant interactions between breed and forage type for any of the response variables and for all three age groups. All breed types had similar LWs (Table 1). Sheep offered GN had higher LW, DM intake (DMI) ($P < 0.001$) and CH_4 emissions (g/d) ($P < 0.001$), but lower CH_4 output per DMI and per GE intake (GEI) when compared to those given G or GS. There were no significant differences between breeds in terms of DMI, CH_4 emissions (g/d) and CH_4 output per DMI and GEI. Within each diet type, CH_4 emission factors appeared similar across age groups (e.g. for GN: between 4.0 and 4.7 % of GEI).

Table 1 Effects of breed and forage type on live weight and methane emission factors

	Diet				Breed				
8 mths:	GN	G	s.e.d	P	B×	H×	T×	s.e.d	P
LW (kg)	40.1	29.6	1.14	< 0.001	34.4	35.0	35.2	1.37	0.64
CH_4/DMI (g/kg)	16.0	24.6	1.36	< 0.001	19.6	19.7	21.6	1.64	0.13
CH_4-E/GEI (MJ/MJ)	0.047	0.073	0.0040	< 0.001	0.057	0.058	0.064	0.005	0.12
12 mths:	GN	GS			B×	H×	T×		
LW (kg)	53.8	43.4	3.20	0.003	50.0	46.9	48.9	4.75	0.95
CH_4/DMI (g/kg)	13.6	22.8	1.48	< 0.001	17.1	18.4	19.2	2.20	0.63
CH_4-E/GEI (MJ/MJ)	0.040	0.068	0.0045	< 0.001	0.051	0.054	0.057	0.0067	0.50
19 mths:	GN	G			B×	H×	T×		
LW (kg)	60.4	51.5	2.62	0.002	57.4	54.1	56.3	3.88	0.42
CH_4/DMI (g/kg)	13.9	23.0	1.48	< 0.001	19.9	18.4	17.1	2.20	0.40
CH_4-E/GEI (MJ/MJ)	0.040	0.069	0.0043	< 0.001	0.059	0.055	0.050	0.0064	0.34

Conclusions There was no difference in CH_4 emissions between breeds. Methane emission factors for ewes offered fresh grass were on average 7.3 and 6.9% for 8 and 19 months olds respectively, which are higher than the value currently used in the national GHG inventory (6.5%, IPCC 2006). Further studies with both young and adult ewes are required to assess whether emission factors for adult ewes can also be used for growing sheep, as suggested in this study.

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Effect of starting date and length of lambing season on dairy sheep profitability in Greece – a simulation study

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Implications Profitability of the dairy sheep sector can be greatly improved by enhancing reproductive efficiency. Farmers, nutritionists and veterinarians should intensify their efforts to achieve this goal.

Introduction Intensive dairy sheep production is a high input enterprise that requires a high output in order to be profitable. Reproductive efficiency is essential in this respect (Scott 2010). An early start and a short duration of lambing season maximizes milk production as milking is generally discontinued after the end of July and heavier lambs are sold during the most favourable market period (Christmas and Eastern). The aim of this simulation study was to assess the combined effects of starting date and length of lambing season on dairy sheep profitability under Greek conditions.

Material and methods A typical intensive system was simulated including two lambing seasons, autumn (AS) for adult ewes (70% of total) and winter (WS) for ewe-lambs and adult repeat breeders (30% of total). Reproductive patterns were established based on official lambing data from 64 Chios sheep flocks (80 AS and 91 WS) spanning a 2-year period. Lambing starting in early October, late October and mid November was classified as early (E), medium (M) and late (L), for the AS lambing season, respectively. The corresponding dates for WS were mid January (E), early February (M) and late February (L). Length of lambing season was classified as short (S, >80% of ewes lambing during the first 17-day period), medium (M, <60% of ewes lambing during the first 17-day period but > 90% lambing during the first two 17-day periods) and long (L, all other cases). The combination of these cases is denoted by four letters, for example, the ESMM scenario stands for early/short AS and medium/medium WS. A flock having all its ewes lambing during the first 17-day period of both AS and WS was considered to have a mean production of 300 kg of milk per ewe (330 kg and 230 kg, for AS and WS, respectively). Either AS or WS had a total of six 17-day periods of lambing. Milk production for each scenario was adjusted on actual lambing patterns as follows: for AS, milk production corresponding to the six 17-day periods was 100%, 92%, 84%, 76%, 70% and 66%, respectively. Corresponding values for WS were 100%, 86%, 73%, 65%, 56% and 46%. This is based on official milk recording data and pertains to all Greek dairy sheep breeds. Profitability of extra milk production was estimated as income over feed costs: 1.0 kg of concentrates (0.35€/kg) was considered necessary to produce 1.4 kg of milk (0.95€/kg). Regarding lamb production, prolificacy was considered similar in all scenarios (2 lambs per ewe) and after accounting for replacements and mortality, 98 and 48 lambs were available for slaughter from AS and WS, respectively. Comparisons were based on a standardized 8-week old, 10-kg carcass of milk-fed lamb. Costs were equal in all six 17-day periods; however, the 2010-2013 average prices for lambs slaughtered after Christmas and Easter were 1.05€/kg and 1.00€/kg lower, respectively, than those for lambs slaughtered before the holiday seasons. The ([3x3] x [3x3]) factorial arrangement of the variables resulted in 81 scenarios. Analysis of variance was used to estimate the effects of variables on profitability. Both factors were fitted as fixed effects in the analysis model.

Results Compared to the best scenario (ESES), milk production and income loss ranged from 10.1% and 2.4€ (ESEM) to 26.6% and 70.1€ (LLLL) per ewe per year. Some selected scenarios and means are presented in Table 1. The mean loss of 37.0€ represents 10.3% of the total output per ewe. Losses in milk production accounted for most (77%) of the income loss; however, and opposite to common farmers' beliefs, a considerable loss (23% of total) resulted from slaughtering lambs at lower prices. Both starting date and length of lambing season had a statistically significant effect (P<0.001) on profitability.

Table 1 Milk production and income losses compared to the best scenario (ESES) – Selected scenarios and means

				Total income loss compared to ESES		
Selected scenarios	Milk per ewe, kg	Milk per ewe, %	Lambs sold at lower prices, %	per ewe, €	from milk, %	from lambs, %
ESES	295.0	0.0	0.0	0.0		
ESLS (rep. the best 3rd)	277.3	-6.0	32.9	-17.2	72.1	27.9
MSLM (rep. the middle 3rd)	256.0	-13.2	47.3	-38.6	81.6	18.4
MLLM (rep. the worst 3rd)	235.7	-20.1	100.0	-56.6	73.3	26.7
LLLL	216.4	-26.6	100.0	-70.1	78.5	21.5
Mean (all except ESES)	254.9	-13.6	58.6	-37.0	77.0	23.0

Conclusion Improvements in reproductive efficiency can have a considerable effect on dairy sheep profitability. Farmers and their advisors (veterinarians, nutritionists etc) should focus their attention to adequate nutritional and health management that greatly influence reproductive outcome in intensively reared dairy flocks.

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The effect of weaning system on the milk fatty acid profile of Chios sheep breed

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Implications The rearing system of lambs during the first 5 weeks post-partum, affects both the fat content and the fat quality (profile of fatty acids) of the commercial milk produced by their dams.

Introduction The weaning/rearing system that a dairy sheep farmer applies, affects the quantity and the fat content of the commercial milk yield and hence, the economic return. This becomes quite important considering that approximately 30% of the total milk yield is produced during the first 35 days of lactation in Chios sheep (Hadjipanayiotou and Louca 1976). The objective of the present study is to compare milk composition and quality (fatty acid profile) in two different rearing systems of Chios sheep ewes.

Material and methods Forty ewes of the Chios breed, were assigned, before parturition, in two balanced groups of twenty animals and allocated to the following two different rearing/weaning systems: (a) ewes were weaned from their lambs at 48 hours postpartum and machine milked twice per day (NL - no lambs group) and (b) beginning 48 hours postpartum, ewes separated from their lambs during the night, and machine milked once daily in the following morning (WL -with lambs group). At 5 weeks of age, lambs from the WL group were weaned, and ewes were machined milked twice daily. From each ewe, milk samples were collected once weekly for the first 10 weeks of lactation and also on weeks 12, 16, and 20. Samples were analysed for milk composition (fat, protein, lactose, solids non-fat), using established ISO methods and for fatty acid profile by applying a modified GC-MS method according to Feng *et al*. (2004). All animals were fed the same ration to meet their requirements. The treatment effect was tested using a mixed linear model for repeated measurements.

Results During the first 5-week treatment, the composition of the sheep milk obtained was similar between the different groups except for the total fat content (Figure 1), which was approximately 42% higher in the NL group ($p<0.001$, for weeks 1 to 5).The lipid profile analyses of milk obtained during the first 5 week period demonstrated also significant differences in specific fatty acids such as conjugated linoleic acid (9c,11t, $p < 0.01$ for week 2 and 5), and linoleic acid ($p < 0.05$ for weeks 1, 2, 4 and 5; Figure 2), while no significant differences observed in other fatty acids such as lauric (mean ± SD: 7.6 ± 0.95), myristic (mean ± SD: 15.2 ± 0.99), or stearic acid (mean ± SD: 8.4 ± 1.68). After weaning of WL lambs, differences in total milk fat and fatty acids content between the two groups were small and statistically not significant. There was a significant interaction between time and treatment effects ($p < 0.001$) in all tested variables.

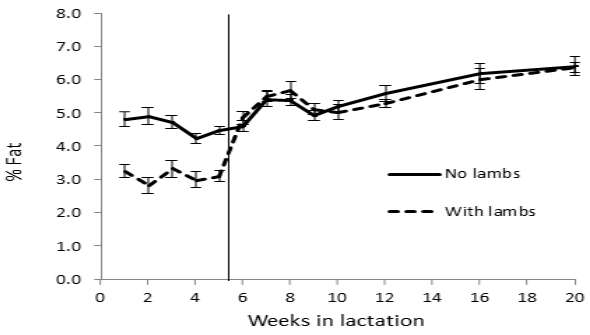

Figure 1 Sheep milk fat content in two different weaning systems (vertical line - lamb weaning)

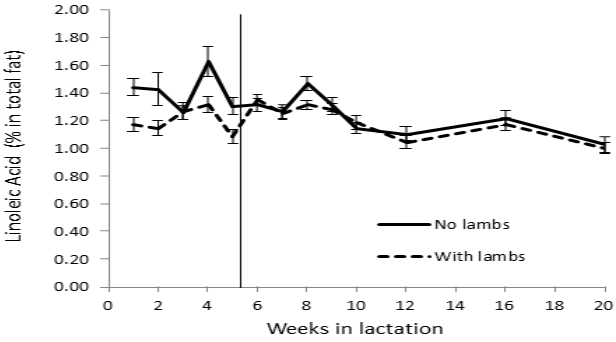

Figure 2 Linoleic acid content of sheep milk fat in two different weaning systems (vertical line - lamb weaning)

Conclusion Partial suckling of lambs, during the first 5 weeks of lactation, adversely affected both the total fat content of milk obtained by machine milking of their dams, and the fatty acid profile of the milk fat. Among other fatty acids, linoleic acid, an essential fatty acid, is particularly affected.

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Effects of forage and breed types on the efficiency of utilisation of nitrogen in hill replacement ewes

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Implications Manure nitrogen output in hill replacement ewes can be predicted from N intake. The accurate prediction of manure N output is essential to reduce the environmental impact of sheep production system.

Introduction Nitrogen (N) excretion from sheep production systems is a considerable source for groundwater pollution and global warming. The efficiency of utilisation of N can be used to calculate N excretion as an environmental footprint. The aims of this experiment were to investigate how different forage and breed types affected the efficiency of utilisation of N and to use these data to develop relationships between N intake and output in hill replacement ewes.

Material and methods Thirty six hill replacement ewes (18 pure Scottish Blackface and 18 Swaledale x Scottish Blackface (75:25)) aged approximately 12 months and weighing 42 ± 4.0 kg were allocated to 3 treatments balanced for breed and live weight. Each breed was offered 3 forages *ad libitum*: grass nuts, fresh grass and grass silage (CP = 152, 114 and 100 g/kg DM, respectively). Grass nuts were sourced from a commercial supplier (Drygrass South Western Ltd, Burrington, UK). Fresh grass was harvested daily from the primary regrowth of perennial ryegrass, while the grass silage was made from the 2nd harvest of perennial ryegrass ensiled with Ecocyl as an additive. The animals were individually housed and fed experimental forages for at least 14 days before being transferred to individual calorimeter chambers for a further 4 days with N intake and output measured. Feed intake and live weight were reported by Zhao *et al*. (2013). Data were analysed using two-way ANOVA for effects of forage type, breed and their interaction, with a probability level of P = 0.05 for significance between treatments, using the Genstat statistical package.

Results The effects of forage and breed types on the efficiency of utilisation of N are presented in Table 1. There were no interactions between breed and forage types on any variable of N intake, output or retention. Sheep offered grass nuts had higher N intake, faecal N output, manure N output and faecal N as a proportion of N intake (P<0.001) compared to those given fresh grass and grass silage. However, N retention as a proportion of N intake was not affected by diets. Moreover, Scottish Blackface produced more urinary N than Swaledale x Blackface (P=0.05). There were no significant differences between the 2 breeds in any other variable. The statistical analysis using all data found that there was a significant linear relationship (P<0.001) between manure N (g/d, y) and N intake (g/d, x), $y = 0.76x - 0.41$, $R^2 = 0.84$.

Table 1 Effects of forage and breed types on the efficiency of utilisation of nitrogen

	Swaledale x Blackface			Scottish Blackface				Probability		
	Grass Nuts	Fresh Grass	Grass Silage	Grass Nuts	Fresh Grass	Grass Silage	s.e.	Breed	Forage type	Interaction
N intake (g/d)	39.3^{b}	17.7^{a}	13.2^{a}	38.3^{b}	17.1^{a}	15.0^{a}	3.15	0.910	< 0.001	0.823
Faecal N (g/d)	20.1^{b}	3.7^{a}	4.9^{a}	18.7^{b}	4.3^{a}	5.3^{a}	1.84	0.941	< 0.001	0.710
Urinary N (g/d)	7.2^{a}	7.7^{a}	4.8^{a}	13.0^{b}	7.7^{a}	6.5^{a}	2.13	0.050	0.023	0.172
Manure N (g/d)	27.3^{b}	11.4^{a}	9.7^{a}	31.7^{b}	12.0^{a}	11.8^{a}	2.90	0.132	< 0.001	0.668
N retention (g/d)	12.0^{b}	6.3^{a}	3.5^{a}	6.6^{a}	5.0^{a}	3.1^{a}	2.46	0.103	0.011	0.348
Faecal N/N intake	0.50^{c}	0.23^{a}	0.39^{b}	0.49^{c}	0.26^{a}	0.36^{b}	0.046	0.912	< 0.001	0.681
Urinary N/N intake	0.19^{a}	0.43^{b}	0.42^{b}	0.34^{ab}	0.45^{b}	0.45^{b}	0.096	0.274	0.036	0.582
Manure N/N intake	0.69	0.66	0.81	0.83	0.71	0.81	0.114	0.331	0.350	0.739
N retention/N intake	0.31	0.34	0.19	0.17	0.29	0.19	0.114	0.331	0.350	0.739

^{a,b,c} means within rows with same superscripts are not significantly different (P>0.05)

Conclusion Sheep offered grass nuts had higher N intake and excretion than those given fresh grass or grass silage. However, N retention rate is not affected by the forage type. There was no difference in the efficiency of utilisation of N between Scottish Blackface and Swaledale x Scottish Blackface. Manure N output in hill replacement ewes can be predicted from N intake.

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029

Effects of breed and forage types on methane emission factors for hill replacement ewes aged between 9 and 18 months

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Implications The methane emission factor for replacement ewes offered fresh grass was on average 5.7%, which is lower than the value currently used for adult ewes in the UK national greenhouse gas inventory (6.5%, IPCC 2006).

Introduction Breeding from replacement hill ewes rarely takes place until they are approximately 18 months old, representing a significant overhead cost in terms of greenhouse gas (GHG) emissions compared to the lowland sector where some replacement ewes can be mated when they are about 8 months old, depending on the animal size and weight. Within the Tier 1 methodology of IPCC (2006), methane (CH_4) emission factors of adult ewes are also applied to replacement hill ewes, despite their smaller body size, resulting in an overestimate of total GHG emissions. The aims were to establish CH_4 emission factors specific to growing hill ewes and investigate the effects of different breeds and forage types.

Material and methods Animal and gas data were measured over three periods, using animals at approximately 9, 13 and 17 months of age respectively. For each period, 36 replacement ewes were allocated to two or three diet groups balanced for breed and live weight (LW). Three breed types were studied: Scottish Blackface (B, all age groups), Blackface × Swaledale × Blackface (BSB, 13 and 17 mths) and Texel × Blackface (TB, 9 mths). Animals were offered *ad libitum* either grass nuts (GN) as a common diet between age groups or a diet representative of standard practice, i.e. fresh grass (G), grass silage (GS) or hill grass (HG). Grass nuts were sourced commercially (Drygrass South Western Ltd, Burrington, UK). Fresh grass and grass silage were obtained from a predominantly perennial ryegrass sward. The hill grass was obtained from an upland sward predominated by *Holcus lanatus*, *Carex spp* and *Juncus effusus*. The animals were individually housed and fed experimental diets for at least 14 days before being transferred to individual calorimetric chambers for a further 4 days, with feed intake (measured as DM offered minus refusals) and CH_4 emissions measured for the final 3 days. Live weight was measured at the beginning of the study and after leaving the chamber. Data were analysed for each period using a REML variance components analysis with breed, diet and their interaction as fixed effects.

Results There were no significant interactions between breed and forage type for any of the response variables and for all three age groups. Blackface ewes had higher LWs than BSB, but lower than TB ewes (Table 1). Sheep offered GN had higher LW, DM intake (DMI) ($P < 0.001$) and CH_4 emissions (g/d) ($P < 0.001$), but lower CH_4 output per DMI and per GE intake (GEI) when compared to those given G, GS or HG (Table 1). There were no significant differences between breeds in terms of DMI, CH_4 emissions (g/d) and CH_4 output per DMI and GEI. Within each diet type, CH_4 emission factors appeared similar across age groups (e.g. for GN: between 4.4 and 4.7 % of GEI).

Table 1 Effects of breed and forage types on live weight and methane emission factors

	Breed				Diet				
9 mths:	B	TB	s.e.d	P	GN	GS		s.e.d	P
LW (kg)	35.2	41.3	1.30	< 0.001	41.1	35.3		1.30	< 0.001
CH_4/DMI (g/kg)	19.9	19.2	1.37	0.64	15.2	23.9		1.34	< 0.001
CH_4-E/GEI (MJ/MJ)	0.058	0.056	0.0040	0.56	0.044	0.069		0.0040	< 0.001
13 mths:	B	BSB			GN	GS	G		
LW (kg)	46.8	42.7	1.43	0.006	48.9 [a]	42.6 [b]	42.9 [b]	1.75	0.001
CH_4/DMI (g/kg)	17.6	18.7	1.07	0.30	15.1 [a]	20.3 [b]	19.0 [b]	1.31	< 0.001
CH_4-E/GEI (MJ/MJ)	0.053	0.057	0.0033	0.27	0.044 [a]	0.065 [b]	0.057 [b]	0.0040	< 0.001
17 mths:	B	BSB			GN	G	HG		
LW (kg)	51.7	46.4	1.52	< 0.001	54.7 [a]	47.8 [b]	44.6 [b]	1.84	< 0.001
CH_4/DMI (g/kg)	17.9	18.6	1.15	0.45	16.2 [a]	19.2 [b]	19.4 [b]	1.40	0.049
CH_4-E/GEI (MJ/MJ)	0.053	0.055	0.0034	0.42	0.047 [a]	0.058 [b]	0.057 [b]	0.0041	0.024

Conclusions Feeding grass nuts, rather than fresh grass or grass silage can reduce enteric CH_4 emissions as a proportion of feed intake in sheep, while there is no difference in CH_4 emissions between breeds. Methane emission factor for ewes at 13 and 17 mths offered fresh grass was on average 5.7%, which is lower than the value currently used (6.5%, IPCC 2006).

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030

Benefit of by-pass based protein supplementation on periparturient ewe performance

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Implications The greater sensitivity of ewe performance to digestible undegradable protein (DUP) supply over that of crude protein (CP) and metabolizable protein (MP) *per se* suggests that ewe MP supplementation is more effective when based on by-pass protein.

Introduction It was recently observed that MP supplementation using a faba-bean based ration failed to increase ewe performance, whilst the latter was achieved through the use of a xylose-treated soya bean meal (SoyPass) based ration that was calculated to supply similar levels of additional MP supply over a low MP control (Sakkas *et al*., 2012). As that SoyPass ration would have supplied more DUP than that faba bean ration, it is hypothesized here that ewe performance may be more sensitive to DUP than to CP or MP *per se*.

Material and methods Thirty-two twin-bearing Texel-tupped 2-3 parity Scottish mules, with average body weight (BW), condition score (CS) and faecal egg count (FEC with 95% CI) of 77.3±2.1 kg, 2.85±0.05 and 225 (194-261) epg at day -41 (day 0 is parturition) were housed individually and allocated to one of four feeding treatments until day 30 into lactation. The feeding treatments 1, 2, 3 and 4 were formulated to supply 137, 157, 178 and 157 g CP, 87, 93, 107 and 107 g MP, and 29, 34, 41, and 49 g DUP per kg restrictedly fed mixed ration. The latter consisted of 30% hay and 70% concentrates, in which additional soya bean meal or SoyPass were included through replacing appropriate amounts of beet pulp, soya hulls and protected fat to formulate feeding treatments 2 to 4. Ewes were not dewormed at housing, to assess feeding treatment effects on naturally acquired gastrointestinal nematode infections. Ewes were condition scored, faecal sampled for FEC fortnightly and weighed weekly, whilst lambs were weighed at birth, twice daily until day 3 and weekly thereafter. Performance and log-transformed FEC data were analysed using ANOVA with orthogonal contrast statements to assess linear and quadratic effects of feeding treatments, using initial BW, CS and FEC as covariates.

Results Table 1 shows that feeding treatment linearly affected ewe body weight gain during late pregnancy and early lactation, with a similar tendency for litter weight gain from day 3 onwards. Feeding treatments did not affect litter birth weight and weight gain over the first three days, i.e. a proxy of colostrum production. Feeding treatment and its interaction with time were not significant for FEC (P>0.50), which averaged 356 (314 to 404) epg during late pregnancy and 366 (316-424) epg during lactation.

Table 1 Ewe performance on feeding treatments, calculated to supply different levels of CP, MP and DUP (see above).

	Feeding treatments					P-values	
Parameter	1	2	3	4	sed	Linear	Quadratic
Ewe BW (kg)							
Day -4	83.1	84.6	85.8	85.1	1.30	0.096	0.246
Day 0	70.3	72.4	73.2	72.6	1.56	0.132	0.246
Day 30	62.1	63.2	65.7	66.3	1.68	0.013	0.811
Ewe BCS							
Day -7	2.65	2.70	2.72	3.01	0.14	0.017	0.233
Day 8	2.41	2.63	2.61	2.70	0.14	0.075	0.520
Day 30	2.27	2.51	2.30	2.52	0.12	0.151	0.901
Litter BW (kg)							
Day 0	9.8	10.5	10.1	9.3	0.65	0.321	0.114
Litter BW gain (g/day)							
Day 0 – 3	860	837	786	888	128	0.933	0.497
Day 3 – 30	618	645	686	664	30	0.068	0.253
Day 0 – 30	645	665	702	685	29	0.106	0.375

Conclusion These data supports the view that ewe performance may be more sensitive to DUP nutrition than to CP or MP nutrition *per se*. Effects on ewe body weight gain and condition scores were more pronounced than those on litter weight gain, which is consistent with the view that milk production may take priority over maintenance of body reserves (Houdijk *et al*., 2001). However, the magnitude of feeding treatment effects, especially on litter weight gain, were smaller than those observed in earlier studies (e.g. Kidane *et al*., 2010), which likely arose from a smaller degree of MP scarcity for feeding treatment 1. It cannot be excluded that the latter may have contributed to the lack of feeding treatment effect on ewe FEC.

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031

The effect of iron supplementation on hepatic copper content of growing lambs

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Implications Dietary iron increases hepatic iron storage while decreasing copper. However, there was no effect on other minerals.

Introduction Copper (Cu) is considered to be one of the most important trace elements that are required for normal biological function in all living higher organisms (Suttle, 2010). Clinical copper deficiency is recognised as an important nutritional problem in ruminant animals resulting in loss of performance along with a decrease in health. The main cause of deficiency is due to interactions with other minerals, and the main factors influencing Cu metabolism in sheep are molybdenum, sulphur and iron. The interaction between molybdenum (Mo) and sulphur (S) has been well documented and shown to be mediated via the production of thiomolybdates in the rumen (Gould and Kendal, 2011). The mechanism by which Fe alters Cu metabolism has not been well characterised or quantified. Therefore, the aim of this study was to investigate the effects of dietary Fe and S on hepatic Cu storage.

Material and methods Thirty-six Texel-cross lambs (33.2 kg liveweight, s.e.d 0.41) were blocked by weight and sex and then randomly allocated to one of four treatments, housed in individual pens. Lambs were fed an isonitrogenous, isoenergetic diet based on dried grass, formulated for them to gain 200g /day (AFRC, 1993) over a 6 week study. All lambs were fed the basal diet, with the control lambs having no additional supplements, lambs on the Low Fe diet were supplemented with 250 mg Fe per kg DM, lambs on the Mid Fe diet were supplemented with 500 mg Fe per kg DM and lambs on the High Fe diet were supplemented with 750 mg Fe per kg DM. Lambs were weighed and blood sampled weekly. After 6 weeks, the lambs were slaughtered in a commercial abattoir and liver samples collected. Mineral levels of liver and blood plasma were analysed using ICP-MS (Cope *et al.*, 2009). All data was analysed by analysis of variance (ANOVA) as a random block design (GenStat, 15th edition) and for blood data week one was used as a covariate. Daily liveweight gain (DLWG) was calculated by regression analysis and analysed by ANOVA. Significance differences between means were tested using the protected least significant difference (LSD).

Results Control and low Fe lambs trended to have higher hepatic Cu concentrations compared with the Mid and High Fe fed groups (P=0.067). In addition, the total liver Cu store was significantly greater (P=0.016) in control and Low Fe fed groups compared with the Mid and High groups. Lambs fed High and Mid Fe diet had significantly greater (P <0.001) liver Fe concentration than Control and Low groups (Table 1). There was no effect (P>0.05) of dietary treatments on DLWG or feed intake. Plasma Fe was significantly higher (P <0.05) in week 4 and 6 of the experiment in Fe supplemented lambs. However, plasma Cu concentration did not show any significant influences though-out the 6 weeks experimental period. There was no effect (P >0.05) of dietary treatments on the haematology parameters and superoxide dismutase activity (SOD) at any time point to the end of trial.

Table 1 Effect of dietary level of Fe on hepatic Cu and Fe concentration in growing lambs (µg/g DM)

Minerals	Treatment				s.e.d.	P Value
	Control	Low (250 Fe mg/kg $^{-1}$)	Mid (500 Fe mg/kg $^{-1}$)	High (750 Fe mg/kg $^{-1}$)		
Cu	313.30	322.30	242.80	205.00	49.250	0.067
Fe	362.30^{a}	407.00^{a}	551.00^{b}	631.90^{b}	37.040	<0.001

Conclusion With increasing dietary Fe level, liver Cu concentration decreased but Fe concentration increased in accordance to inclusion dose. Dietary Fe levels had no effect on lamb performance parameters and blood components values. The mechanism by which dietary Fe reduces liver Cu needs to be elucidated.

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Pathogenesis of Pestivirus infections

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Implications Pestiviruses are livestock pathogens capable of causing severe outbreaks of disease, particularly amongst cattle, sheep and pigs. The viruses are highly mutatable and present problems for their diagnosis and control. Although pathogenesis can be complex, our research has unravelled some of its fascinating pathways.

Introduction The major pestiviruses are grouped into four clusters; bovine virus diarrhoea Group 1 (BVDV 1), bovine virus diarrhoea Group 2 (BVDV 2), Border disease virus (BDV) of sheep and Classical Swine Fever virus (CSFV). In this short lecture, I will deal only with the pathogensis of BVDV.
BVDV is an economically important important disease affecting cattle worldwide. In infected herds, losses are due to decreased fertility, secondary infections following immunosuppression and decreased milk production in acutely infected animals (Edwards and others 1986, Brownlie *et al* 2000). In 1998, it was estimated that 65 per cent of UK herds had experienced recent BVDV infection and 95 per cent of the national herd had been exposed to the virus (Paton and others 1998). In 2003, BVDV was calculated to cost the UK cattle industry £40 million per year placing the disease as the third largest loss after mastitis and lameness (£180 & £54 million per year, respectively) (Bennett and Ijpelaar 2003).

Acute disease Acute infection (first time infection of naive animals) can be variable from the sub-clinical or mild through to severe and fatal. This depends on the virus variation and virulence but, also, partly on level of exposure to virus. There is little doubt it can be highly immunosuppressive and exacerbate dual infections with other viral or bacterial agents. This is apparent with acute respiratory and enteric infections in calves. Interestingly, eradicating BVDV can significantly reduce the incidence of respiratory disease.

Persistent Infection (PI) This is an exception illustration of a viral persistent infection. If BVDV infects a dam in early pregnancy, the virus can pass across the placenta to infect the unborn foetus. The foetus, if before 120 days of its 280-day gestation, is not immune-competent to recognise this 'foreign invader' and allows it to establish in its tissues. When it does reach the point of immune-competence at around 110-120 days, the immune system recognises the virus as 'self' and become tolerant to the virus. From this point onwards on into neonatal and even adult life, the virus is accepted and, thus, no immune responses (antibody or cell-mediated) are generated. These PI calves thereafter shred enormous amounts of virus and become the major reservoirs of virus infection.

Mucosal disease This is a fatal syndrome of cattle, first described in 1953 (Ramsey and Chivers 1953). It was not until 1984 that the complex pathogenesis of this fatal syndrome was understood (Brownlie *et al* 1984). The trigger for this process started with the foetal infection to produce a PI neonate and then the mutation of the persisting virus to a cytopathogenic biotype that, under the cover of the BVDV tolerance, was able to infect and fatally damage the tissues. This will be explained within this presentation. The mechanism of the mutation and its impact on the immune system are now better understood. The importance of antigenic 'homology' between the persisting and the super-infecting virus has become evident after a series of critical experimental infections and its consequent pathology. These studies have given further insights into the tropism of the virus.

Conclusions It is hoped, that in a short lecture, I can unravel this extraordinary pathogenesis that, as yet, has not been seen with other viral infections.

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033

The epithelial-endothelial barrier in influenza virus pathogenesis

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Implications Our data provide new insights into the pathogenesis of influenza A virus.

Introduction Influenza A virus (IAV) is a major cause of worldwide morbidity and mortality. The natural reservoirs of IAV are wild waterfowl and shorebirds. In these species, IAV typically causes a mild or subclinical infection. Following infection of wild birds, some IAV subtypes can spread to domestic poultry. In domestic poultry, IAV can cause a mild or subclinical infection and is thus referred to as low pathogenic avian influenza (LPAI). Some LPAI can subsequently evolve in poultry to become into highly pathogenic avian influenza (HPAI), which typically causes a fatal and systemic infection. Humans can be directly infected with both LPAI and HPAI strains, with later often causing a systemic infection. HPAI can also spread to other mammals including tigers, leopards, domestic cats and dogs. Here, HPAI can also spread beyond the respiratory tract and cause systemic disease.

In humans, IAV can cause an infection of the lower respiratory tract resulting in viral pneumonia, which can then give rise to acute respiratory syndrome (ARDS). Clinically, ARDS is characterised by severe pulmonary lesions, respiratory insufficiency and a high case fatality rate. Central to the development of these pulmonary lesions is damage to the epithelial-endothelial barrier. Destruction of this barrier results in fluid leakage from interstitium, fibrin deposition and pulmonary haemorrhaging. Currently, much of our understanding of endothelial-epithelial damage during ARDS is derived from studies of bacterial sepsis, where the endothelium is the first site of damage. However, there are limited models available to describe how IAV, a pathogen which first infects the pulmonary epithelium, damages the endothelial-epithelial barrier. Here, we seek we seek to summarise and analyse the available literature regarding how IAV damages the epithelial-endothelial barrier in humans in order to provide a new paradigm for disease pathogenesis. We then support this paradigm using *in vitro* studies and we compare and contrast our proposed model of pathogenesis in humans to other animal species.

Material and methods Relevant articles were identified by searching PubMed and Google Scholar. *In vitro* studies were performed by seeding human epithelial cells seeded on the upper half of a transwell membrane whilst endothelial cells are seeded on the lower half. These cells are then grown in co-culture for approximately seven days and then IAV is added to the upper chamber. IAV-induced damage is then measured at 24 hours post-infection.

Results Our model suggests that damage to the epithelium plays a major role in IAV induced ARDS. Very early during infection IAV can directly target tight junctions on the pulmonary epithelium and induce alveolar fluid accumulation. Later, IAV can also induce epithelial apoptosis and necrosis, further exacerbating lung injury. Epithelial cells can also produce various chemokines in response to IAV. Neutrophils subsequently enter the alveolus and produce toxic granules which damage both the alveolar epithelium and endothelium. Chemokines can also recruit macrophages to the lung where they damage the epithelial-endothelial barrier by the production of a wide-array of cytokines and toxic molecules. Finally, whilst there have been some suggestions that IAV can infect, and damage, human endothelial cells, perhaps of greater importance in humans the ability of the endothelium to act as a driving force behind the ‘cytokine storm’ seen in severe IAV infections. This is marked contrast to the pathogenesis of HPAI in chickens and cats (following intestinal inoculation) where IAV infection of the endothelial is a key aspect of the viral pathogenesis.

Conclusion Damage to the pulmonary epithelial-endothelial barrier during IAV infection is a complex and multi-factorial event that involves primarily damage to the pulmonary epithelium. This is in contrast to models of ARDS derived from (e.g.) septic shock, and IAV infection of other animals species where there is a more prominent role for damage to the endothelium.

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034

Host response to footrot in sheep

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Implications Footrot is an endemic bacterial infection of the interdigital skin of the sheep foot that results in lameness. This disease, present in >95% of flocks, is the key priority of the UK sheep industry due to its health, welfare and economic impact, with an annual cost of £24-£84m. Elucidating the inflammatory response will ultimately contribute to a more targeted development of vaccines and their adjuvants for the prevention of ID and footrot.

Introduction The pathogenesis of footrot is complex and multifactorial. Physical damage to the interdigital skin is required to initiate disease. This is often caused by prolonged exposure to moisture. Bacterial replication in this damaged skin leads to interdigital dermatitis (ID) where the superficial epidermal layers are inflamed, damaged and slough off irregularly. This may progress to footrot with separation of the hoof horn capsule from the underlying sensitive tissue. The bacterial cause is *Dichelobacter nodosus*, an obligate anaerobe, without which hoof horn separation does not occur. A second bacterium, *Fusobacterium necrophorum*, is also implicated in the disease process. Since much of the pathology is as a result of the host response, the aim of this research was to investigate this in interdigital dermatitis (ID) and footrot focussing on the histological appearance and the expression of Toll-like receptors (TLR), molecules crucial for host recognition of bacterial infections, and pro-inflammatory cytokines of clinically normal, ID and footrot affected tissues.

Material and methods Six mm necropsies (at abattoir) from healthy feet and across lesions in ID and footrot scored feet were sampled for RNA isolation or fixed for histopathology. RNA was isolated and cDNA synthesised. Transcript levels of *TLR1,2,4* and *6, IL-1β* and *TNFα* were measured by quantitative PCR and expressed as relative expression compared to the housekeeping gene *β-actin* (M value < 0.05) (Hughes *et al*., 2007). Histopathology was scored on H&E stained tissue sections and in tissue Gram stain (Sigma) was used to visualize bacteria.
Statistical analysis: The biopsy data were modelled in a mixed effect two level model which incorporated autocorrelation of feet within sheep. ANOVA followed by Turkey's multiple was carried out on clinical score vs. histopathological score.

Results Expression of *TLR2*, *TLR4* and *IL-1β* was significantly increased in severe ID (CI: TLR2 1.41-4.45, TLR4 1.41-4.8, IL-1β 1.35-6.27) and footrot (CI: TLR2 1.71-4.36, TLR4 4.65-4.63, IL-1β 4.37-8.68). In contrast, *TLR6* expression was decreased in mild ID (CI: (-2.81) - (-0.22)) but not in severe ID and footrot. Expression of *TLR1* and *TNFα* was similar in all samples. ID and footrot lead to progressive chronic-active pododermatitis with a mixed lymphocytic and neutrophilic infiltration, developing from a mild form in clinically normal feet, to moderate in ID and to a focally severe form in footrot, with frequent areas of purulence. There is a significant correlation between clinical score and histopathological score ($p=0.0009$).

Conclusion We present here the first study of innate immune responses to ovine ID and footrot, linking immunopathology, inflammatory mediator expression localised to the interdigital skin.

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Investigating the bovine caruncular epithelial cell line as a model for *Listeria monocytogenes* invasion of reproductive tissues in ruminants

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Implications Listeriosis is of major veterinary importance due to its impact on animal welfare and its designation as a food-borne pathogen. As the cause of bovine abortion, encephalitis and keratoconjunctivitis, Listeriosis must be addressed in order to sustain the farming industry financially, with every abortion costing the dairy farmer £630.

Introduction Over the last 17 years the percentage of bovine abortions caused by *Listeria* has risen by 2.9%. This bacterium has particular tropism for the gravid uterus, whereby infection usually leads to abortion in late gestation. The route by which *Listeria* infects the ruminant placentome is relatively unknown; most research has focused on infection of human and rodent, where comparisons are hard to draw due to differences in placental structures. *Listeria* invasion is mediated by the interaction of InlA and InlB with host E-cadherin and c-met tyrosine kinase (Met) receptors, respectively. These interactions have been shown as species specific when analysing rodent and human sequences; therefore we aimed to align ruminant amino-acid sequences for these host receptors to uncover whether ruminant internalins are permissive to interaction. Secondly, we aimed to investigate the ability of L. monocytogenes to infect cells of the bovine foeto-placental barrier, using a range of environmental and clinical *Listeria* isolates and two cell types: Caco-2 (a human colon epithelial cell line used routinely to investigate *Listeria* pathogenesis) and Bovine Caruncular Epithelial Cells (BCECs, maternal cells of the foetal/maternal interface of the placentome).

Material and methods A range of environmental and clinical *L. monocytogenes* isolates were used to infect Caco-2 and BCECT-1 cells ($2x10^5$ cells/well or on gelatine coated glass coverslips). Following 1h infection, extracellular bacteria were destroyed using 100µg/ml gentamycin for 1h, then after cell lysis, bacteria were plated on HI agar or fixed using 4% (v/v) paraformaldehyde. CFUs were enumerated after 17h incubation at 37°C. Fixed cells were stained with Tetramethylrhodamine B isothiocynate labelled phalloidin (actin) and DAPI (DNA/nuclei) and analysed by fluorescent microscopy. E-cadherin and Met protein sequences (amino acids 382-455 and 151-200, respectively) from different species were used to create multiple sequence alignments. From the Met alignments a dendogram was created. Statistical analysis was performed using a one or two-way analysis of variance (ANOVA), followed by Holm-Sidak's multiple comparisons test and Tukey's test, respectively.

Results Sequence alignment showed a proline at residue 16 of ruminant E-cadherin, essential for interaction with InlA. Sequence alignments and dendogram for Met demonstrated that cow and sheep are most closely related to InlB permissive species (e.g. human) than to the rabbit Met which is unable to interact with InlB. All *Listeria* isolates tested were able to invade Caco-2 and BCEC cells, however the level of invasion was significantly lower ($p<0.0001$) for BCEC cells, compared to Caco-2 cells. This finding was confirmed using flourescent microscopy with the average number of cells infected as 31.0% and 11.9% (total cells counted: 1105 and 195), for Caco-2 and BCEC cells, respectively. Of the cells infected, the majority of infected Caco-2 cells contained >10 bacteria (52.3%) whereas the majority of infected BCEC cells had a single *Listeria* invading (81.8%). These results show that *Listeria* invasion was unevenly distributed amongst Caco-2 cells 2h post-infection.

Conclusion Our results suggest that ruminants are permissive to InlA and InlB interactions with their host receptors, mediating *Listeria* invasion. *Listeria* is capable of invading BCEC cells but to a lesser extent than Caco-2 cells, even for bovine abortion isolates, capable of causing Listeriosis at the placentome *in vivo*.

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036

Microbiota of cow's milk; distinguishing healthy, sub-clinically and clinically diseased quarters

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Implications Using a culture independent metagenomic approach we were able to better describe bovine milk microbiota in health and disease. Studies of this type have the potential to improve our understanding of mastitis etiopathogenesis and can eventually lead in a better confrontation of the disease.

Introduction Intramammary infections are currently defined as identified predominantly by aerobic culture, modified anaerobic and in some cases by molecular diagnostics. However, despite advances in diagnostics approximately 25% of clinical mastitis samples are culture negative or show no significant pathogens. Hunt *et al*. (2011) used barcoded pyrosequencing to characterize the diversity of bacterial communities in milk samples taken from human mammary glands and showed that this technique identified a much greater diversity of bacteria in milk than what had previously been reported. However, similar studies using bovine milk are scarce. In a previous study, our group argued that sequencing and analysis of hyper variable regions within the 16S rRNA gene provided relatively rapid and cost-effective methods for assessing bacterial diversity in mammary secretion from healthy and affected cows (Oikonomou *et al*. 2012). The objective of the present study was to use pyrosequencing of the 16S rRNA gene to describe the microbial diversity of bovine milk samples derived from clinically unaffected quarters across a range of somatic cell counts (SCC) values or from clinical mastitis, culture negative quarters. The obtained microbiota profiles were used to distinguish healthy, subclinically and clinically affected quarters.

Material and methods Two dairy farms were used for the collection of milk samples. A total of 177 samples were used. Fifty samples derived from healthy, culture negative quarters with a SCC of less than 20,000 cells/ml (group 1); 34 samples derived from healthy, culture negative quarters, with a SCC ranging from 21,000 to 50,000 cells/ml (group 2); 26 samples derived from healthy, culture negative quarters with a SCC greater than 50,000 cells/ml (group 3); 34 samples derived from healthy, culture positive quarters, with a SCC greater than 400,000 (group 4, subclinical); and 33 samples derived from clinical mastitis, culture negative quarters (group 5, clinical). Bacterial DNA was isolated from these samples and the 16S rRNA gene were individually amplified and pyrosequenced. The obtained sequences file was uploaded in the Ribosomal Database Project (RDP) pipeline initial processor that trimmed the 16S primers, tag sorted the sequences, and filtered out additional sequences of low-quality. DECIPHER was used for chimera sequences identification. RDP Classifier at the RDP's Pyrosequencing Pipeline was used to assign 16S rRNA gene sequences of each sample to the new phylogenetically consistent higher-order bacterial taxonomy using an 80% confidence threshold, providing information regarding different genera prevalence in each sample. Different genera prevalence in each sample derived from the above described analysis were used as covariates in stepwise discriminant analysis models. The five groups of samples were used as the categorical variable in these analyses.

Results All samples analyzed revealed great microbial diversity. Four bacterial genera were present in every sample obtained from healthy quarters (*Faecalibacterium* spp., unclassified Lachnospiraceae, *Propionibacterium* spp. and *Aeribacillus* spp.). Discriminant analysis models showed that samples derived from healthy quarters were easily discriminated based on their microbiota profiles from samples derived from clinical mastitis, culture negative quarters; that was also the case for samples obtained from different farms. *Staphylococcus spp.* and *Streptococcus spp.* were among the most prevalent genera in all groups while a general multivariable linear model revealed that *Sphingobacterium* and *Streptococcus* prevalences were associated with increased 10 log SCC. Conversely, *Nocardiodes* and *Paenibacillus* were negatively correlated, and a higher percentage of the genera was associated with a lower 10 log SCC.

Conclusion The current study presents a cross-sectional description of the milk microbiome in healthy and subclinically and clinically affected quarters. Although this provides a first evaluation of the bacterial flora in milk, more interesting developments may be expected from longitudinal studies. The dynamics of the milk microbiome in healthy quarters during different phase of early mammary growth, gestation and lactation will provide more insight in the mechanisms that lead to the establishment of a healthy gland. Given that DNA sequencing technology has advanced at an incredible pace in recent years, leading to astonishing decreases in sequencing cost, such culture independent approaches based on next generation sequencing may also eventually provide with powerful diagnostic tools for mastitis and subclinical mastitis.

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Veterinarians' perceptions of mycotoxicosis and other silage-related diseases in ruminant livestock

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Implications Difficulties in differential diagnosis make mycotoxicosis a disease of uncertain prevalence. Metabolomic research is needed to improve diagnosis and to confirm the efficacy of mitigation measures.

Introduction Mycotoxicosis and other diseases linked to contaminated silage and moist animal feeds are associated with reduced ruminant livestock performance reflecting altered digestion and metabolism, endocrine malfunctions and immunosuppression (Scudamore and Livesey, 1998; Wilkinson, 1999; Fink-Gremmels, 2008; Kiyothong *et al*., 2012). Diagnosis of mycotoxicosis is not easy due to multi-causality and lack of metabolomic indicators. There is a need for epidemiological information to assist in determining the scale of the problem on farm.

Material and methods To gain information on the diagnosis, prevalence, incidence and mitigation of mycotoxicosis and other silage-related diseases, a postal survey was undertaken of eighty-six large animal veterinary practices in the south-west of England between July and September 2013.

Results Thirty-eight responses were received (0.44 return rate). One practice reported that mycotoxicosis had been seen in approx. 0.8 of dairy herds, three said that mycotoxicosis occurred in at least 0.5 of herds and seven estimated that 0.1 of dairy herds were affected. The disease had not been seen by three practices (0.08 of responses). Eleven practices (0.29 of responses) had observed mycotoxicosis in beef herds and five (0.13) had seen the disease in sheep flocks. In most cases of suspected disease there was no further confirmation by laboratory analysis. Seventeen practices (0.45 of responses) said that differential diagnosis was confirmed ex *post* by observing the response of livestock to the addition of a mycotoxin binder to the diet. Signs used in diagnosis are as presented in Table 1. Nineteen practices (0.50 of responses) were of the opinion that the incidence of mycotoxicosis in dairy herds in the south-west of England was increasing; ten (0.26 of responses) considered that the disease was over-diagnosed. The incidence of analysis of silage for mycotoxins was less than 0.10. Nineteen practices (0.50) indicated that 0.23 of clients (range 0.02 to 0.60) always used a mycotoxin binder, whilst twenty-four (0.63) practices had clients who occasionally added a binder (average 0.25, range 0.05 to 0.60). Twenty practices (0.53) responded that they had clients who never used a binder (average 0.59, range 0.20 to 0.90). Of the other silage-related diseases, 0.82 reported listeriosis with 0.50 citing it as the most prevalent. Increased incidence of silage-related diseases may be the result of increased understanding and recognition of mycotoxicosis and other conditions.

Table 1 Signs used by veterinarians to aid the diagnosis of mycotoxicosis in farm livestock

	n	Proportion
Reduced milk yield	34	0.89
Visual evidence of contaminated silage	29	0.76
Reduced appetite	26	0.68
Reduced reproductive performance	23	0.61
Increased incidence of other diseases	18	0.47
Confirmed contamination of silage with mycotoxins	17	0.45
Increased incidence of laminitis, lameness and inflammatory issues	14	0.37
Diarrhoea	12	0.32
Reduced growth rate	7	0.18
Other factors	4	0.11

Conclusion Mycotoxicosis and listeriosis are considered by veterinarians to be significant potential disease hazards to livestock. Emphasis should be on efficient management of silage and other feeds with the goal of minimising moulds and undesirable bacteria. Metabolomic research is needed to aid the diagnosis of mycotoxicosis on farm.

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038

Study of the milk metabolome profile to describe rumen function and diet utilization in dairy cows fed different types of carbohydrate and protein concentrations.

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Implications Analysis of milk using flow infusion electrospray ionization mass spectroscopy (FIE-MS), in contrast to Fourier transform infrared spectroscopy (FTIR), could help nutritionists to predict the efficiency of N utilization under on-farm conditions. This could be done by developing prediction equations to identify inefficient animals or feeding situations.

Introduction Unbalanced diets can lead to inefficient feed utilization by the ruminant and high N losses that often provoke environmental problems. To optimize ruminant nutrition, measurements of rumen fermentation, nutrient flow to the intestine and overall efficiencies of diet utilization are required. These measurements are not feasible under on-farm conditions and there is a need for simple and cost-effective techniques that could provide this information. Milk represents the most accessible biological sample from dairy cows. Therefore, the aim of this report was to investigate whether milk metabolite profiles can provide insight into the efficiency of diet utilization by dairy cows. Two high-throughput techniques were considered: FTIR spectroscopy and FIE-MS.

Material and methods Four diets combining 2 protein levels (10.8% vs. 14.4% CP) and 2 energy sources (1.3 vs. 2.9 NDF/starch ratio) were compared in four Holstein cows, fitted with rumen and duodenum cannulas, using a 4x4 Latin square design to generate variability in nutrient utilization. Experimental details and performances have been reported previously (Fanchone *et al*., 2013). Milk samples (n=96) were taken from morning and evening milking for 3 non-consecutive days at the end of each experimental period. For FTIR analysis, milk was defrosted and infrared spectra were collected by transmission spectroscopy using an Equinox 55 FTIR spectrometer fitted with HTS-XT 96 well plate reader (Bruker Optik GmbH, Germany) which collected spectral information in the range 4000-500cm^{-1}. For FIE-MS, milk was skimmed, deproteinized, and injected in a surveyor liquid chromatography system and data were acquired (range from 15-1200 m/z) in altering positive and negative ionization profile mode on a LTQ linear ion trap (Thermo, Electron Corporation, CA, US). Correlations between the FTIR or FIE-MS profiles and production data were performed using partial least square regressions (PLS) using Matlab. Prediction models were cross-validated and their fit was assessed by the coefficient of determination (R^2) and the root mean square error of the cross validation ($RMSE_{CV}$).

Table 1 Prediction of cow performance by milk analysis.

	Range	**FTIR**		**FIE-MS**	
	Min-Max	$RMSE_{CV}$	R^2_{CV}	$RMSE_{CV}$	R^2_{CV}
DM intake (kg/d)	18.6-21.8	1.09	0.04	0.63	0.58
Rumen data					
pH	6.26-6.86	0.14	0.16	0.11	0.46
NH_3 (mg/l)	16.3-173	49.5	0.00	38.9	0.37
VFA (mM)	88.2-120	9.44	0.01	6.42	0.53
Duodenal flow (kg/d)					
DM	9.89-15.0	1.66	0.00	1.43	0.20
Non-NH_3-N	0.21-0.47	0.14	0.00	0.12	0.26
Microbial N	0.21-0.49	0.65	0.09	0.75	0.03
Milk (kg/d)					
Yield	18.6-27.7	2.67	0.09	1.55	0.65
Fat	0.63-1.10	0.14	0.01	0.85	0.49
Lactose	0.90-1.38	0.13	0.12	0.63	0.78
Urea (g/d)	1.40-9.28	2.48	0.00	1.40	0.59
N balance (g/d)					
N intake	0.30-0.46	0.06	0.00	0.03	0.60
Milk N	84.8-134	13.0	0.14	7.99	0.66
Faecal N	135-182	14.7	0.09	13.00	0.22
Urinary N	45.9-152	41.9	0.02	24.5	0.52
N use ratios					
Urinary N/N int.	0.14-0.36	0.07	0.10	0.05	0.40
Milk N/Urinary N	0.75-2.34	0.51	0.00	0.41	0.37
Milk N/N intake	23.2-34.1	3.36	0.00	2.29	0.56
Manure N/Milk N	1.71-2.84	0.35	0.00	0.27	0.47

*Grey cells indicate coefficients of determination above 0.5

Results Milk metabolomic profiles, in comparison to plasma (Belanche *et al*., 2014), showed a lower correlation with all productive parameters studied. FTIR spectroscopy showed poor predictions for all parameters investigated. Similarly, data from FIE-MS analysis were poorly correlated with rumen fermentation patterns and duodenal flows of nutrients (R^2<0.53). However, FIE-MS data gave moderate correlations with N intake, milk N and urinary N outputs (R^2 from 0.52 to 0.66). The best correlations were found between milk FIE-MS spectra and yields of milk, milk urea, and lactose (R^2 from 0.59 to 0.78).

Conclusion Plasma is preferable to milk as sample to analyse for the efficiency of diet utilization. However, milk is more accessible and when analysed by FIE-MS can provide some insight into N partitioning in the animal. More research is needed using a greater number of samples to confirm whether milk analysis by FIE-MS can truly been used to identify animals or dietary situations which led to low efficiencies of N utilization.

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Potential use of Fourier transform infrared spectroscopy (FTIR) and flow infusion electrospray ionization mass spectroscopy (FIE-MS) as fast fingerprinting methods to predict efficiency of nitrogen utilization in cows fed different diets

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Implications In order to predict efficiency of N utilization by dairy cows, 2 bio-fluids (plasma and milk) and 2 fingerprinting techniques were evaluated (FTIR and FIE-MS). Our results showed that plasma analysis using FIE-MS provided the most accurate estimates and could be used to identify inefficient animals in on-farm conditions.

Introduction Nitrogen pollution represents a major challenge for water quality and the EU already has several directives based on inspections, regulations and fines to manage this problem. Considering that ruminants habitually have a lower efficiency of N utilization than monogastrics, ruminant farmers should be prepared to minimize the N pollution derived from their activities. To this end, a challenge for the farmer is to determine the efficiency of N utilization for each individual animal in on-farm conditions. Thus, the aim of this report was to investigate the potential use of the plasma and milk samples to provide some insight on the N utilization by ruminants using 2 high-throughput techniques (FTIR and FIE-MS).

Material and methods Nine lactating multiparous and fistulated Holstein cows were used. To generate variability in the N utilization, animals were fed 3 different diets: standard protein level (0.155 kg/kg), low protein (0.122 kg/kg), and low protein supplemented with niacin (6 g/d). Experimental details and production parameters have been reported previously (Aschemann *et al*., 2012a, 2012b). Plasma samples (*n*=36) were taken before and 2.5h after feeding while milk (*n*=36) was sampled in 2 non-consecutive days. FTIR and FIE-MS analysis were conducted as previously described in this meeting. Prediction models based on partial least squares regressions were cross-validated and their fitting was assessed by the determination coefficient (R^2_{CV}) and the root mean square error of cross validation ($RMSE_{CV}$).

Table 1 Prediction of animal performance based on plasma and milk analyses.

	Dataset	Plasma FTIR		Plasma FIEMS		Milk FTIR		Milk FIEMS	
	Min-Max	$RMSE_{CV}$	R^2_{CV}	$RMSE_{CV}$	R^2_{CV}	$RMSE_{CV}$	R^2_{CV}	$RMSE_{CV}$	R^2_{CV}
Intake (kg DM/d)	15.4-17.1	0.47	0.00	0.46	0.02	0.46	0.07	0.43	0.25
Duodenal flow									
OM (kg/d)	8.46-11.0	0.64	0.07	0.70	0.21	0.67	0.01	0.55	0.13
Non-NH_3-N (kg/d)	0.31-0.44	0.23	0.52	0.27	0.43	0.47	0.01	0.32	0.20
Microbial N (kg/d)	0.22-0.33	0.13	0.42	0.15	0.31	0.25	0.03	0.15	0.28
Utilizable CP (kg/d)	1.72-2.47	0.14	0.50	0.15	0.47	0.28	0.00	0.20	0.18
Urea N									
Blood (mg/l)	8.26-37.7	4.98	0.66	5.12	0.63	9.14	0.00	9.63	0.19
Milk (g/d)	1.73-7.21	1.29	0.61	0.98	0.75	1.82	0.33	1.97	0.17
N balance									
N intake (g/d)	297-429	32.6	0.52	0.02	0.80	49.2	0.02	46.8	0.17
Milk N (g/d)	124-163	12.1	0.12	11.5	0.23	11.8	0.08	9.79	0.42
Faecal N (g/d)	95.3-129	8.40	0.04	7.49	0.21	6.50	0.48	7.78	0.19
Urinary N (g/d)	42.3-135	23.3	0.49	14.5	0.80	34.7	0.00	35.4	0.03
Urinary N/N int.	0.13-0.31	0.04	0.48	0.03	0.77	0.06	0.01	0.07	0.03
Milk N/Urinary N	1.02-3.66	0.64	0.41	0.46	0.68	0.80	0.11	0.85	0.13
Milk N/N intake	30.9-48.7	4.01	0.44	4.01	0.41	5.06	0.13	5.52	0.09
Manure N/Milk N	0.98-1.88	0.21	0.45	0.17	0.63	0.27	0.10	0.30	0.00

*Grey shadows indicate parameters with high coefficient of determination (R^2>0.50)

Results Plasma analyses using either FTIR or FIE-MS were able to predict most parameters related with N metabolism, such as N intake, duodenal N flow, urinary N excretion, N partitioning and urea concentration in milk and plasma. In general, FIE-MS prediction models were more accurate than those derived from FTIR using plasma samples, especially for those predicting the efficiency of N utilization by the cow. On the contrary, milk metabolomic profile was poorly correlated with most of the parameters investigated, independently of the fingerprinting method used.

Conclusion Analysis of the plasma metabolome using FTIR or FIE-MS as high-throughput techniques has potential to estimate the efficiency of N utilization in cows in on-farm conditions. On the contrary, milk metabolomic profile seems to be less useful. More research in needed by using a greater number of samples to develop further prediction equations.

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Responses in blood met-haemoglobin (Met-Hb) concentrations of steers during adaptation to inclusion of nitrate in the diet and subsequent long-term feeding of nitrate

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Implications Consistently elevated blood Met-Hb concentration were recorded over a 100 day period but with no adverse effects on performance; Met-Hb therefore may not be a good indicator of toxicity when nitrate is fed to adapted cattle.

Introduction Nitrate poisoning in cattle is associated with acute ingestion of large quantities of forage containing high nitrate concentrations. The ingested nitrate is reduced to nitrite in the rumen and nitrite absorbed into the bloodstream reacts with haemoglobin (Hb) to generate met-haemoglobin (Met-Hb) thus reducing oxygen carrying capacity. Blood Met-Hb is used as a marker of nitrate poisoning with a value of 30% of total Hb associated with clinical symptoms. Recent interest in the controlled feeding of nitrate has been stimulated because the reduction of nitrate to ammonia in the rumen of adapted animals provides an alternative hydrogen sink to production of methane and is therefore a potential route for mitigating methane emissions. Here we report the long-term health and production implications of using nitrate as a means of reducing methane production in cattle.

Material and methods Charolais x (n=14) or Luing (n=14) steers were fed one of two complete diets consisting (g/kg, DM basis, forage:concentrate) of either 480:520 (Forage diet) or 75:925 (Concentrate diet), respectively. Diets were offered *ad libitum* to steers once daily. Nitrate was added progressively to the diets (up to 19 g nitrate / kg DM) over 4 weeks (0.25, 0.50, 0.75 and 1.00 of maximum inclusion). Blood samples for Met-Hb analysis were obtained approximately 3 h after fresh feed was offered on the day after dietary nitrate inclusion was increased (days 1 (0.25), 8 (0.50), 15 (0.75) and 22 (1.00)), respectively, and then 8 days after maximum nitrate inclusion was achieved (day 29). Feed intake and live-weight gains were then recorded over an 8 week period. Finally after completion of 8 wk period, on days 114 and 128, blood, samples were obtained to establish long-term responses in Met-Hb concentration. Met-Hb in blood was measured within 2 h of sampling by co-oximetry (Critical Care Express, NOVA Biochemical, Runcorn, UK). Data were analysed in Genstat using a repeated measures ANOVA including the effects of breed, diet, sampling day and their interactions. Data are reported as means and standard error of difference (Table 1).

Results Met-Hb concentrations (Table 1) increased with time ($P<0.001$; day 1 < (days 8 and 15) < (days 22, 29, 114 and 128) and there was an interaction between diet and sampling day ($P<0.001$) such that Met-Hb concentrations on the Concentrate diet were consistently greater from Day 29 onwards. There was consistent animal to animal variation in Met-Hb concentrations when offered the maximum nitrate intake (days 22 to 128). Of 28 steers, 6 always had Met-Hb concentrations less than median Met-Hb whilst 9 steers had Met-Hb concentrations greater than the upper quartile. Maximum values for Met-Hb (Table 1) were always less than the value considered clinically significant.

Table 1 Changes in blood Met-Hb concentration (% total Hb) in relation to nitrate intake and long-term nitrate feeding

Day	1	8	15	22	29	114	128	
Nitrate[1]	0.25	0.50	0.75	1.00	1.00	1.00	1.00	SED
Forage	0.26	0.78	0.80	3.50	2.16	1.29	3.54	0.820
Concentrate	0.32	0.62	0.98	2.80	4.53	6.46	4.95	
Maximum	0.50	2.00	3.20	9.50	11.60	15.40	10.30	

[1] Nitrate as proportion of maximum level of intake (19 g/kg DM)

Conclusion Increases in blood Met-Hb concentrations in response to feeding up to 14 g nitrate / kg DM were low. When 19 g nitrate / kg DM was fed, Met-Hb concentrations were persistently increased and the response was animal dependant; thus there were consistently low and high responders. However, throughout the 100 day period when 19 g nitrate / kg DM was fed, when feed intake and live-weight gains were compared with a control cohort of steers fed the experimental diets without nitrate, no adverse effects on performance were recorded.

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Evaluation of charcoal and honey as anti-aflatoxin feed additives in maize based broiler diets

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Implications Addition of 2% charcoal into contaminated feed has the capacity of binding aflatoxins while 2% honey did not prevent absorption of aflatoxins across the GIT but heals any lesions caused by toxicity

Introduction Recent trend in research for animal nutritionists has been directed to solving the issue of contamination of animal feeds with mycotoxins (AF) since they pose serious threat to both humans and animals (Zhang and Cauper, 2012). Several approaches have been employed in the research of aflatoxin decontamination. The use of charcoal has been reported to be enterosorbent as it has the capacity to binds to several mycotoxins (Whitlow, 2006) due to its large surface area with negatively charged site. Wellford *et al.* (1978) reported that honey had an antifungal effect against *Aspergillus flavus* and *Aspergillus parasiticus* and stronger anti-aflatoxigenic effect. Honey has therapeutic potential against both internal and external lesions. This makes it a good ameliorating agent against the detrimental effects of AF on the organs of broiler birds. The study evaluated the use of cahrcaol and honey on gut morphology and organ attribute of broilers

Material and methods In a completely randomised design, two hundred and forty one-week old Arbor acre broiler birds were distributed randomly to six dietary treatments with four replicates of ten birds per replicate. The treatments were as follows: T1= Normal diets (diet formulated with normal maize) (positive control, with 15ppb AF); T2= Rejected maize diets (negative control, with 32ppb AF); T3=Positive control plus 2% charcoal; T4=Rejected maize diets plus 2% charcoal; T5=Positive control plus 2% honey; T6=Rejected maize diet plus 2% honey. The birds were raised on deep litter housing system. On the 42nd day, nine birds per treatments were selected for gut morphometric attributes (villus height, crypt depth) of duodenum, jejunum and ileum part of the gut were evaluated .Tissues from these parts were, sectioned using rotary microtome (LEICA RT 2115), stained using haematoxylin and Eosin staining technique and viewed under microscope magnification of x100. The histopathology of liver, kidney and bursa of fabricus were also evaluated. All data obtained were subjected to statistical analysis of variance of SAS 1999 while significant means were separated using the Duncan Multiple range test of the same software

Results Significant ($p<0.05$) differences were observed in the villus height for all the treatments in each of duodenum, jejunum and ileum. However, the histopathology of liver, kidney and bursa of fabricius shows healing power of honey as no visual lesions were seen on the slides of the organs prepared for birds on the 2% honey.

Table 1 Gut parameters of broiler birds fed AF contaminated feed supplemented with charcoal and honey

Treatment	T1	T2	T3	T4	T5	T6	sem	P value
Villus height (Duodenum)	269.50^{ab}	180.80^{d}	280.65^{a}	235.13^{c}	243.03^{bc}	214.00^{c}	9.9	0.4
Crypt depth (Duodenum)	31.47^{b}	44.90^{a}	23.55^{c}	44.90^{a}	30.30^{bc}	45.30^{a}	2.4	0.6
Villus height (Jejunum)	162.15^{d}	204.70^{a}	157.15^{d}	167.07^{cd}	183.90^{bc}	202.63^{ab}	5.9	0.4
Crypt depth (Jejunum)	23.77^{bc}	24.88^{bc}	29.60^{ab}	28.40^{ab}	32.33^{a}	19.77^{c}	1.9	0.1
Villus height (Ileum)	147.17^{b}	138.20^{bc}	174.45^{a}	182.10^{a}	117.90^{c}	115.80^{c}	7.8	0.3
Crypt depth (Ileum)	24.80^{ab}	19.17^{b}	26.33^{a}	19.63^{b}	22.30^{ab}	25.48^{ab}	2.0	0.1

Conclusion This study revealed that charcoal was able to prevent the absorption of the toxins into the enterocyte giving birds better gastrointestinal tracts attributes. However, honey was therapeutic by healing sours generated by mycotoxins absoption.

Ethical statement Authors declare that principles of farm and laboratory animal care were followed.

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Hepatotoxicity of Cassia fistula extract in experimental chicks and assessment of clinical parameters

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Implications *Cassia fistula* is an important medicinal plant with diverse biological activities such as antimalarial, antimicrobial, and analgesic etc. Biochemical analysis of the plant may provide useful information for health professionals
.

Introduction *C. Fistula* is a well known plant used for many ailments throughout Asia. Two bioactive compounds benzyl 2-hydroxy-3,6-dimethoxybenzoate and its dimer dibenzyl 2,2'-dihydroxy-3,6,3",6"-tetramethoxy-biphenyl-1,1'-dicarboxylate, being active against *Cladosporium cladosporioides* and *C. sphaerospermum* have been identified from *C. Fistula*. The plant has also yielded an antileishmanial sterol identified as clerosterol which is 3.6 times less toxic than pentamidine (pentostam), a recommended drug used for the treatment of leishmaniasis. In the present study we investigated the nutritional and medicinal aspects of the plant.

Material and methods Fruit seeds from mature plants present on GC University campus, Lahore, Pakistan were collected during April 2009 and used in this study. A 0.5% w/v aqueous extract of powdered seeds was prepared by soaking the powdered seeds in distilled water for 12 hr. The extract was recovered by passing through cheese cloth and seed powder was reextracted twice with distilled water. The pooled extract was centrifuged at 3000 rpm for 20 min and the clear supernatant was taken, freeze dried and used for animal studies. Five day old 20 broiler birds were randomly divided into four groups (A-D), with five birds in each group. All birds were kept together for one week for acclimatization, followed by housing each group of birds in separate cages placed at 37^{o}C, 40% relative humidity and 12 hr light/dark period using candescent lights. All birds were provided *ad libitum* homemade feed consisting of corn, wheat, hulled barley, sunflower seeds, peanuts, wheat bran, split peas and lentils, mixed in equal amounts. To study time and dose response, experimental birds were fed by gavage i.e Group A birds received normal saline (1mL) while groups B-D daily received plant freeze dried seed extract redissolved in normal saline (1mL) at 30, 40, 50 mg/kg/BW for one, two and three weeks respectively. At the end of the study, birds were injected with intraperitoneal sodium pentothal, blood was collected from jugular vein with a 1 mL syringe, left on ice for 2 hr, centrifuged at 150g and serum was taken for measurement of biological parameters. Total protein and cholesterol were measured. Birds were sacrificed and liver after removal was sectioned by microtomy, gram stained and microscopic study was carried out. All experiments were done in triplicate and the data was validated by using SPSS v 16 at 95% confidence level. Animal rights ethical committee rules of GC university were strictly followed.

Results Most studied parameters remained at normal level at 30mg/kg/BW and 40mg/kg/BW dose of the extract after treatment for one-three week. However, at 50mg/kg/BW dose for one week and more, both histological and biochemical parameters were altered. The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), RBCs, WBCs, leukocytes count such as monocytes, neutrophils, lymphocytes and eosinophils proteins, urea and creatinine all significantly ($p \leq 0.05$) decreased compared with the control group [Mueller *et al*., 2013]. The data suggest that plant extract impairs hematological and biochemical parameters with mutilation of immune system at 50 mg/kg dose for three weeks of treatment. Serum hepatic enzymes (ALAT, ASAT) indicative of hepatic fitness of the birds were all significantly reduced ($p \leq 0.001$) indicating hepatoprotection at 50mg/kg dose, compared with the control group [Ali *et al*., 2013]. A non-significant decrease in cholesterol level parallel with a significant ($p \leq 0.05$) decrease in blood urea in group D birds was observed indicating potential therapeutic value of the plant extract.

Table 1 Measurement of hepatic enzymes(3rd week)

Serological Parameters	Group A	Group B	Group C	Group D
ALAT Conc. in U/l	23.94^{a}± 0.06	23.49^{b}± 0.55	22.75^{c}± 0.53	20.73^{d}± 0.43
ASAT Conc. in U/l	18.57^{b}± 0.85	18.69^{a}± 0.45	16.62^{c}± 0.44	15.57^{d}± 0.53

Conclusion *C. fistula* extract has slight serological implications with hepatoprotective and renal protective characteristics. The plant can be thus safely used as a herbal medicine and can be delivered through saline water and also a feed additive for chicks. These data suggest further studies for the chemopreventive efficacy of *C. fistula.*

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Effect of protease supplementation on ileal crude protein digestibility of feather meal in broiler chickens

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Implications Exogenous enzymes improve digestibility of feedstuffs/nutrients in animals lacking such enzymes improving nutrient availability, reducing nutrient voided and reducing cost of feed/production.

Introduction Protease enzymes have been used in improving the digestibility of some protein sources in poultry feeds. These enzymes as biological catalysts help to break specific peptide bonds that the animal otherwise cannot break thereby making the amino acids available for metabolic processes which is reflected in improved performance. Feather meal is a cheap and available source of protein but with low digestibility in broiler chickens and protease was added in this study to help make the amino acids more available to broiler chickens. Pre-caecal or ileal digestibility of proteins is generally considered a more reliable measure for feed protein evaluation in poultry compared to total tract digestibility. The study was to determine the crude protein ileal digestibility of feather meal (FM) and the effect of supplementation with a protease.

Materials and methods 480 one-day-old broiler chicks were brooded for 3 weeks in a well ventilated and illuminated poultry brooding pen. They were fed a commercial broiler starter diet *ad libitum* to supply 23%CP and 3000Kcal/kg ME, clean water was also given for 3 weeks. On day 21, the birds were randomly allotted to 8 treatment diets of 6 replicates with 10 birds per replicate in a 4x2 factorial arrangement (0, 20, 40, and 60g FM/kg diet as only source of varying Nitrogen and 0 or 0.5g protease/kg of diet).Diets were fed to the birds till day 26. All diets had similar ME of between 3444-3529Kcal/kg diet and Titanium dioxide was added as an indigestible marker. On day-26, the birds were asphyxiated using carbon dioxide and ileal digesta harvested. Ileal digesta were pooled according to replicates, frozen, freeze-dried and milled for analysis. Data were analysed using the GLM procedure in SAS of 1999 and a 2-way ANOVA was used to determine the main effects of protease, FM and their interaction. True CP digestibility values were estimated by regressing digested CP(g/kg DMI) against CP intake (g/kgDMI) per block of 6 replicates for 0 or 5g Protease/kg diet using PROC GLM linear regression model and solutions (Bolarinwa and Adeola, 2012). The slopes of the regression represented estimates of true CP digestibility without and with protease.

Results There was a significant ($p<0.01$) linear increase in CP digestibility by FM and by Protease ($p<0.05$). there was no response of DM digestibility to Protease. The same response criterion was significantly ($p<0.05$) affected by FM but without a linear effect. FM and Protease interaction significantly ($p<0.05$) increased digestibility of CP but not that of DM suggesting that the CP digestibility in diets with increasing levels of FM will be improved by addition of Protease. The true digestibility of CP in FM was increased from 56.7% without Protease to 58.2% with Protease supplementation

Table 1 Effect of protease, FM and their interaction on apparent crude protein digestibility (%) when broiler chickens were fed with FM as only Nitrogen source.

	Protease			FM					*P-Anova*		
Parameter	0	5	SEM	0	2	4	6	SEM	Protease	FM	FM*Protease
CP digestibility	65.9^{a}	76.1^{b}	2.3	58.8^{a}	64.1^{ab}	75.8^{bc}	85.3^{c}	3.3	0.0061	<0.0001	0.0323
DM digestibility	69.6	69.9	2.8	83.2^{a}	69.5^{ab}	58.5^{b}	67.7^{b}	3.9	0.9461	0.0015	0.7613

^{a,b,c} Values along the row for a specific factor with different superscript are significantly different ($P<0.05$). FM= Feather Meal, DM= Dry Matter, CP= Crude Protein, SEM= Standard Error of Mean.

Conclusion Protease supplementation improved apparent and true CP digestibility of FM in broiler chickens, indicating less Nitrogen being voided in the faeces of broiler chickens fed diets containing FM.

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Precaecal digestibility of phosphorus and crude protein of sesame seed meal supplemented with phytase in broilers using regression method

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Implications Supplementation of broiler diets containing sesame seed meal with phytase resulted in higher digestibility of phosphorus (PHOS) and amino acid thereby reducing the amount of these nutrients that are excreted into the environment by the birds.

Introduction Sesame seed meal (SSM) is considered a good alternative to soybean meal (SBM) for poultry feeding having about 440 g/kg CP, an amino acid profile close to that of SBM and total PHOS content of 12 to 15 g/kg about 60-70% of which is bound as phytate. In practical poultry production dietary PHOS and CP over requirement leads to excretion of the undigested nutrients into the environment. Supplementation of such diets with phytase can result in increased digestibility and utilization of PHOS and CP of SSM (Sebastian *et al.,* 1998).

Material and methods Seven dietary treatments were formulated: Diet 1 was the control diet and contained no SSM; Diets 2, 3 and 4 had SSM in place of SBM at inclusion levels of 200, 250 and 300 g/kg diet with no phytase added. Diets 5, 6 and 7 contained the same levels of SSM, respectively with 1500 FTU phytase (Ronozyme NP, DSM Nutritional products, Switzerland Basel) added. The diets contained a minimum metabolizable energy (ME) of 3204 kcal/kg and CP of 233 g/kg. Titanium dioxide was added at the rate of 5g/kg diet as an indigestible marker. Two hundred and ten one-day-old broiler chicks were wing-branded and fed a starter diet till d 14 when they were weighed and assigned in a randomized complete block design by body weight to the 7 diets with 5 replicates per diet and 6 birds per replicate. The birds were fed for a further 7 days. At d 21 all birds were sacrificed by asphyxiation in carbon dioxide to obtain digestea on replicate basis (30 birds per treatment) from the precaecal section for estimation of nutrient digestibility. Data were analysed statistically using the PROC GLM of SAS (SAS Inst. Inc., Cary, NC) to determine the effect of phytase supplementation on performance and digestibility of PHOS and CP. Means were separated using least significant difference and α level of 0.05 (significant) was used. Regression of PHOS or CP digested against PHOS or CP intake respectively was done using Graphpad Prism 4.0 (Rodehutscord *et al.,* 2004)

Results Phytase supplementation of the SSM diets significantly (P<0.05) improved the weight gain (from 161.8 to 215 g/bird), feed intake (from 456.6 to 572.7 g/bird) and feed conversion ratio (from 2.80 to 2.63). The apparent digestibility was significantly (P<0.05) increased by phytase supplementation (from 35.8 to 55.3% for PHOS and from 74.9 to 91.7% for CP). Interaction of SSM and phytase significantly (P<0.01) improved the response criteria. When digested PHOS or CP (g/kg DMI) was regressed against PHOS intake (g/kg DMI), the slopes of the curves, which represented the estimates of true digestibility of PHOS and CP of SSM, were 93% and 84%, respectively (Figure 1).

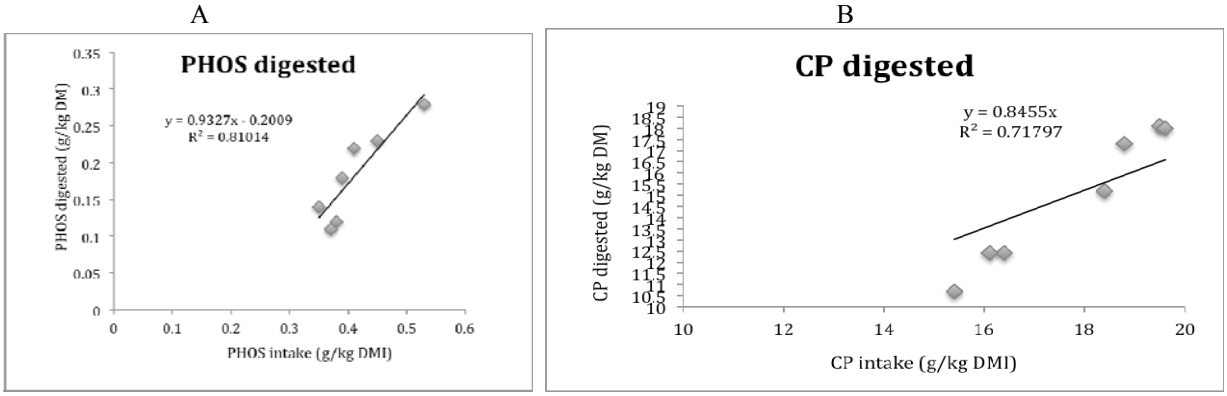

Figure 1 Relationship between digested PHOS (A) and CP (B) and PHOS and CP intake in broiler chickens.

Conclusion Phytase supplementation improved the performance of broiler chickens and the apparent digestibility of PHOS and CP of the SSM. The true digestibility values of PHOS and CP in SSM were 93 and 84% respectively

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Influence of pharmacological zinc oxide, phytase and phosphorus on weaned piglet growth performance and apparent faecal phosphorus digestibility

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Implications Pharmacological ZnO reduced phosphorus digestibility and average daily gain when fed at 3500 mg/kg. This effect was mitigated with supplemental phytase or excess digestible P, especially when ZnO was reduced to 1750 mg/kg.

Introduction Pharmacological doses of zinc oxide (ZnO) are commonly fed to piglets to reduce post-weaning scour (Carlson *et al.,* 1999) and in some cases improve growth performance. However, previous authors have reported a reduction in average daily gain (ADG) and feed conversion ratio (FCR) as ZnO supplementation increased above 2000 mg/kg (Hill *et al.,* 2001; Walk *et al.,* 2013). Walk *et al*. (2013) suggested this may be associated with a reduction in phosphorus (P) digestibility. Furthermore, phytase has been found to improve P and Zn digestibility in weanling pigs (Revy *et al.,* 2004; Kies *et al.,* 2006). The aim of this study was to evaluate the interaction between pharmacological ZnO, phytase and excess dietary P on piglet growth performance and apparent faecal P digestibility.

Material and methods Piglets (n=720; 9.5±0.3 kg) weaned at 28±3 days post-farrowing were allocated to 9 dietary treatments with 8 replicate pens and 10 piglets/pen from d 0 to 21 post-weaning. Treatments were arranged as a 3 x 3 factorial with 0 or 2500 FTU/kg phytase or an extra 1.0 g/kg digestible P (dgP) from monosodium phosphate and 3 levels of pharmacological ZnO (120, 1750 or 3500 mg/kg). The phytase was Quantum® Blue (AB Vista Feed Ingredients, Marlborough, UK). Treatment diets contained (on an as-fed basis) 682 g/kg micronised wheat, barley, maize and oats, 72.5 g/kg fishmeal, 140 g/kg whey powder and 60 g/kg soy oil plus micro-ingredients and were formulated to be adequate in all nutrients, including calcium and dgP. Titanium dioxide was added to the diets as an inert marker and faecal samples were collected and pooled/pen from d 18 to 20 and for determination of apparent faecal P digestibility. Data were analysed in JMP v. 11 and significant means were separated using Tukey's HSD test.

Results The analysed chemical composition and phytase recoveries in the diets were as expected. Performance and P digestibility data are presented in Table 1. ZnO supplementation significantly increased average daily feed intake (ADFI). ADG and FCR were improved in pigs fed 1750 mg/kg ZnO with phytase or 3500 mg/kg ZnO with 5.5 g/kg dgP, which resulted in a significant Zn×P interaction. Apparent faecal P digestibility decreased as ZnO increased, except when pigs were fed 5.5 g/kg dgP or phytase with 120 or 1750 mg/kg ZnO, which resulted in a significant Zn×P interaction.

Table 1 Growth performance and apparent faecal P digestibility in piglets from d 0 to 21 post-weaning

ZnO, mg/kg	120	1750	3500	120	1750	3500	120	1750	3500		
Phytase	-	-	-	+	+	+	-	-	-		
dgP, g/kg	4.5	4.5	4.5	4.5	4.5	4.5	5.5	5.5	5.5	SE	P-value
Final BW, kg	15.3^{d}	17.6^{ab}	16.8^{bc}	16.7^{bcd}	18.4^{a}	17.0^{abc}	15.7^{cd}	17.4^{abc}	17.8^{ab}	0.35	Zn×P*
ADFI, kg/d	0.38	0.44	0.43	0.41	0.48	0.42	0.39	0.44	0.44	0.01	Zn***
ADG, kg/d	0.29^{c}	0.41^{ab}	0.37^{b}	0.36^{bc}	0.45^{a}	0.38^{ab}	0.33^{bc}	0.40^{ab}	0.41^{ab}	0.02	Zn×P*
FCR	1.28^{a}	1.09^{c}	1.16^{bc}	1.15^{bc}	1.08^{c}	1.13^{bc}	1.21^{ab}	1.11^{bc}	1.07^{c}	0.03	Zn×P**
P digestibility, %	48.1^{bcd}	35.9^{de}	32.6^{e}	62.5^{a}	54.9^{ab}	39.2^{cde}	51.8^{ab}	47.3^{bcd}	50.1^{abc}	2.72	Zn×P**

Zn = main effect of ZnO, P = main effect of phosphorus source, Zn×P = interaction. *P<0.05, ** P<0.01, *** P<0.001

Conclusion Feeding pharmacological ZnO at 1750 mg/kg increased growth performance, especially in the presence of phytase. Supplementing piglet diets with 3500 mg/kg ZnO numerically reduced ADG compared to pigs fed 1750 mg/kg ZnO and decreased apparent faecal P digestibility. Therefore, high levels of Zn from pharmacological ZnO may interfere with dietary P and reduce P digestibility. This can result in decreased piglet growth performance, especially if dgP is limiting. Supplementation of piglet diets with 1750 mg/kg ZnO plus phytase or excess dgP mitigated the negative effect of high dietary Zn from ZnO on piglet performance and P digestibility.

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The effect of phytase on grower pig growth performance

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Implications These results show that grower pigs are able to compensate for a period of phosphorus (P) restriction by exhibiting compensatory growth when provided with diets supplemented with phytase. This observed compensatory response may present an opportunity to optimise performance, reduce feed costs and P excretion.

Introduction Phytase catalyses the de-phosphorylation of the anti-nutritional factor phytate. Phytases are commonly used in pig diets to improve P availability of grain based diets and to minimise P excretion. High doses of phytase have been shown to enhance pig performance (Braña *et al*., 2006), presumably due to the quick removal of ingested phytate and its associated anti-nutritional effects. However, there is a paucity of information available on the effects of high doses of phytase in grower-finisher pigs, therefore this study was designed to evaluate a conventional and a high dose of phytase on grower pig growth performance. It was hypothesised that superior growth performance would be observed in pigs receiving the high dose.

Material and methods A total of 576 eight week old pigs were used in this 4 x 2 factorial 49d experiment. Pigs were allocated to pens of 9 balancing for live weight and litter of origin. A total of 8 replicate pens were used. The first factor (D1) was offered to pigs (*ca.* 17kg BW) from d0-30 (phase 1) and consisted of one of four dietary treatments, including: (**PC**) a high dig-P diet (0.05% above the BSAS recommendation); (**NC**) a basal diet with Ca and dig-P 0.16% and 0.124% below the recommended levels respectively; (**500**) NC + 500 FTU/kg; (**2000**) NC + 2,000 FTU/kg. The second factor was another diet (D2, with Ca 0.54% and dig-P 0.35%) which was offered immediately after D1 (from *ca*. 40kg BW) from d30-49 (phase 2). D2 was formulated to contain one of two levels of phytase: a conventional dose (500 FTU/kg) or a high phytase dose (2,500 FTU/kg). Both D1 and D2 were offered *ad libitum*. Pig weights and feed consumption were weighed biweekly throughout phase 1 and weekly throughout phase 2 for the determination of average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). The growth data were analysed as a 4 x 2 factorial design using the General Linear Model procedure of SPSS with the pen serving as the experimental unit.

Results Pigs initially fed the NC diet had a lower ADG ($P<0.01$) and ADFI ($P<0.05$) than pigs fed the PC, 500 or 2000 diets throughout phase 1 (data not shown). The treatment effects on pig growth performance throughout phase 2 and overall (d0-49) are presented in Table 1. There was a significant D1xD2 interaction for ADG throughout phase 2 and the total experimental period. The pigs offered the NC diet had the lowest BW ($P<0.01$) at the end of phase 1, however upon the subsequent provision of 500 FTU/kg, these pigs were able to catch up in weight to pigs fed P adequate diets throughout due to marked improvements in their FCR and ADG. No D1xD2 interactions were observed for ADFI or FCR. Pigs receiving the high phytase D2 diet had a higher ADFI ($P<0.01$) and FCR ($P<0.1$) than those receiving the conventional dose.

Table 1 Effect of dietary treatments on growth performance throughout phase 2 and the total experimental period

D1		PC		NC		500		2000			*P*-value		
D2		2500	500	2500	500	2500	500	2500	500	SEM	D1	D2	D1xD2
ADG (g)	d30-49	907	825	842	902	872	805	842	842	23.9	0.404	0.192	0.015
	d0-49	913	873	853	885	890	859	881	891	10.5	0.106	0.352	0.003
ADFI (g)	d30-49	1932	1749	1809	1767	1901	1766	1928	1778	63.2	0.748	0.005	0.713
	d0-49	1604	1530	1516	1498	1602	1525	1596	1529	27.0	0.086	0.003	0.672
FCR	d30-49	2.13	2.12	2.16	1.96	2.18	2.21	2.29	2.17	0.07	0.120	0.068	0.250
	d0-49	1.76	1.75	1.78	1.69	1.80	1.78	1.81	1.72	0.03	0.265	0.008	0.228

Conclusion Grower pigs initially offered a P deficient diet had a reduced ADG and ADFI. However, after the introduction of phytase these pigs exhibited compensatory growth due to improvements in ADG and FCR. More research is necessary to ensure this response is consistent and to address any long term effects of P restriction on growth or health status. The higher dose of phytase increased ADFI but in this experiment delivered no further significant benefit.

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Energy metabolizability by finishing turkeys fed malted sorghum sprouts supplemented with enzyme or yeast

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Implications Increased levels of MSP diet reduced energy utilisation. Enzyme supplementation improved energy metabolisability than yeast supplementation as MSP level increased.

Introduction Livestock feed production has been on the increase and feed price have continued to rise steadily. This is caused in turn by steep increase in cost of conventional feedstuffs coupled with competition for the limited resources between human, other animals and industries. The rising cost has necessitated research efforts into unconventional feedstuffs such as Malted Sorghum Sprouts (MSP). MSP has prospects as a livestock feed but its usefulness is limited by its tannin content and non-starch polysaccharides. Oke *et al.* (2010) reported that a commercial enzyme supplementation in MSP diets improved broiler performance. Furthermore, yeast supplementation in poultry diets successfully promoted growth by improving the digestion of lipids and carbohydrates (Spring, 2002). This research work therefore determined the energy metabolisability of finishing turkeys fed MSP diets supplemented with enzyme or yeast.

Material and methods One hundred twenty (120) 28d day old British United Turkeys (BUT) were randomly allocated to 6 dietary treatments. The six experimental diets were formulated such that each treatment contained either enzyme or yeast at 200ppm supplemented with MSP at 0g/kg, 50g/kg and100g/kg respectively. The exogenous enzyme used is a commercial blend of xylanase (EC3.2.1.8), β glucanase (EC3.2.1.4) and protease (EC 3.4.2.1). The yeast used is the commercially available baker's yeast. At day 112, 6 groups of 6 turkeys each were allocated to 6 experimental diets while an additional group of 6 turkeys were used for estimation of endogenous losses. All the turkeys were housed individually in steel metabolic cages. Known weights of feed were given to the birds contained in the metabolic cage daily. Leftovers were also measured and discarded. Daily excreta collection was done for three days, while the second group of birds were given 0.1N glucose solution each so as to estimate the endogenous losses. Apparent metabolisable energy (AME), AME corrected for nitrogen retention (AMEn), true metabolisable energy (TME) and TME corrected for nitrogen retention were determined according to the methods of Sibbald (1979). Data generated were analysed using ANOVA. Polynomial contrast (Linear and quadratic) was used to determine effects of enzyme or yeast inclusion using SPSS (1999).

Results The AME reduced linearly ($P < 0.05$) with inclusion of MSP. This was not so for TME and TMEn. Turkeys fed enzyme supplemented diets irrespective of MSP inclusion level had higher ($P < 0.05$) values of AME AMEn, TME and TMEn. The interaction between MSP inclusion and supplementation (enzyme or yeast) showed significant effect ($P < 0.05$) on AME (linear), TME (quadratic) and TMEn (quadratic).

Table 1 Effects of MSP inclusion and enzyme or yeast supplementation on the apparent and true metabolizable energy of finishing turkeys

					P value					
Measurements	0	5	10	SEM	L	Q	Enzyme	Yeast	SEM	Pvalue
AME (MJ/kg)	15.92[a]	15.84[b]	15.83[b]	0.02	0.048*	0.075[NS]	15.91	15.82	0.02	0.04
AMEn (MJ/kg)	15.85	15.79	15.77	0.01	0.087[NS]	0.202[NS]	15.85	15.75	0.02	0.02
TME (MJ/kg)	16.43[b]	16.64[a]	16.57[a]	0.03	0.075[NS]	0.019*	16.62	16.48	0.03	0.09
TMEn (MJ/kg)	16.35[b]	16.52[a]	16.60[a]	0.04	0.056[NS]	0.008*	16.56	16.42	0.03	0.03

[abc] Means on the same row having different superscripts are significantly different ($P < 0.05$)
NS- Not significant**,** *Significant, L- Linear, Q-Quadratic

Conclusion MSP inclusion decreased AMEn, AME. Furthermore, enzyme supplementation led to higher ($P < 0.05$) indices of energy utilisation than yeast supplementation irrespective of MSP levels.

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Effect of enzyme and yeast supplementation on energy metabolisability by broiler chickens fed diets containing malted sorghum sprouts and wheat bran

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Implications Malted Sorghum Sprout can alternatively be used in place of Wheat bran if supplemented with enzyme alone and combination of enzyme and yeast to improve utilization of the low quality feedstuff.

Introduction Commercial poultry production in developing country like Nigeria is hindered by increasing cost of feed ingredients such as wheat bran coupled with the steep food-feed competition existing between livestock and man. This scenario necessitated the exploration into cheap, unconventional feedstuffs such as malted sorghum sprouts (MSP).This MSP can offer a viable alternative to wheat which has become scarce and very expensive in Nigeria. MSP proximate composition is almost similar with wheat bran (WB) but MSP contains tannin and non-starch polysaccharides (NSPs) which limit its nutritive value and inclusion level (Elkin *et al.,* 1995). Previous findings showed that addition of either exogenous (Acamovic, 2001) enzyme or yeast (Spring, 2002) supplementation improved the nutritive value of low quality feedstuffs for poultry birds. An experiment was therefore carried out to investigate the effect of yeast and enzyme supplementation on energy metabolisability by broiler chickens fed diets containing MSP or WB.

Material and methods Diets were formulated such that MSP replaced WB contained (150g/kg) in a broiler finisher diet according to NRC, 1994 for broiler finishers in the tropics with 19.80 % CP and ME of 11.43MJ/Kg while other ingredients remained unaltered. Six additional diets were formulated such that each of MSP and WB was supplemented with yeast (+Y), enzyme (+E) or a combination of yeast and enzyme (+Y+E). The unsupplemented diets (-Y-E) served as the control making 8 diets in all. The commercial enzyme cocktail used is a blend of xylanase (EC3.2.1.8), β glucanase (EC3.2.1.6) and protease (EC 3.2.1.4) and was added at the rate of 10g/100kg diets, while the yeast *Saccharomyces cerevisiae* was added at 10g/kg diet. Sixty-four 56d old Ross broiler chickens were divided into 8 groups of 8 birds each and were allocated to 8 experimental diets while an additional group of 8 birds were used for estimation of endogenous losses. The birds were acclimatized for three days in steel metabolic cage individually and daily excreta collection was done for three days to determine the apparent metabolisable energy (AME), AME corrected for nitrogen (AMEn), true metabolisable energy (TME) and TME corrected for nitrogen (TMEn) as described by Sibbald (1979). The experiment was arranged in a 2 × 4 factorial design made up of factors (MSP, WB) and 4 levels. Data generated were subjected to analysis of variance. Significant means were separated using Duncan's multiple range tests at 5%.

Results The energy metabolisability of birds on the MSP and WB diets were similar, although WB recorded higher values of AME, AMEn, TME and TMEn. Combination of yeast and enzyme supplementation recorded the highest ($P<0.05$) AME, AMEn, TME and TMEn respectively, although the values were not significantly higher than those supplemented with enzyme only. Supplementation with yeast showed no improvement ($P > 0.05$) on the energy metabolisability of the birds.

Table 1 Effect of yeast (Y) and (E) supplementation on energy metabolisability(MJ/kg) by finisher broilers fed diets containing Malted Sorghum Sprouts or Wheat bran

	MSP *vs* WB inclusion			Yeast and /or Enzyme Supplementation				
	MSP	WB	SEM	-Y-E	+Y	+E	+Y +E	SEM
AME	12.90	12.96	0.052	12.47^{b}	12.19^{b}	12.98^{a}	13.12^{a}	0.138
AMEn	12.72	12.83	0.054	12.34^{c}	12.45^{c}	12.82^{ab}	13.03^{a}	0.144
TME	15.21	15.08	0.051	15.28^{ab}	15.43^{ab}	15.57^{a}	15.46^{ab}	0.088
TMEn	15.33	15.45	0.052	14.18^{c}	15.55^{ab}	15.52^{a}	15.49^{a}	0.186

^{a,b} Means on the same row with different superscripts differ significantly ($P<0.05$)

Conclusion The use of enzyme alone and combination of enzyme and yeast showed improvement on AME and AMEn, TME and TME. Similar energy are metabolised from MSP and WB by the finisher broilers.

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Effect of two different fibre sources on growth and immune function in layer pullets

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Implications Supplementing pullet grower diets with fibre may be a useful replacement for antibiotics in order to enhance growth and disease resistance and thus improve sustainable egg production.

Introduction Restrictions or complete bans to the use of antibiotics as feed additives in rations of poultry have resulted in negative impacts on their health and productivity. Knowledge of alternatives to antibiotics for enhancement of immune function is important if sustainable and economic production is to be achieved. Different types of dietary fibres may be effective alternatives to antibiotics for enhancement of immunity and performance of layers. In broilers and ducks increasing fibre to the diet has been shown to increase T and B lymphocyte proliferation as well as improve growth of their lymphoid organs (Dong *et al.* 2007; Shi-bin and Hong 2012). The aim of this study was to determine whether dietary fibre would enhance layer pullets' innate immune function and growth of lymphoid organs.

Material and methods Thirty-six 4 week old Hy-line brown pullets were weighed and randomly placed, four/ pen, in slatted floor pens (1.8 x 0.9 m), three pens/ treatment. The three dietary treatments were Control, a commercial starter and grower feed with no additive; Group MF, given control diet with 1g of a commercial mixed soluble/insoluble fibre supplement (~59% crude fibre, ~85% mixed soluble and insoluble fibres, ~30% lignin, manufacturer's data) per 100g diet; Group IF, given control diet with 1g of a commercial insoluble fibre supplement (65 – 70% crude fibre high in insoluble cellulose and >20% lignin, manufacturer's data) per 100g diet. At 8 weeks of age blood samples were taken over 4 consecutive days from the brachial vein of 8 pullets/ treatment: pullets were taken randomly from the pens and not all pullets in a treatment came from the same pen. Heterophils were isolated using Ficoll-Hypaque discontinuous gradients (Andreasen and Latimer 1989) and heterophil oxidative burst measured (Wan *et al.* 1993). All pullets were then weighed and killed on the same day with intravenous pentobarbitone sodium under La Trobe University Animal Ethics Committee approval (No. AEC12-68) and guidelines. From each pullet the spleen, bursa of Fabricius, and combined left and right thymus glands were collected and weighed. Data were analysed using one-way analysis of variance (ANOVA, SPSS, 2012). Statistical significance between means of different treatment groups was compared by Turkey's test at $P<0.05$.

Results Heterophil oxidative burst in both MF and IF pullets was significantly increased at 8 weeks of age (Table). Live body weight and relative weights of bursa of Fabricius and thymus glands of pullets in Groups MF and IF were significantly ($P<0.05$) higher than the Control group, while those in Group IF group had significantly ($P<0.05$) higher relative weights of the spleen compared to those in the Control group (Table).

Table Mean live weight (lwt) and relative weights (% of lwt) of immune organs (Mean ± SE, N = 12) and heterophil oxidative burst (Mean ± SE, N = 8, ΔRFU = relative fluorescence units) of pullets fed different diets.

Group	Live weight (g)	Spleen (% of lwt)	bursa of Fabricius (% of lwt)	Thymus (% of lwt)	Heterophil oxidative burst (ΔRFU)
Control	647.9±12.3^{a}	0.40±0.02^{a}	0.50±0.01^{a}	0.50±0.01^{a}	3509±207^{a}
MF*	696.3±9.9^{b}	0.42±0.02^{ab}	0.57±0.01^{b}	0.77±0.02^{b}	4330±183^{b}
IF*	717.5±14.7^{b}	0.47±0.02^{b}	0.57±0.02^{b}	0.78±0.01^{b}	5264±199^{c}

* See methods for definition. a-c Values with different superscripts in the same column are significantly different ($P<0.05$).

Conclusion Compared to controls, live weight and innate immune function were increased in pullets fed a mixture of soluble and insoluble fibre or insoluble fibre alone. The benefits may have been the results of improved immune function in the gut or decreased gut pathogens but further work would be needed to confirm this and to identify whether insoluble fibre contributed more to improvement than soluble fibre.

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050

Use of thyme (*Thymus vulgaris*) essential oil or plants to control the poultry red mite (*Dermanyssus gallinae*) in domestic fowl

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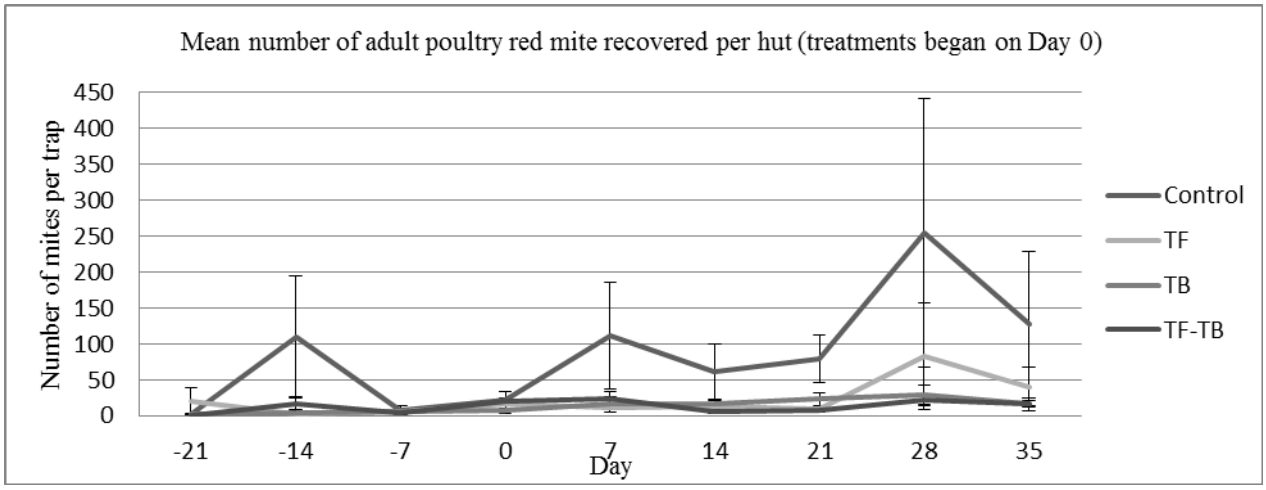
Implications With further developmental work, this study suggests that supplementation with thyme essential oil could be used as a practical means to control poultry red mite which is the most prominent ectoparasite of laying hens in Europe.

Introduction Poultry red mite (*Dermanyssus gallinae*) is the most prominent ectoparasite in laying hens across Europe, impacting on the economics of egg production as well as reducing hen welfare. Chemical acaricides are often used in red mite control, but these may have damaging effects on both the environment and human health. Furthermore, overuse of some of these products has resulted in development of resistance to them, increasing the need for novel approaches to red mite control. Laboratory-based research has shown plant-derived essential oils, such as that from thyme (*Thymus vulgaris*), to be toxic to red mite but there is a lack of information on application of this science to practical poultry keeping. The aim of this study therefore was to assess whether supplementation with different forms of thyme could control red mite.

Materials and Methods The study was set up as a 2x2 factorial design, comprising two factors, namely dietary supplementation (with or without thyme essential oil at a concentration of 1% in the feed), and enrichment of bedding material (with or without chopped fresh thyme in the bedding, by twice-weekly addition of 2% chopped thyme leaves to the woodshavings). Thus the 4 treatments comprised: control (C), thyme in feed (TF), thyme in bedding (TB) and thyme in both feed and bedding (TB-TF). Four replicates of each treatment were run concurrently, where each replicate consisted of four huts (mini-barns containing space for perching, exercise, feeding and drinking and nest boxes) each containing 3 laying hens of a commercial genotype. On Day -28, one week after the hens arrived, approximately 30,000 red mite were released into each hut (10,000 per bird), and the population allowed to develop for 4 weeks before application of treatments began on Day 0. To estimate red mite population, two cardboard traps were placed in each hut each week, and left for 24 hours. Mites were recovered from each trap, euthanised and then counted. Data relating to red mite, egg production, hen body weight, mean feed intake and feather condition was collected on a weekly basis, and analysed by analysis of variance.

Results The graph below shows that in every week of the study the control huts had a higher population of red mite than huts provided with thyme. However, the number of mites recovered from the traps was extremely variable, so that there were few significant effects of treatment. When treatments were combined in an analysis of mite population in huts 'with thyme in feed' versus those 'without thyme in feed', only on Day 21 were there significantly fewer red mite ($P<0.05$) in huts which received thyme in the feed. Mean feed intake per bird for treatments TF and TB-TF decreased significantly on Days 7 and 14 compared to the other treatments, with a concomitant reduction in bodyweight (significant on Day 7). However, in both treatments, bodyweight subsequently recovered so that by the end of the study there was no effect of treatment on bodyweight. Thus, although treatment with thyme affected palatability/feeding behaviour the birds quickly become accustomed to it, deeming it only a short-term issue.



Conclusion The results of this study suggest potential for use of thyme essential oil added to the feed or chopped thyme added to bedding to control red mite control. Further study on a larger sample size would be beneficial to confirm these findings and to consider the use of appropriate measures to overcome any negative effects of thyme essential oil on feed intake (e.g. combination with sweeteners when added to feed).

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Nutrient digestibility of diets based on wheat distillers dried grains with solubles supplemented with enzymes by broiler chickens

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Implications The inclusion of wheat distillers dried grains with solubles (wDDGS) in broiler chicken diets lowered the apparent digestibility of protein but increased the digestibility of fibre. The addition of enzymes, such as xylanase, protease, amylase and glucanase, may help improve the nutrient digestibility of wDDGS.

Introduction The availability of wDDGS for inclusion in broiler feeds has recently been increasing as a nutrient rich by-product of biofuel industry in the UK. While wDDGS are rich in fibre, protein, fat, and phosphorus, these nutrients are not efficiently utilized by broilers (Rano *et al.* 2013). Enzyme supplementation of cereal-based diets can significantly improve chick performance by increasing nutrient digestibility and hence efficiency of DDGS-based feed utilization. Therefore, this study aimed to compare the effects of adding two separate enzyme mixtures to wDDGS based diets on the nutrient digestibility by broiler chickens.

Material and methods A completely randomized design with 2 X 3 factorial arrangement with 2 wDDGS levels (0, 15%), and 3 enzyme levels (NE, +EA, +EB) was used. One hundred and eighty day-old broiler chicks were used for the experiment which lasted for 6 weeks. The birds were housed in groups of 6 on wood shavings in circular floor pens. Isonitrogenous diets without (NE) or with one of the two enzymes (+EA, +EB) were formulated by replacing ground wheat and soybean meal by wDDGS, giving varying metabolizable energy and fibre contents, Each enzyme was separately added at 0.5 kg /tonne feed, where one g of enzyme A (+EA) contained 9200U xylanase, 1600U alpha-amylase and 16000 U protease whereas Enzyme B (+EB) contained 12200U xylanase and 1520U glucanase. The diets and clean drinking water were provided *ad libitum*. At the end of the feeding trial, two birds per pen were transferred to metabolism cages for total faeces collection over 4 days to determine apparent nutrient digestibility of each feed. Samples of faecal collections and feeds were dried in a forced air circulation oven at 60^{o}C, pooled for each cage as replicates, ground and then analysed for nutrients to measure nutrient digestibility. The data were statistically compared by using analysis of variance procedures in Minitab 16. Tukeys test was used if there were more than two means to compare for significant difference at $P<0.05$.

Results Table 1 show the main effects of wDDGS and enzymes on nutrient digestibility of the experimental diets. The dry matter (DM) digestibility did not differ significantly for wDDGS inclusion. While digestibility of protein and phosphorus decreased, the digestibility of fibre fractions were increased with the inclusion of 15% wDDGS. Both ether extract and phosphorus digestibilities were significantly better with enzyme B than enzyme A.

Table 1 Means for the main effects of wDDGS & Enzyme on nutrient digestibility of the diets

	DIETS								
	wDDGS Levels (%)				Enzyme Inclusion				
Digestibility (g/kg)	0	15	SEM	P value	NE	+EA	+EB	SEM	P value
Dry Matter	770.7	752.3	6.90	0.071	757.4	752.8	774.2	8.45	0.190
Protein	713.0	669.6	11.73	0.015	687.1	685.0	701.8	14.37	0.670
Fat (Ether Extract)	799.2	800.6	10.35	0.924	795.6^{ab}	772.5^{b}	831.5^{a}	12.68	0.011
Neutral detergent fibre	386.7	477.1	14.98	0.001	426.0	430.8	438.8	18.34	0.884
Acid detergent fibre	81.5	157.0	8.37	0.001	131.2	119.6	106.9	10.25	0.264
Calcium	296.9	331.1	9.31	0.016	304.1^{b}	291.8^{b}	346.1^{a}	11.39	0.007
Phosphorus	383.2	356.1	9.75	0.001	327.8^{b}	349.7^{b}	431.4^{a}	9.75	0.001

a,b,c Means bearing different letters within rows for enzyme inclusion are significantly different ($P<0.05$).

Conclusion Enzyme supplementation of wDDGS based diets did improve the nutrient digestibility of fat, calcium and phosphorus. This demonstrates the efficacy of using enzyme B over enzyme A to improve the digestibility of nutrients and hence the utilisation of wDDGS based diets by broilers.

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The impact of enzyme inoculation on fermentation of ensiled maize cobs

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Implications Maize cobs (MC) are a valuable feed resource that are underutilised in the face of high feed costs. Cost effective ways of improving the maize cobs such as ensiling with exogenous enzymes will greatly improve their utilisation

Introduction Maize cobs (MC) are readily available and can be incorporated into pig diets to reduce feed costs (Kanengoni *et al*., 2004) and minimize nutrient losses to the environment (Bindelle *et al*., 2008). Although unprocessed MC have been included in pig diets, poor animal performance was reported due to high fibre content (Kanengoni *et al*., 2004). Utilisation of the MC by pigs can be improved if the lignocellulosic bonds can be disrupted sufficiently by fermentation and use of exogenous enzymes (Zhang *et al*., 2010). The addition of cell wall degrading enzymes to maize forage at ensiling improved the chemical characteristics of silages and reduced fibre content (Colombatto *et al.,* 2004). The aim of this study was to evaluate the impact of ensiling maize cob with exogenous enzymes on silage quality and nutritive value.

Material and methods Maize cobs (920 g/kg DM) were ground to pass through a 5 mm sieve and water was added to achieve 60% moisture before ensiling. The MC was treated with or without enzyme inoculation, as follows: CON (no additives), Porzyme 9302 enzyme, which was applied either at 0.5 g/kg MC (ENZ1) and 1 g/kg MC (ENZ2). The mixtures were ensiled in 1.5 L anaerobic glass jars for 32 days. Sampling was done on days 0, 1, 4, 15 and 32 for determination of fermentation characteristics and nutritive value of the silage. Samples of day 32 were also exposed to air for 5 days to determine aerobic stability. Data were analysed in a completely randomized design by ANOVA using SAS (2012).

Results After 32 days of ensiling, ENZ1 and ENZ2 had higher (P<0.001) NDF concentrations (Table 1). Also, ENZ1had higher (P<0.001) ADF concentrations. Although there were no differences (P > 0.05) in DM and GE MJ/kg DM between the treatments, control had higher (P<0.001) CP and EE than ENZ1 AND ENZ2.

Table 1 Nutrient composition of MC after 32 days of ensiling (n=3)

	Treatments				
Parameters	CON	ENZ1	ENZ2	P	SEM
DM g/kg	44.5	43.5	43.6	0.615	1.86
Crude Protein g/kg	36.2^{a}	34.1^{b}	33.2^{b}	0.0004	0.936
GE MJ/kg DM	18.1	18.2	18.1	0.274	0.026
EE g/kg	6.1^{a}	5.2^{b}	5.7^{b}	<0.0001	0.113
NDF g/kg	768.0^{b}	798.0^{a}	788.0^{a}	<0.0001	5.832
ADF g/kg	424.7^{b}	455.0^{a}	436.0^{a}	<.0001	4.257

^{a,b} Means followed by different superscripts within rows differ significantly (P<0.05),DM = Dry Matter; GE = Gross energy; EE = ether extract; NDF= neutral detergent fibre; ADF =acid detergent fibre

Conclusion Addition of porzyme 9302 in two levels did not improve the nutritive value and fermentation quality of ensiled MC.

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The health and welfare implications of selection, population structure and breeding in pedigree and other dogs

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Implications Minimisation of the effects of domestic dog population structure on inherited disease prevalence and morbidity requires an understanding both of the genetic structures underlying diseases, and better disease recognition and ascertainment.

Introduction Dogs breeds have a greater variety of morphology, size, pelage and behaviour than any other species, but dogs also have a larger number of described genetic diseases than other veterinary species. Pedigree dogs have low effective population sizes, often with substantial founder effects and relatively high levels of inbreeding. For the last five years there has been considerable debate about the health effects this population structure on both pedigree dogs and the non-pedigree population. In this talk I will review the effect that population structure has on inherited disease prevalence for monogenic and polygenic disease; giving estimates of numbers and prevalence of disease genes in particular populations, of coefficients of inbreeding, and thus of additional morbidity from this source compared with outbred populations. I will look at how exaggerations of conformation lead to disease, and at the uncertainties in estimating real prevalence for diseases in dogs. I shall consider breed health surveys and primary practice surveys as sources of data. Also in this regard I shall review the importance of accurate phenotypic description, touching on work to develop objective measurement of brachycephalic obstructive airway syndrome (BOAS). This type of work will allow genetic approaches to complex polygenic diseases. Lastly I will briefly review recent work on cancer predispositions and their genetics and consider how breeding schemes could be modified to take account of DNA testing for polygenetic disease.

Material and methods Objective measurements of respiratory function have been performed on French Bulldogs, Pugs and other breeds using whole body barometric plethysmography (QDA). A clinical grading scheme based on exercise tolerance, audible breathing problems and medical history was used with the WBBP results to train a quadratic discriminant analysis (QDA) tool to predict BOAS status. MicroRNA profiling and whole genome array studies (GWAS) were used in the cancer studies described in flat-coated retrievers and cocker spaniels.

Results Central estimates of the proportion of pedigree dogs that could suffer extra morbidity through the effects of inbreeding in recessive disease amount to around one dog in six. But on the other hand the genetic load in many breeds has been reduced by the purging of disadvantageous alleles. Although most breeds are not themselves in danger individual animals are at increased risk.
In studying BOAS we have shown that although brachycephaly is a necessary precondition for upper airway respiratory disease, it is not sufficient, and that populations of both French Bulldogs and Pugs do exist with relatively normal respiratory function. WBBP used with a QDA tool has high specificity and sensitivity in discriminating BOAS affected animals.
GWAS analysis of a number of cancer types is yielding results that show that high incidence tumours in several breeds are the result of characteristic inherited mutations. It is feasible to use such results in breeding schemes based on estimated breeding values.

Conclusion It is important to recognise that the isolated sub populations in breeds have had important effects on increasing morbidity from particular diseases, but can also act as protective barriers to inherited disease. In considering the importance of breeding schemes in controlling inherited disease we need to consider the individual rather than the breed. At the moment our conclusions are based on limited prevalence data but improvements to this are beginning to accumulate through health surveys and through primary practice monitoring schemes. More work in this regard is still needed.

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Five misconceptions about companion animals that affect welfare

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Dogs are wolves Dogs' DNA clearly indicates their derivation from the grey wolf *Canis lupus*, but domestication has profoundly affected their cognitive abilities, and, perhaps most critically, their capacity to form emotionally-driven attachments. The notion that a wolf "lurks" inside every dog is widely used by those who advocate physical punishment (check-chains, shock-collars, physical beatings) as an essential element of dog-training. In this conception, wolves are portrayed as establishing social relationships primarily through violence and intimidation, which therefore has to be imitated by any owner wishing to keep their dog under control, otherwise the dog will succeed in controlling the owner. Such ideas persist even though it has been known for well over a decade that wolf society is based upon amicable associations between family members, and that the aggression-based "hierarchy" is an artefact seen only in captivity (Bradshaw *et al*., 2009).

Cats are sociable Domestic cats are descended from a solitary, territorial species, *Felis silvestris lybica*, so it is remarkable that during domestication they have evolved a rudimentary sociality that enables them to live in colonies. However, the default reaction of one unfamiliar cat to another is to either attack or flee, and even cats that appear to live "together" in a household may in fact be avoiding one another all or most of the time. It is becoming increasingly evident that much problematic behaviour in cats, especially inappropriate urination and defecation, is caused by unresolved tensions between individuals, either within one household, or living in adjacent households. Moreover, such conflict is now known to be an important contributory factor to stress-related illnesses such as idiopathic cystitis and dermatitis (Cameron *et al.*, 2004).

Dogs can feel guilt Dogs (and cats) are increasingly anthropomorphised, including the assumption that their emotional capacities are identical to our own. However, there is no evidence that either species is capable of experiencing self-conscious emotions such as guilt and embarrassment, although it can be easy to unwittingly interpret their behaviour as evidence for such feelings. Dogs' "guilty looks" are more parsimoniously explained by the anticipation of punishment (Horowitz, 2009).

A well-fed cat shouldn't need to hunt Conservationists are placing cat owners under increasing pressure to prevent their cats from hunting: even though there is little evidence that pet cats have a significant impact on populations of wild animals, there is undoubtedly a welfare impact on the individual animals that are killed or maimed. Moreover, cats' hunting is increasingly portrayed as "cruel", as if they were hunting to satisfy some perverse desire for violence for its own sake. Cats may appear to "play" with their prey, but this is an anthropomorphic interpretation, the behaviour arising from a conflict between the cat's need to obtain food and fear that it will be injured in the process (Biben, 1979) . Historically, cats have been valued for their abilities to suppress the impact of vermin on food stores, and abhorrence of this once-desired characteristic appears to be a recent phenomenon. However, advances in understanding of feline nutrition during the past half-century have finally rendered cats' hunting instincts superfluous, and the cat as a species may therefore change over the next few decades.

Dogs are happy to be left on their own Dogs form very powerful emotional attachments to people, but research has revealed that many are incapable of ignoring such attachments when those people are absent. A large proportion of pet dogs - possibly as many as half - feel chronically anxious whenever they are left alone, and because this is a problem that is only expressed when there is no-one present to observe it, in the past its prevalence has been substantially underestimated. Some dogs express or attempt to reduce their anxiety by being destructive, urinating or defecating, vocalising, or pacing: others may remain trapped in a state of learned helplessness until their owner returns. The welfare of pet dogs could be significantly improved if owners understood the benefits of training their dog how to cope with being left alone, using the method of progressively building up an association between departures and reunions (Blackwell *et al.,* 2006).

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An investigation into relationships of horse and rider pelvic asymmetry

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Implications This study provides positive evidence of a relationship between horse and rider pelvis asymmetry. This has implications in awareness of related asymmetries and in chiropractic treatment of horse and rider.

Introduction Asymmetry of the horse and rider partnership is a challenging and complex area. Previous research has investigated effects such as axial rotation of the pelvis in horses with clinically diagnosed back problems. Asymmetry of the human pelvis is also thought to contribute to back pain. There is limited scientific research on the occurrence of misalignments in the horse and rider, and whether there is any relationship between the two, thus making asymmetry of the horse and rider a challenging but potentially insightful topic of investigation. The aim of this study was to investigate asymmetry of single horse and rider combinations focusing on pelvis asymmetry.

Material and methods 14 single horse and rider combination subjects (together for at least 6 months), kept on the same daily routine, at the same yard were used. Horse and rider combinations were assessed for misalignments of the pelvis, spine, and neck on the same day by a fully qualified human and animal (McTimoney) practitioner. A pilot study confirmed reliability of method and equipment for rider and horse measures. Triplicate measurements were recorded of rider pelvis asymmetry (mean values for distance (nearest 0.2cm) between iliac crests and degrees of tilt), sitting and standing, using a PALM palpation meter. Triplicate tuber coxae height (left, right) of the horse's pelvis was measured using a plumb line. The difference between the two means gave a measurement for rotation of the pelvis. A value < 0 indicated rotation to the left, a value > 0 indicated rotation to the right, and a height difference of zero indicates equal height of the tuber coxae and thus no asymmetry of the pelvis. The coefficient of Kurtosis was used to test data for normality, >0.05 assumed data was not significantly different from that of a normal distribution. Pearson's correlation analysis and regression examined relationships between horse and rider pelvis asymmetry. A paired T-test was used to compare measures of rider iliac crest height in the standing versus sitting positions. Chi-square was used to assess pelvis misalignment to other misalignments in horse and rider.

Results Degree of asymmetry of the horse pelvis with tuber coxae height discrepancy ranged from 0.2cm to 6.6cm. The mean height difference between left and right tuber coxae was 1.3 ± 1.6cm (1.d.p). 64% of horses showed asymmetry of the pelvis to the left and 36% to the right.
The mean height discrepancy of the rider iliac crests in a standing position was 0.89 ± 0.64cm (2.d.p) and 1.00 ± 0.66cm (2.d.p) in the sitting position. Of those with a tilt present in the sitting position (93%), 46% had a tilt left and 54% a tilt right. The mean iliac crest height discrepancy for riders with a left tilt in the sitting position was 0.64 ± 0.77cm (2.d.p). Those with a right tilt had a mean iliac crest height discrepancy of 1.36 ± 0.26cm (2.d.p).
Asymmetry of the pelvis was a feature of 93% of horse/rider combinations. Of those combinations where both had asymmetry of the pelvis, 85% occurred in the same direction, 15% in opposite direction. There was a significant positive correlation between horse tuber coxae and rider iliac crest height discrepancies ($r=0.64$, $n=14$, $p<0.05$). Horse and rider pelvis asymmetry relationship suggested that 40% of the variance in rider iliac crest height discrepancy is due to changes in horse tuber coaxae height discrepancy ($R^2 = 0.4$, $F = 8.24$, $P < 0.05$). There were no significant trends between misalignments of the spine and neck of the horse or rider when compared to pelvis asymmetry.

Conclusion This study provides positive evidence of a relationship between horse and rider pelvis asymmetry.
The hypothesis was accepted that if the horse had a left ventral rotation of the pelvis the rider had a left tilt of the pelvis; if the horse had a right ventral rotation the rider had a right tilt of the pelvis. The causal effect relationship between the two variables is indeterminate but the knowledge that a significant relationship is present could have fundamental implications for physical therapy treatment of horse and rider and awareness of asymmetry.

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Inheritance patterns of primary closed angle glaucoma, an emerging disease in UK Border Collies

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Implications This study aims to model the inheritance pattern of the ocular developmental abnormality goniodysgenesis, which is strongly associated with an increased risk of developing primary closed angle glaucoma, an emerging genetic disease of concern in the Border Collie dog breed.

Introduction Goniodysgenesis is a developmental abnormality of the eye which is strongly associated with an increased risk of developing primary closed angle glaucoma (PCAG) in humans and dogs. PCAG is associated with a reduced angle between the iris and the sclera, and can be due to a developmental abnormality termed goniodysgenesis. Glaucoma was rarely reported in Border Collies until recently. Four related cases that resulted in eye loss were known in Australia prior to 2000, including a parent and offspring. In the last few years, there has been a marked increase in the number of Border Collies affected with goniodysgenesis and/or glaucoma in the UK, all of which can be traced back to two champion affected individuals. Goniodysgenesis in these lineages is associated with increased likelihood of developing glaucoma, similar to the situation in humans, where 10% of people diagnosed with anatomically closed (or narrow) angles will develop PCAG (Wang *et al* 2002) Restriction to specific lineages, shared ancestry in pivotal individuals, and the relatively high level of inbreeding in the Border Collie population, appears to be consistent with single recessive gene aetiology. The increased incidence of glaucoma, and the parallel rise in the predisposing factor goniodysgenesis, has impelled breeders to look for solutions to this emerging problem of concern in the breed. Through the Pastoral Breeds Health Foundation (PBHF), breeders in the UK have set up a database for dogs affected with goniodysgenesis and organized workshops with veterinary ophthalmologists to ensure consistency of classification of disease severity.

Material and methods World wide data (Border Collie database) were analysed to ascertain the numbers of dogs that have been tested by an ophthalmologist (including evaluating the iridocorneal angle of the eye) and found to have goniodysgenesis or glaucoma. An extensive pedigree containing affected and unaffected Border Collies and their relatives was developed from online databases containing pedigree information for registered Border Collie breed lineages (Anadune Border Collie Database) and worldwide goniodysgenesis test results for Border Collies (Border Collie database). The heritability and genetic trend of goniodysgenesis is currently being investigated in the Border Collie lineages using ASReml (Gilmour *et al* 1995). We are currently evaluating two alternate inheritance models for goniodysgenesis: (Wang *et al* 2002) Mutation at a single gene could cause goniodysgenesis if heterozygous and glaucoma if homozygous (i.e., "partial dominance" with glaucoma manifesting as recessive), (Border Collie database) Goniodysgenesis may be recessive, resulting from homozygosity for a mutation in a single gene, and the development of glaucoma in animals with genetic goniodysgenesis would be associated with additional mutations in another gene(s). Otherwise stated, the condition may be polygenic as is found in humans.

Results Of 1,328 dogs tested worldwide, 89 (7%) had goniodysgenesis and 8 (0.6%) had glaucoma (Wang *et al* 2002); as of 22 October 2013). This reflected figures for the UK population (47 with goniodysgenesis and 3 with glaucoma of 651tested). There appears to be a strong primary genetic cause, since some lineages of the breed had a high number of affected animals. The two key animals with goniodysgenesis have a common ancestor, in both the maternal and paternal lines.

Conclusion The increased incidence of PCAG in the Border Collie breed over the last 10 years is associated with the recent extensive use of two popular sires with shared ancestry, suggesting single recessive gene aetiology. However, the inheritance pattern of goniodysgenesis and eventual development of glaucoma has been difficult to determine due to incomplete ascertainment, missing individual and phenotype information, and the difficulty working with inbred lines where there is a high probability of homozygous-heterozygous matings, which can produce apparent dominant inheritance patterns. We are currently evaluating two alternate inheritance models for goniodysgenesis to elucidate the relationship between goniodysgenesis and glaucoma, and hence understand the underlying genetic architecture responsible for the development of glaucoma in Border Collies, as well as providing important information on the aetiology of the disease applicable to both canine and human health.

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Quantification of acute phase proteins in interstitial fluid from equine body and limb wounds by the use of mass spectrometry

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Implications This study indicates that chronic inflammation is involved in impaired wound healing on the limb of horses. The results were obtained by mass spectrometry, and our study reports a robust quantification method applicable for future research and diagnostics in the horse.

Introduction In horse, the tendency for pathological wound healing with formation of exuberant granulation tissue (EGT) is a particular problem on limbs, whereas body wounds heal without complications (Wilmink and van Weeren 2005). Our studies of wound healing in horses aims to investigate the role of inflammation in the pathogenesis of EGT formation by analysis of acute phase proteins (APPs) local in the wound environment. Limited access to validated antibodies for protein identification and quantification remains a major challenge in veterinary research (Soares *et al.* 2012). We here report a mass spectrometry (MS) based method that offers a valuable alternative for quantification of the acute phase response in horse.

Material and methods From experimental wounds on the body and limb of five horses interstitial fluid was collected by microdialysis (microdialysate) on day 1, 2, 7 and 14 after wounding. Thirteen APPs were selected for quantification by MS (one major APP, six minor APPs, and six proteins known with APP properties in other species). Since analysis by MS rely on identification of peptides that map uniquely to the protein of interest, MS data from multiple equine tissues and body fluids were generated and assembled into The Equine PeptideAtlas (Bundgaard *et al.* 2013) to assist the selection of suitable reference peptides for the proteins. Quantification was enabled by the use of chimeric peptides of the reference peptides labelled with heavy isotopes. These chimeric peptides were developed by the use of a quantification concatamer (QconCAT) strategy. A targeted MS method was established and the quantotypic properties of the isotope-labelled chimeric reference peptides were validated in microdialysate and serum. Robustness of the MS method was confirmed by comparing the absolute concentrations of APPs measured in serum from both healthy and inflamed animals. Moreover, the measured values were correlated to previous biochemical measures. The optimised targeted MS method was finally used to map the orchestration of APPs in microdialysate from wounds healing normally and wounds healing with EGT.

Results Of the 13 APPs eight proteins could readily be quantified in equine serum and interstitial fluid obtained by microdialysis from a wound, while the expression levels of the five remaining proteins were too low for reliable quantification within the dynamic range of the assay. The concentrations obtained by this QconCAT-MS based method revealed close accordance with concentrations obtained by other methods. The concentration of fibrinogen, haptoglobin, ceruloplasmin, prothrombin, and α-1-antitrypsin were significantly higher (2.5 – 3.5 folds higher day 14) in wounds healing with EGT than wounds healing normally.

Conclusion The QconCAT-MS based method provides a valuable approach for concurrent quantitative analysis of multiple APPs in microdialysate from equine wounds and serum. The orchestration of APPs in microdialysate indicates that non-resolved inflammation is implicated in the pathogenesis of EGT formation. This is the first report to demonstrate the absolute protein levels of APPs quantified by a MS based approach in the horse. This approach can circumvent the considerable challenge associated with lack of validated antibodies for protein identification and quantification. Hence, we expect that MS is a valuable approach for both research and diagnostics in the horse.

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The validation of a novel protocol for the culture of equine endometrial epithelial and stromal cells

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Implications Uterine inflammation (endometritis) is ubiquitous in the mare post-mating but subsequent persistent mating-induced endometritis (PMIE) has implications for fertility and affects approximately 15% of Thoroughbred broodmares (Zent *et al*., 1998). A cell culture model was established to investigate the innate immune response of endometrial cells to inflammatory stimuli, negating the need for whole-animal studies.

Introduction Current methods for investigating the immune pathways that underlie PMIE and potential therapeutic targets uses the whole (live) animal. Whole animal model data accurately reflects the *in vivo* scenario, accounting for the interplay between internal organ and tissue systems. However, whole animal models require large numbers of animals and the application of invasive procedures such as repetitive artificially-induced endometritis and uterine biopsy. Therefore, alternative *in vitro* models are essential tools if synonymous of the whole animal. *In vitro* cell culture models are commensurate with the 3Rs of animal research; Reduction, Refinement and Replacement. Equine endometrial epithelial and stromal cells have been cultured twice previously (Watson *et al*., 1992; Szostek *et al*., 2012); however, methodological challenges were apparent. This study aimed to adapt a bovine endometrial cell culture protocol (Singh *et al*., 1999) and validate its use for equine endometrial epithelial and stromal cells.

Material and methods Equine uteri were collected post-slaughter from a commercial abattoir (n=17). All uteri were in the luteal phase of the oestrous cycle. Endometrium was dissected from underlying tissue, chopped into $1mm^3$ sections, and plated in culture flasks. Tissue remained in each flask until a corona of mixed endometrial cells had formed. Cultures were purified via a process of differential trypsinisation. Williams complete media was used for cell culture and 0.1% and 0.25% trypsin to lift stromal and epithelial cells respectively. Once confluent; cells were challenged with control (media alone), oxytocin (OT; 1nM), or *E. coli*-derived lipopolysaccharide (LPS; 1µg/ml), to determine their response to physiological and immunological conditions. The response was assessed by measuring cellular prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) secretion by radioimmuno assay.

Results Stromal cells secreted significantly more ($P<0.05$) $PGF_{2\alpha}$ and PGE_2 when challenged with OT than control treatment. Although no other significant response to any treatments were seen when the raw data was assessed it became clear that in every case control treated cells secreted less prostaglandin than either OT or LPS treated cells. Passage number had a significant ($P<0.05$) effect on prostaglandin secretion, with both EC and SC secreting more PGE_2 in passages four and five respectively. Stromal cells secreted more $PGF_{2\alpha}$ in passage five, whereas EC had a tendency ($P<0.08$) to secrete most in passage two. There was a large degree of inter-mare variability ($P<0.001$).

Conclusion The protocol was established, optimised and validated for the culture of equine endometrial epithelial and stromal cells. The model may be used for future studies to investigate the response of equine endometrial cells to immunological challenge and the effect of potential anti-inflammatory compounds. The protocol negates the need for live, whole animals to be used in endometritis research, and will facilitate the understanding of PMIE and potential treatment strategies for improving mare health and welfare.

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An investigation into the relationship of pelvic misalignment on forelimb hoof size

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Implications This study demonstrated a relationship between direction of pelvic rotation and forelimb hoof size (width and length). This should raise potential awareness when assessing pelvic or feet asymmetries.

Introduction Pelvic misalignment or pelvic rotation occurs when the most dorsal aspect of the tuber coxae is higher on one side of the horse than it is on the other rather than being level and in alignment. It is a common problem in horses and is a significant cause of lameness, performance breakdown and diminished activity (Weeren and Crevier-Denoix, 2006). Studies investigating the science of lameness and resulting compensation mechanisms suggest that a lame horse, or with a pelvic rotation, attempts to take the weight off the lame limb by redistributing locomotor forces to other limbs, mainly the diagonal limb, Clayton *et al*, (2001). The occurrence of differently shaped and sized front feet is a commonly encountered fault of which the clinical significance is unclear, Heel *et al* (2006). The aim of this study was to determine whether there is a relationship between pelvic misalignment in the horse and uneven forelimb hoof size.

Material and methods 30 healthy, sound, multidiscipline horses, regularly shod/trimmed every 6 weeks, were assessed; (10.3years±7.3), height (14.3hh±2.1). Pelvic height measures were taken from the dorsal aspect of the tuber coxae to level ground when stood square, using a plumb line and measuring stick. Ventral pelvic rotation was calculated, left or right. A 300mm digital vernier calliper measured foot dimensions. Hoof width (lateral to medial edge) and hoof length (dorsal to palmar edge), were measured of both fore feet, prior to and after farrier trim, at two consecutive 6 week shoeing intervals by the farrier blinded to pelvic measures. Both methods were tested for reliability and repeatability. Statistical analyses of data sets included chi-squared, symmetry indices and one-way ANOVA.

Results Left ventral pelvic rotation was evident for 13 horses, right ventral pelvic rotation for 12 horses and no rotation for 5 horses. Horses with a ventral pelvic rotation resulted in more hoof growth (width) on the contralateral forelimb and more hoof length on the ipsilateral forelimb over a 6 week shoeing interval period.

Table 1 Pelvic rotation direction relationship to mean hoof growth measures(mm) ± s.e for 6 week period

Ventral pelvic rotation	Increase in hoof width		Increase in hoof length	
	Near fore	Off fore	Near fore	Off fore
Right	3.9±0.3	1.8±0.4	3.4±0.5	5.7±0.5
Left	3.2±0.3	5.3±0.6	4.5±0.5	2.8±0.4

There was a significant relationship between uneven hoof width and pelvic rotation directional ($P<0.001$); a significant relationship between uneven hoof length and pelvic rotation directional ($P<0.001$). Over a 6 week period, there was a relationship between amount of hoof width growth ($P<0.001$) and hoof length growth ($P<0.001$) with pelvic misalignment. Symmetry indices demonstrate how the horses near or off fore-hoof was wider or longer in relation to the pelvic rotation.

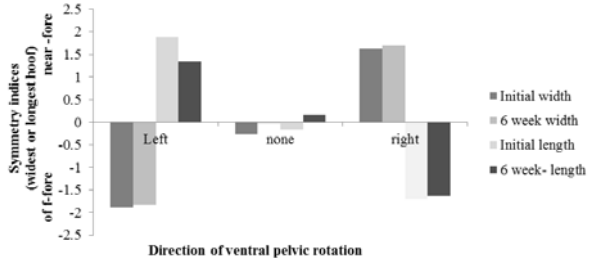

Figure 1 Symmetry indices of hoof length and width in relation to ventral pelvic rotation

Conclusion This study suggests statistically that alignment of the pelvis does have a significant relationship on width and length growth of horse's fore-hooves. Further study would be beneficial in understanding more the chain of compensatory effects on the equine body and in relation to performance parameters.

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The effect of live yeast supplementation on the canine hindgut microbial population in a continuous culture fermentation

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Implications The *in vitro* model described provides a stable simulation of canine hindgut fermentation, and can be used to assess the impact of feed additives on digestibility, bacterial structure/pathogen load or metabolite production profiles here.

Introduction There is growing commercial interest in developing nutritional supplements such as probiotics for the pet nutrition market. With dogs the largest pet market sector in the UK, this presents a need to develop cost effective, non-invasive systems, which allow the effect of diet and dietary supplements on digestive function in the dog to be studied. The aim of this experiment was to investigate the effect of supplementation with a live yeast on metabolite production profiles and the bacterial population composition of the canine hindgut represented using a simple *in vitro* fermentation model.

Material and methods Freshly voided faeces were collected from 7 dogs, sealed in plastic bags with air expelled, and incubated at 37°C until preparation of a pooled fermentation inoculum (max 4 hours). All donors were adult dogs (2-8 years) fed commercial dry dog foods, none had received antibiotic therapy in the preceding 3 months. The fermentation was carried out using 8 x 2L bioreactor vessels (4 per treatment – live yeast or control), working volume of 750 ml, with automatic pH (6.5 ± 0.1), temperature (37°C) and agitation (100 rpm) control. Anaerobic conditions were maintained by bacterial fermentation and redox continuously measured. A sterile nutritive culture medium (Macfarlane *et al*, 2005) provided the fermentation substrates, supplied continuously by peristaltic pump to the vessels at 0.5 ml/min, with equal outflow. Treatment vessels were dosed with 0.3 g of live yeast per vessel each day, with control vessels given no yeast. The yeast product tested was a commercial strain (ActiSaf Sc 47) supplied by Lesaffre Feed Additives, France. Daily fermenter samples were analysed for VFA concentration as an indicator of fermentation activity. Day 13 and 14 fermenter samples were analysed for VFA, lactate and ammonia concentrations, and quantitative PCR (qPCR) measurement of bacterial cell numbers. Massive parallel sequencing of the 16S rRNA V1-V2 gene region (400 bp amplicon) using Next Gen techniques (Ion Torrent PGM™, Life Technologies) was performed to investigate the bacterial composition of each vessel. Metabolite concentrations were tested by one-way ANOVA in Genstat. Principle Co-ordinate analysis (PCoA) and pairwise MANOVA was performed in 'R' with the Operational Taxonomic Unit (OTU) data for each day 13/14 fermenter and faecal sample.

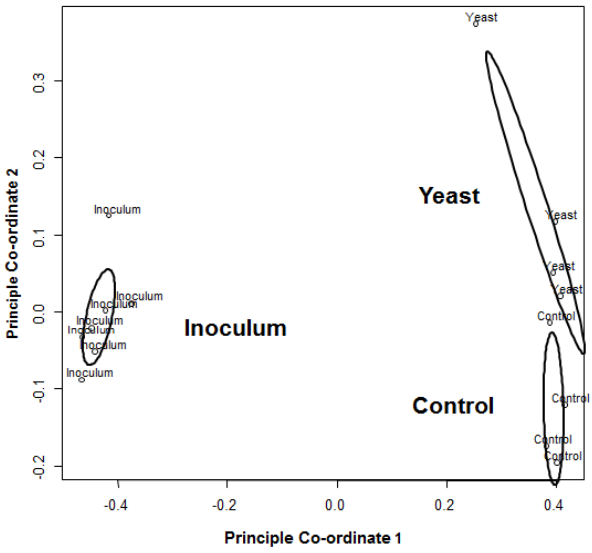

Figure 3 PCoA plot based on Bray-Curtis dissimilarity measures, 95% confidence limit intervals of live yeast and control treatment vessels (4 per treatment) after a 14 day *in vitro* fermentation trial, and the 7 canine faecal samples used to form the fermentation inoculum

Results Total VFA, ammonia and lactate levels were found to be consistent between all vessels and values similar to previous studies (Macfarlane *et al*, 2005), with no significant treatment effects. qPCR analysis indicated a stable number of bacterial cells surviving in culture, comparable to *in vivo* values (Suchodolski, 2011). Sequencing of the 16S rRNA gene identified 600 OTUs at the 97% similarity (species) level, from 6.5 million sequence reads within the final library sample set. The average Goods coverage value was 99.83% (s.d. 0.121) per sample. PCoA and pairwise MANOVA analysis revealed separate grouping of the yeast and control treatments at a 95% confidence interval, showing a significant (P=0.031) difference in the bacterial composition between each *in vitro* treatment group (Figure 1).

Conclusion The *in vitro* system used here showed remarkable stability throughout the 14 day fermentation period, and there was a clear response to the yeast supplement given *in vitro* in terms of the bacterial composition of the fermenter contents. However the greatest difference was seen between the faecal donor samples and the fermenter vessels regardless of treatment. *In vitro* models are a useful tool for the initial study of how dietary supplements effect the bacterial microbiome and fermentation activity within the gut.

Acknowledgements KMH acknowledges funding via the KESS program, and Lesaffre Feed Additives, France for provision of the live yeast product. We are very grateful to E. Jones and Dr S. E. Girdwood for their technical assistance.

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061

A preliminary study to investigate the passive range of motion of the proximal limb joints and relationships to British Veterinary Association/Kennel Club (BVA/KC) hip score in dogs

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Implications Joint range of motion (ROM) data may be an effective, low cost, monitor of susceptibility to hip dysplasia (HD) in dogs and for evaluation of any progression.

Introduction Hip dysplasia continues to be one of the most common orthopaedic diseases in dogs. Dogs afflicted with HD can often show minimal to no clinical signs but it can be a highly debilitating condition. Currently, prediction or measurement of HD is only available through orthopaedic examination and pelvic radiographs. It would be beneficial to be able to assess any progression of the disease on a more regular basis. Joint ROM describes the limits of which a particular joint can move, in its physiological planes of motion, either actively or passively. Passive ROM demonstrates the integrity of the joint capsule, ligaments, fascia and articular surfaces of the joint without the influence of muscle activity. The aim of the present study was to provide scientific data on passive joint ROM of the shoulder, elbow, hip and stifle joints and whether there are relationships between particular joint ROM and to BVA/KC hip score in dogs.

Material and methods 16 KC registered, healthy, sound, single breed (Siberian Husky) dogs of mixed gender(9 female, 7 male) and age >1year (mean±s.d.:7.6±3.8 years), that live and work together, were used to minimise genetic and environmental effects. Goniometry was used to measure joint range of motion as it is has been validated in dogs (Jaegger *et al*, 2002) as a reliable, repeatable, non-invasive method of measuring passive ROM and is economical and easy to learn. Dogs were conscious, placed in lateral recumbency and specific boney landmarks identified. Triplicate goniometer measures of joint flexion and extension were measured on both sides of each dog for the shoulder, elbow, hip and stifle joints. All measures were taken by the same investigator who was previously tested for acceptable repeatability of measurement as per Jaegger *et al*, 2002. Wither height and body weight were also measured. Mean values of the triplicate measures were computed for each dog. Data was analysed using Students t-test and Pearson Moment Correlation statistics to investigate gender, age (<6yrs vs. >6yrs) and laterality effects on joint range of motion and any relationships to BVA/KC hip score.

Results Gender and age had no significant effect ($p>0.05$) on joint range of motion measures for flexion or extension of the shoulder, elbow, hip and stifle joints.

Table 1 Joint ROM measures for all dogs (mean±s.d., (range))

Joint	Joint flexion (degrees)		Joint extension(degrees)	
	left side	right side	left side	right side
Shoulder	61.4±8.3 (46.7-72.7)	65.6±9.0 (51.0-81.3)	128.3±12.8 (106.3-158.7)	114.3±7.8 (102.3-127.0)
Elbow	29.9±4.9 (19.7-37.7)	26.2±5.0 (19.7-38.7)	132.1±7.3 (118.0-143.7)	133.6±7.5 (120.0-146.0)
Hip	71.9±8.7 (57.0-91.7)	65.2±8.1 (45.7-77.7)	121.3±9.8 (106.0-139.0)	126.8±7.7 (113.7-141.7)
Stifle	34.7±4.7 (26.0-45.7)	42.5±4.5 (35.0-51.7)	125.7±7.7 (107.3-137.7)	125.0±5.9 (115.7-135.0)

There were statistically significant differences between the left and right side ROM measures of the hip ($p<0.01$), stifle ($p<0.01$), shoulder ($p<0.001$) and elbow ($p<0.05$) joints with differences in joint flexion being the dominant effect.
There was a significant correlation between total BVA/KC hip score and joint ROM means for left elbow extension ($r=0.559$, $p=0.02$) and right hip extension ($r=0.518$, $p=0.04$). There was a significant correlation between right side BVA/KC hip score and left elbow extension ($r=0.606$, $p=0.01$) and right hip extension ($r=0.704$, $p=0.002$). There was no significant ($p>0.05$) laterality effect for neither hip nor elbow extension ROM.

Conclusion Bilateral ROM measurement is important to consider. Dogs tested radiographically to have a higher hip score on a particular side, may predispose to an increased extension range of that hip joint and the contralateral forelimb elbow extension. This could have implications in monitoring limb joint function of dogs, with and without a BVA/KC hip score test, as an indicator of sub-clinical changes related to movement dysfunction.

Acknowledgements There was no external funding for this project

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062

Breed-specific health results from surveys of three pedigree dog breeds – Otterhounds, Manchester terriers and Hungarian wirehaired vizslas

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Implications Cross-sectional studies such as these are well-suited to the goal of describing variables and their distribution patterns, in this case, prevalence of disease conditions in different breeds of dogs.

Introduction Most studies on canine morbidity and mortality use pet insurance data (Egenvall *et al.*, 2009). A recent study reported morbidity from a survey of members of the Danish Kennel Club (Proschowsky, *et al*., 2003). As very little is known about the prevalence of disease in UK dogs, these surveys were undertaken to obtain baseline and follow-up data.

Material and methods Data for 2004 was collected from a first survey undertaken with the UK Kennel Club that included anonymous responses from owners of pedigree dogs for 169 breeds. The aim was to identify important breed-specific problems for future genetic research and to provide baseline information against which the success of future control schemes could be measured. The second surveys were carried out between 2009 and 2010 as separate breed-specific confidential surveys. The aim was to gather further health information on a larger number of individually identified dogs. Disease occurrence is reported as breed-specific prevalence rates, estimated as (number of affected dogs)/(total number of live dogs), with 95% confidence intervals (CIs).

Results The overall response rate (RR) in 2004 for the 169 breeds was 24% and the RRs were 14% for Otterhounds (20/139), 33% (59/178) for Manchester Terriers and 40% (54/136) for Hungarian Wirehaired Vizslas. In the breed-specific surveys, the RRs were 59% (359/612) for Otterhounds, 57% (158/277) for Manchester Terriers and 57% (159/281) for Hungarian Wirehaired Vizslas, and these were each significantly higher (P<0.0001) than in 2004. The first survey shows breed differences in lifespan, causes of death and prevalence of disease, and the results support previous evidence that smaller breeds tend to have longer lifespans compared to larger breeds. Long-lived breeds died of diseases appropriate to their longevity with cancer, old age and chronic renal failure representing the highest proportional mortalities for these breeds. The results of the 3 breed-specific surveys were similar to those of the 2004 survey for each breed with respect to the most commonly reported conditions although the estimated prevalences of specific conditions varied (Table 1).

Table 1 Breed-specific prevalence of disease (95% confidence intervals) for 3 UK breeds.

Otterhounds	2004 (N=56)	2009 (N=122)	
1 Sebaceous cysts	11% (4 – 23)	36% (28 – 45)	
2 Ear infections	9% (3 – 20)	27% (20 – 36)	
3 Bloat/GDV	9% (3 – 20)	23% (16 – 32)	
4 Other Dermatologic	7% (2 – 18)	10% (5 – 17)	Anal gland problems
Manchester Terriers	2004 (N=117)	2009 (N=216)	
1 False pregnancy	16% (8 – 27)	29% (21 – 38)	
2 Kennel cough	8% (4 – 15)	24% (18 – 30)	Anal gland conditions
3 Gastroenteritis	4% (2 – 10)	8% (5 – 13)	Conjunctivitis
4 Heart murmur	4% (2 – 10)	6% (3 – 10)	Gastroenteritis
Hungarian Wirehaired Vizslas	2004 (N=102)	2009-2010 (N=240)	
1 Aural	7% (1 – 9)	36% (30 – 43)	
2 Dermatological	7% (1 – 9)	29% (18 – 30)	
3 Immune-mediated	6% (1 – 9)	10% (7 – 15)	Ocular
4 Ocular	6% (1 – 9)	7% (4 – 11)	Immune-mediated

Conclusion The 3 participating breed clubs are using the results of these surveys to prioritise future research and to help plan breeding strategies. Further analysis of the data will include pedigree analysis to assess potential inheritance of reported health conditions. Otterhounds are still being followed.

Acknowledgements These surveys were generously funded by The Kennel Club Charitable Trust with additional contributions by the individual breed clubs involved. The research was carried out at the Animal Health Trust.

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063

Demonstrating the value of carrying out randomised clinical trials in small animal practice: the example on the use of medetomide and/or butorphanol as preanaesthetic agents

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Implications Studies that investigate anaesthetic agents can be performed in practice and the results of such studies can inform clinical decisions regarding efficient and cost effective anaesthetic regimens.

Introduction Randomised blinded controlled clinical trials (RCTs) are considered the gold standard method for investigating efficacy of treatments. In veterinary medicine RCTs remain relatively rare and often involve multicentre studies that tend to be funded by pharmaceutical companies with specific interests. The aim of this paper is to show the value of carrying out RCTs in small animal practice by examining the effects of two preanaethetic agents, medetomidine and butorphanol, used either alone or in combination.

Material and methods Medetomidine and butorphanol are commonly used preanaesthetic medications evaluated in 2 RCTs performed at the Animal Health Trust in the UK as part of anaesthesia residency training. The first study evaluated the sedative effect of low dose intravenous medetomidine and butorphanol, alone and in combination and a placebo (saline) control group in 60 client owned dogs randomly assigned to treatment group. The second study investigated the effects of intramuscular medetomidine and butorphanol, alone and in combination, on the required induction dose of alfaxalone in 85 dogs client owned dogs block randomised to treatment group according to temperament.
Sedation was assessed before and after drug administration using a validated numerical scoring system. Heart rate, respiratory rate, pulse quality, capillary refill time and rectal temperature were also recorded in the first study and induction dose, induction quality, intubation score and the occurrence of any adverse events (sneezing, twitching, paddling, excitement, apnoea and cyanosis) were recorded in the second study. Treatment groups were compared using one-way analysis of variance and significant overall differences were further evaluated using post-hoc mu ltiple pairwise testing.
In the second study 0.5 mg/kg of alfaxalone was administered intravenously over 60 seconds 30 minutes after intramuscular sedative injection and tracheal intubation was attempted. If tracheal intubation was not possible after 20 seconds, further boluses of 0.2 mg/kg were given every 20 seconds until intubation was achieved. All observations were made by an anaesthetist blinded to the treatment group assignment. The level of significance was set at $p<0.05$.

Results Average pre-sedation scores were similar in all groups while post-sedation scores were significantly higher in the group that received medetomidine and butorphanol. Sedation score differences were also significantly higher in the combined drug group (Figure 1). The average dose of alfaxalone required for induction in the second study was similar for the groups that received medetomidine or butorphanol alone and were significantly lower for the group that received medetomidine and butorphanol combined (Table 1). Induction dose of alfaxalone was not influenced by temperament. Induction and intubation scores did not differ between groups. Sixteen dogs experienced adverse events and these were not associated with treatment group, temperament or level of sedation.

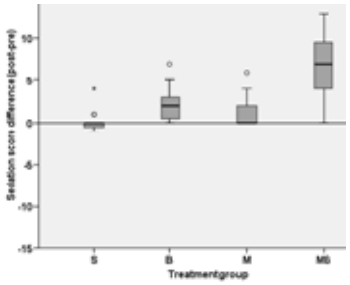

Table 1 Induction dose of Alfaxalone following sedation with butorphanol and/or medetomidine

Tx group	Mean dose (sd)	Mean difference (95% CI)*	P-value
Medetomidine	1.19 (0.36)	0.43 (0.20, 0.66)*	<0.0001
Butorphanol	1.20 (0.38)	0.44 (0.21, 0.67)*	<0.0001
But + Med	0.77 (0.33)		

* Compared to butorphanol (But) + medetomidine (Med) given together

Figure 1 Sedation score difference after IV sedation with saline (S),butorphanol (B), medetomidine (M) or medetomidine and butorphanol (MB)

Conclusion Medetomidine combined with butorphanol induces good sedation and reduces the induction dose of alfaxalone. Medetomidine and butorphanol administered in combination reduce the anaesthetic induction dose of alfaxalone compared to either agent alone. This difference was clinically significant and should be taken into account when using this combination of drugs in a clinical setting. Studies that investigate anaesthetic agents can be performed in practice and the results of such studies can inform clinical decisions regarding efficient and cost effective anaesthetic regimens.

Acknowledgements The research was carried out at the Animal Health Trust.

064

Updates on equine grass sickness

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Introduction Grass sickness (GS, equine dysautonomia) is a multi-system neuropathy of equidae characterised by damage to autonomic, enteric and somatic neurons. It has an extremely high mortality rate (>95%) and significant welfare, emotional and financial consequences. While there is increasing evidence that GS is a toxico-infection with *Clostridium botulinum* types C or D, definitive proof of this is currently lacking. An identical disorder occurs in hares, dogs, cats and rabbits, and possibly also in sheep and llamas. This presentation will review recent information and ongoing work relating to GS.

Vaccine trial Arguably the most significant development regarding GS is the start of a multicentre (AHT, Universities of Edinburgh, Liverpool & Surrey) field trial to determine the efficacy of a killed *Clostridium botulinum* type C toxoid vaccine (BotVax C, Neogen Coroporation) in the prevention of GS. This randomised, placebo controlled, triple blinded trial will last 24-36 months and involve 1100 horses on premises with a previous history of GS. A secondary objective is to use pre- and post-vaccination serological data to evaluate the immunological response to vaccination. The UK-wide trial follows a successful pilot study completed in Scotland in 2013.

Investigation of GS aetiopathogenesis
While there is increasing evidence that GS is a toxico-infection with *Clostridium botulinum* types C or D, work continues to investigate GS aetiopathogenesis, including investigation of alternative hypotheses.

One collaborative study (University of Liverpool & Reading) is comparing the intestinal microbiome and the metabolome (gastrointestinal contents, urine, blood) of GS and control horses to identify potential causal agents and/or disease biomarkers.

Another study (FERA, University of Edinburgh) is investigating the potential role (causal or trigger) of mycotoxins, particularly those from *Fusarium* species, utilising latest knowledge of pathogen epidemiology and state of the art mycotoxicology techniques. This work follows a pilot study which revealed high levels of several *Fusarium* species, and extremely high levels of *Fusarium* derived mycotoxins, on plants from GS pastures.

A multicentre (Newcastle University, University of Edinburgh) study is examining the neuromuscular junction in horses with GS to determine whether there is evidence of the action of *Clostridium botulinum* neurotoxins. Preliminary data suggest that GS is not associated with significant degenerative pathology to the distal components of the motor innervation of the skeletal muscle or the skeletal muscle itself. Furthermore, the apparent loss of synaptic vesicles at the terminal bouton is inconsistent with the hypothesis that GS is due to the effect of botulinum *neurotoxins*, because botulinum neurotoxins typically inhibit the release of transmitter by hydrolysing the proteins SNAP-25 and syntaxin, leading to accumulation of synaptic vesicles at the nerve terminals. These findings do not, however, preclude involvement of any of the other toxins produced by *C. botulinum*. Additionally, study of the distribution of the target proteins for botulinum neurotoxins (syntaxin-1, SNAP-25, synaptobrevin) in autonomic neurons from horses with GS has also yielded data that are inconsistent with involvement of botulinum neurotoxins in GS. In contrast to horses with paralytic botulism, horses with GS had accumulation of all three proteins within neuronal perikaraya, probably reflecting failure of axonal transport of protein-containing vesicles to the nerve terminals.
Proteomic profiling of cranial cervical ganglia from GS and control horses has identified 2311 unique proteins, with 320 proteins being increased and 186 decreased by greater than 20% relative to controls. This study identified dysregulation in proteins commonly associated with cell stress and protein misfolding/aggregation responses, including Beta-amyloid and the ubiquitin-proteasome systems, as well as alterations in synaptic vesicle proteins, proteins involved in synaptic transmission, acute phase response proteins, neurotransmitter proteins, apolipoproteins and secretogranins.

Copas *et al* (2013) reported that GS was associated with marked increases in the acute phase proteins Activin A, serum amyloid A and fibrinogen. Interestingly, Activin A concentrations were also significantly elevated in co-grazing horses; suggesting that they have subclinical disease as a result of exposure to the aetiological agent.

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065

The usefulness and limitations of metformin as a medical aid alongside dietary management in laminitis-prone horses and ponies

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Implications Metformin impairs systemic glucose absorption and the consequent hyperinsulinaemic response in horses. The drug may be useful in laminitis-prone horses which are returned to limited grazing following effective dieting and weight loss.

Introduction Hyperinsulinaemia triggers laminitis in the absence of insulin resistance or hyperglycaemia (Asplin *et al* 2007). It also appears that laminitis-prone individuals experience an exaggerated hyperinsulinaemia following sugar ingestion compared with non-laminitic individuals (Borer *et al* 2012). Moderation of post-prandial hyperinsulinaemia is best achieved via control of dietary quantity and quality, although it is generally the case that many laminitis-prone individuals will return to some grazing. This study was designed to investigate the possibility that metformin hydrochloride might limit systemic glucose absorption and hyperinsulinaemia in horses, as has been shown in other species (Sakar *et al* 2010).

Material and methods Following clinical observations of an apparent beneficial effect of metformin in laminitis-prone individuals, and an indication of a moderating effect on serum insulin concentrations, an experimental study was designed to investigate these effects further. Seven healthy geldings were fed 1 g/kg BWT dextrose with and without pre-treatment with metformin at 30 mg/kg BWT. Glucose and insulin concentrations in plasma/serum were measured and treatment effects were examined.

Results Clinical cases of Equine Metabolic Syndrome and pituitary *pars intermedia* dysfunction with a history of laminitis were found to have greatly reduced serum insulin responses to oral dextrose challenge and were subsequently maintained on treatment. A similar effect was seen in healthy horses with a statistically significant reduction in peak glucose concentration ($P = 0.002$), area under the glucose curve ($P<0.001$) and insulin concentration 120 min after dextrose administration ($P = 0.011$) (Fig 1).

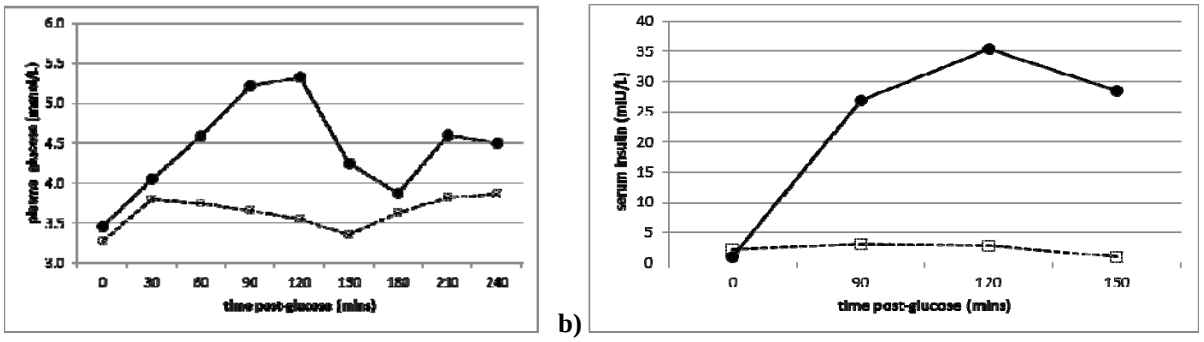

Figure 1 Illustration of a) glycaemic and b) insulinaemic responses to oral dextrose challenge in horses with (dotted lines) and without (solid lines) pretreatment with metformin hydrochloride.

Conclusion Metformin hydrochloride appears to impair enteric glucose absorption in horses and subsequently moderate post-prandial hyperinsulinaemia. Given previous evidence of poor systemic absorption of metformin (Hustace *et al* 2009), the drug might be of little additional benefit when horses are maintained on an appropriate low NSC diet. In contrast, metformin treatment might be most beneficial when applied in a targeted fashion following return to pasture.

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The effects of a high-starch or high-fibre diet on normal equine behaviour

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Implications The study horses demonstrated increased physiological reactivity when fed the high-starch diet compared to the high-fibre diet. This increased reactivity may result in horses that are more difficult to handle.

Introduction Horses have evolved to browse and graze, eating a high-fibre, low-starch diet. It is often thought that the domestic horse needs a change of diet in order to meet the increased energy requirements associated with work. This usually results in a reduction in the forage ration combined with an increase in concentrates, often in the form of high-starch cereal grains. High-starch cereal grains result in a high glycaemic response which may lead to increased reactivity. The aim of the study was to evaluate the behavioural and physiological responses of horses when fed a high-starch (HS) or a high-fibre (HF) diet.

Material and methods A 2 x 28 day cross-over design study was used to evaluate physiological (heart rates) and behavioural responses when horses were fed a high-starch or high-fibre diet. 8 mature working horses were divided into 2 groups. Group 1 received the starch diet for the first 28 day period and group 2 for the second 28 day period. Horses were fed for light work (DE (Mcal/d) = (0.0363 x BW) x 1.2). The HF diet consisted of haylage plus high-temperature molasses free alfalfa (HTA) with a digestible energy (DE) of 11.5 MJ/kg, 27% fibre and 2% starch. The HS diet consisted of haylage plus a high-starch compound mix (HSM) with a DE of 11.3 MJ/kg, 13% fibre and 22% starch. Both the HTA and the HSM were fed to provide the equivalent amount of energy supplementation. The horses on the HS diet received 0.7g of starch/kg BW per meal and the horses on the HF diet received 0.3g of starch/kg BW per meal. During each 28 day experimental period, behavioural and physiological measurements were recorded at 0, 7, 14, 21 and 28 days during behavioural testing. The behavioural tests were based on the initial novel object and handling tests developed by Visser *et al.* (2001) and were conducted using the methodology described by Christensen *et al.* (2005) and Malmkvist & Christensen (2007).
Factor analysis with varimax rotation was used in SPSS® to initially analyse data. There was no effect of day on any of the parameters measured, therefore both FA and raw data was averaged over the 5 testing days. This enabled the total effect of diet over each 28 day period to be analysed. Generalised Linear Mixed Models in Genstat® edition 15 was then used to analyse effects of diet and period on each factor and individual variable.

Results In the handling test, factor 3 (physiological reactivity) showed a significant relationship to diet ($P<0.01$), as did the individual variables of maximum and average heart rates which were both higher when horses were fed the HS diet compared to the HF diet ($P=0.017$, $P=0.01$). Handling scores improved during the second 28 day period ($P=0.05$) suggesting some adaptation to the test. However, there was no difference in average heart rates related to period indicating that horses still demonstrated a physiological response to the test. In the novel test factor 3 (eating reactivity) showed that horses on the HS diet demonstrated more interrupted eating ($P<0.01$). The individual novel test variables showed that when horses were fed the HS diet their maximum heart rates were significantly higher ($P=0.01$) compared to the HF diet.

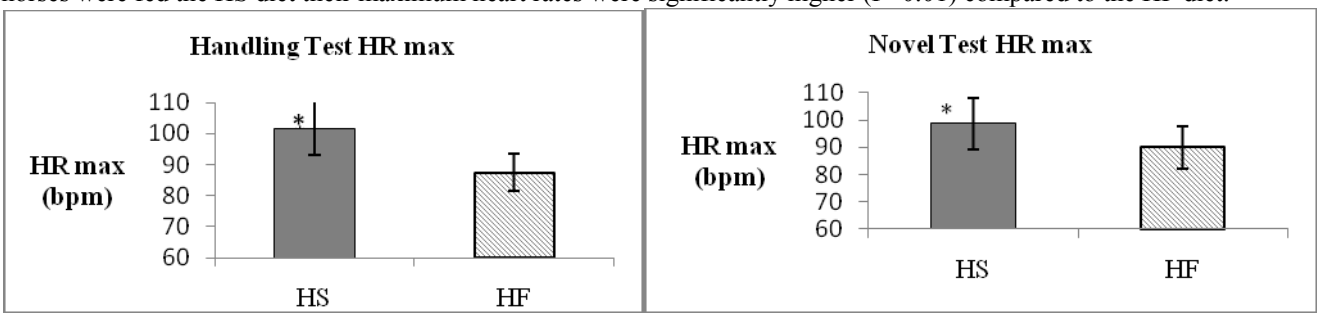

Figure 1a and b Heart rates for the handling and novel tests. * denotes sig diff related to diet (1a)P=0.017 (1b)P=0.01.

Conclusion Results demonstrate that the HS diet had some effect on the normal behaviour of horses, in particular their physiological reactivity. This may increase behavioural reactivity, particularly with less experienced horses and/or horses being handled by an inexperienced handler.

Acknowledgements Dengie Horse Feeds.

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067

Post-transcriptional gene regulation in ageing equine cartilage and chondrocytes

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Implications The results of this study show that the ageing process leads to an increase in the length of time some genetic transcripts remain available for translation into their corresponding protein. These genes have been found to be important for functions such as communication between cells and producing cartilage. The increase in time taken for these transcripts to decay suggests that it becomes increasingly difficult for the cartilage cells to maintain the transcript at optimal levels, as the cells become less rapid at responding to cues which may require acceleration or deceleration of transcription. This may result in abnormal cellular signalling and tissue composition.

Introduction RNASeq studies have shown a difference in gene expression for a number of genes related to cartilage extracellular matrix in non-pathological ageing (Peffers *et al*., 2013).This study had two main objectives. The primary objective was to interrogate the role of mRNA decay in post-transcriptional gene regulation in ageing equine chondrocytes. The secondary objective was to compare the results of explant studies against parallel chondrocyte studies in order to assess chondrocytes as a model for ageing cartilage.

Material and methods Equine articular cartilage from the entire surface of the fetlock joint was harvested from young (n=3) and old (n=4) horses and split into explant and isolated primary chondrocyte work flows. The explants and chondrocytes were maintained in an incubator overnight before being subjected to an actinomycin chase. The cells were harvested at time points over a 5 hour period and subjected to total RNA extraction using the guanidine thiocyanate-phenol-chloroform method. qRT-PCR was used to examine for differential gene expression across the time points and between donors by comparison to GAPDH, using the comparative C_t method. An RNA half-life was generated from these values. Pearson's correlation coefficient and mixed effects linear regression were used for statistical analysis.

Results The results of the study show a significant increase in the half-life of the mRNA for COL2A1 (p=0.007) in older chondrocytes compared to young. The mRNA for DKK1 showed a trend of increasing half-life in old donors compared to young (p=0.07), but this was not statistically significant. There was found to be a statistically significant increase in half-life between old chondrocytes and old explants for COL2A1 (p=0.013) and HAS2 (p=0.022). With combined chondrocyte and explant data, COL2A1 showed a statistically significant increase in half-life (p=0.05).

Conclusion A modification of the RNA decay rates as shown in COL2A1 and HAS2, conferred by the ageing process, may be accountable for the differential gene expression observed for these genes in RNASeq studies of healthy equine articular cartilage. The change in gene expression of genes FGF2, ADAMTS5, COL1A1, and RUNX2 is probably under control of another mechanism, as the half-life of these RNAs was not significantly different between young and old horses. Increasing both young and old sample sizes may clarify the mechanism by which DKK1 mRNA levels are regulated.

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068

The date of return to cyclicity in seasonally anoestrous mares is independent of the endogenous circannual rhythm and is dependent upon daylength which itself is dependent upon latitude

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Implications Breeders and veterinarians often remark that a particular spring is 'late' with mares slow to come into oestrus/ovulation. The date of return to cyclicity is influenced by daylength but what influence does weather (heat, rain etc) have. Could better management advance the date of the first ovulation?

Introduction The period of seasonal or winter anoestrus is characterised by low circulating plasma oestradiol and gonadotrophin levels, and basal progesterone levels. Entry to this period is a response to decreasing daylength, management and the mare's endogenous circannual rhythm. The mare has a photosensitive phase in the hours after the onset of darkness. Melatonin secreted from the pineal gland in the absence of light can adjust rapidly to the light/dark cycle and is inhibitory to the hypothalamic/hypophyseal axis and consequently to gonadotrophin production. Pinealectomy however did not stop mares becoming anoestrus (Sharp *et al,* 1979) suggesting the important influence of the endogenous rhythm. Age, breed, lactation, nutrition and other environmental stressors have strong impact of the date of the last ovulation which can vary in the Northern Hemisphere from July to January. However not all mares experience a seasonal anoestrous phase. This report investigates in three studies by retrospective analysis of accumulated data, the influence of daylength, management, weather and circannual rhythm on the return to cyclicity as determined by the date of the first ovulation.

Material and methods
Study 1. 130 transitional periods in 50 individual mares of mixed breeds aged from 2 to 26 years maintained outside under ambient light at latitude 52N, were studied from the last ovulation of the year until the first ovulation following a period of anoestrus. A retrospective 'weather forecast' from meteorological data was made from March to May for the years 2001-2012.
Study 2. The records of 410 TB mare/years under ambient light conditions but housed at night at latitude 53N were analysed for the years 1991-1997 as in Study 1.
Study 3. The records of 380 Standardbred mare/years maintained under ambient light conditions at latitude 27.5S (Queensland) were analysed for the years 1999-2001 as in Study 1

Results Study 1. The mean date of the first ovulation was April 23rd and only varied in 12 of the 17 years studied between April 20th and April 27th. In the other five years (total 26 mare/years) the variation (April 10th to May 5th) was not significantly different. The mean dates for all years between the earliest and the latest ovulation was April 6th and May 12th respectively. No correlation could be found between 'good' (warm/dry/sunny) spring weather and the mean first ovulation date nor between 'bad' (cold and wet) spring weather. The two seasons with contrasting weather and a large number of mares both had mean dates of April 24th.
Study 2. The mean date of the first ovulation was April 23rd and varied from April 20th to April 24th. No weather data was available for these years.
Study 3. The mean date of the first ovulation was October 24th (=April 24th in NH) and varied from October 22nd to October 25th. 80% ovulated in October and 19% in November. Although no weather data was available, most days were warm (20C to >30 C) and dry.

Conclusion The date of the onset of seasonal anoestrus is extremely variable and although based upon decreasing daylength is influenced by many other factors including circannual rhythms. The onset of the ovulatory season however is almost entirely influenced by day length which is affected by latitude. It may only be modified by breed/type and possibly by circannual rhythm. It does not appear to be much influenced by management or weather conditions. Since at any particular latitude, daylength is the same throughout the seasons from year to year, the mean date of the first ovulation remains constant. Springs are neither early nor late

Acknowledgements Stud managers and my colleagues

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Causes of maternal and neonatal mortality within the equine breeding industry within a 43 year period

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Implications A holistic risk assessment protocol has been proposed that could reduce wastage in the equine breeding.

Introduction Attempts to reduce devastating losses of mares or foals are made through preventative measures, such as management schemes and health precautions; however there is a clear need for further improvements to prevent deaths (Grogan and McDonnell, 2005). The aim of this study was to explore the most common causes of loss of mares and foals that can be classified as preventable.

Material and methods Analyses were conducted on 16,392 records of mare or neonate losses reported to the National Foaling Bank between 1965 and 2007. Data were categorised into 18 causes of foal losses and 14 causes of mare losses. Frequencies of the common causes of loss were compared using Kruskal Wallis analysis, with Mann Whitney U-test for pair-wise comparison of causes. An online survey was used to capture the experiences of equine breeders in the UK in relation to mare and foal losses. Responses (n=118) were used to ascertain how representative the National Foaling Bank data were of the general breeding industry and explore potential factors relating to the causes.

Results Differences were identified in frequencies of mare losses by cause overall (Fig 1a, $P < 0.001$), but the most common causes could not be clearly differentiated from each other terms of incidence (Table 1b).

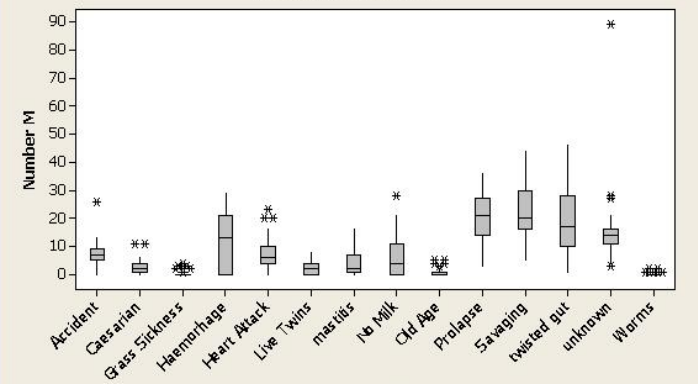

Figure 1a Frequency of common causes of mare loss (median losses per year)

Table 1b Four most common causes of mare loss (causes with distinct letters significantly different $P < 0.05$)

Cause of Mare Loss	Median Incidence / Annum
Prolapse	21^{A}
Savaging	20^{AB}
Twisted Gut	17^{AB}
Unknown	14^{AB}

Differences were identified in frequencies of foal losses by cause overall (Fig 2a, $P < 0.001$), with "Unknown" and Smothering significantly more common than other causes (Table 2b).

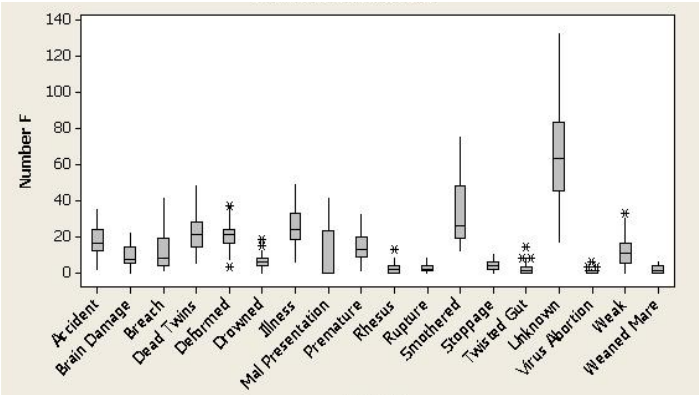

Figure 2b Frequency of common causes of foal loss (median losses per year)

Table 2b Five most common causes of foal loss (causes with distinct letters significantly different, $P < 0.05$)

Cause of Foal Losses	Median Incidence / Annum
Unknown	63^{A}
Smothered	26^{B}
Illness	24^{C}
Dead Twins	21^{C}
Deformed	21^{C}

Questionnaire responses provided some supporting evidence of the types of losses experienced in industry; however, one important finding was that 69.5% reported that their staff were not trained foaling assistants.

Conclusion This detailed analysis of mare and foal losses has informed the development of a holistic risk assessment tool, which will be detailed in the conference presentation. The suggested system provides simple risk management tool prior to active breeding and offers an opportunity for the breeder to decrease the risk and increased potential benefits.

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The effect of mare age and reproductive status on embryo recovery rate and the quality of embryos recovered

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Implications Embryo recovery allows an increase selection intensity and use of fewer breeding horses providing fast assessment of breeding improvements within a breed and across the breeding industry. Despite this limitations in embryo recovery procedure limit its wide spread use within the equine industry. This study supports industry and research based indications that age of the donor mare has an effect on the success of embryo recovery as well as the quality of embryos recovered.

Introduction Equine embryo transfer (ET) has become more widespread in the equine breeding industry, but variable success rates averaging 50% (Squires and McCue, 2007) indicate that there are many limiting factors within embryo recovery reducing the application of the technique. Respective studies have shown that intrinsic factors of the donor mare (Lopes *et al*., 2011) and quality of embryos (Scherzer *et al*., 2008) recovered pose the main limitations within the embryo recovery process. This study hypothesised that first1) age influences the quality and the recovery rate of embryos and, second reproductive status influences the quality and the recovery rate of embryos. The final aim of this study was to investigate whether a combination of age and reproductive status affects the successful recovery of quality embryos.

Material and methods Data was collected from a commercial ET centre, using the records for 156 sport horse mares aging from 2 to 24 years old for the 2007 to 2012 breeding seasons. The mares were allocated a code, and their age, reproductive status, success of embryo recovery and the embryo quality were recorded. Mares were allocated on one of five age groups: 2-5years, 6-9 years, 10-14years, 15-19years and 20 years and older. Reproductive status was grouped into the following four groups: maiden mare, barren mare, foal at foot, flushed in previous season(s). The quality of embryos was graded on a standard industry scale of 1-4 (Camargo *et al*., 2013), with the alteration of nonfertilised oocytes graded as 5 to keep results nominal. The relationship between embryo recovery and quality and the age and reproductive status were analysed using Spearman's Correlation Coefficient on SPSS v. 21. The differences between embryo recovery, embryo quality and reproductive status were analysed using Kruskal-Wallis. The data was grouped by reproductive status then the differences between age group, embryo recovery and embryo quality were analysed using Kruskal-Wallis.

Results The age of the mare was significantly related to embryo recovery (r_s = -.134, P <0.01), and embryo quality, (r_s = -.122, P <0.05), showing a weak, but significant negative relationship. No other significant differences between variables were established as part of this study.

Conclusion In agreement with other studies, there is significant, but weak negative correlation between mare age and the successful recovery of embryos in older mares. The reproductive status was not shown to have a significant effects, which is likely to be due to a number of variables affecting the reproductive status. These were not explored in this study, but could be investigated in future research. This study does not support findings from other studies (Pycock, 2006) which report that older maiden mares are less likely to conceive successfully compared to younger maiden mares. This may indicate that improved knowledge about age related conditions such as endometritis, resulting in improved and more successful management of these conditions, may counteract some of the age related issues which have previously been identified, resulting in higher conception rates in older mares.

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071

Maternal cortisol levels in primiparous and multiparous Thoroughbred mares during abrupt pasture weaning

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Implications The aim of the study was to determine whether the stress response to the weaning process is lower in multiparous mares when compared to maiden mares. This will determine whether specific techniques need to be applied to the individual mare to suit their breeding status, in order to decrease the negative effects of weaning stress. Stress in mares during pregnancy could have adverse effects of the success of the pregnancy, hence the stress induced during the weaning process needs to be limited. Results indicate that abrupt pasture weaning does not induce a significant stress response, based on the cortisol levels observed; indicating that is a weaning method that supports good animal welfare standards.

Introduction The individual stress response has shown to have significant effects on the brood mares' breeding ability in terms of fertility and conception (Baccus *et al*., 1990). The development of knowledge in this area is essential to determine that the welfare of breeding mares is not sacrificed as a result from unsuited weaning techniques causing excess stress. The hypothesis for the study is the maiden mare will display a significantly increased cortisol peak for the post weaning sample due to lack of recent exposure to the stressful process.

Material and methods Saliva samples were collected from 11 TB mares, 4 primiparous and 7 multiparous, at -4hrs, 0hrs (weaning), +4hrs, +24 hrs and +1 week (time point 1,2,3,4,5 respectively) during abrupt pasture weaning. All mares were removed on different days, but between 12noon and 1pm on their respective days. All foals were aged 5 months. The salivary sample was initially centrifuged for 15 minutes at 1500xg to allow separation of saliva and residue phases. The organic phase was then transferred using a plastic pipette into a new test tube. The cortisol was extracted from this organic phase by the use of ethyl ether. The substance was then evaporated by nitrogen stream which then left the remaining cortisol residue at the bottom of the test tube after the ether had evaporated. Extraction buffer (provided in the standard EIA kit) was then added to this residue prior to the assay procedure. Coated microplate, cortisol-HRP conjugate, TMB substrate, cortisol known standard, Extraction buffer, EIA buffer and wash buffer were provided by the commercially purchased EIA kit from Oxford Biomedical Research Inc. from the saliva samples and subsequently analysed using an ELISA to determine cortisol concentration (Moons *et al*., 2005). The cortisol levels for the two groups of mares were compared at the five time points using a Multivariate Analysis of Variance (MANOVA).

Results The MANOVA did not show any significant differences in salivary cortisol level between the two groups of mares or the five time points. When using Pillai's trace, there was no significant effect of mare parity on the cortisol concentrations at the any of the five time points observed, V=0.3, $F(5,5)=1.65$, (ns).

Conclusion Pasture weaning does not seem to elicit a systemic physiological stress response at the time of weaning or thereafter in the broodmares, regardless of their parity. Future research should aim to investigate the effects of age and parity on cortisol levels, using a more representative sample with evenly sized groups to confirm these findings.. The current results indicate that pasture weaning is an appropriate weaning method, causing minimal, if any, welfare concerns for the mares involved in this study, and therefore this would be the recommended weaning procedure from the dams perspective in the equine breeding industry.

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072

The use of eye blink rate and changes in behaviour as potential early indicators of pituitary pars intermedia dysfunction in the horse

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Implications Monitoring eye blink rate (EBR) and behavioural changes are non-invasive techniques that could be used by owners and veterinarians as indicators of alterations in dopamine release and thus be an 'early-warning sign' of pituitary pars intermedia dysfunction (PPID) in horses. Early diagnosis and pharmacological intervention can help to avoid secondary diseases and extend the useful life of a horse.

Introduction Pituitary pars intermedia dysfunction (PPID) is a dopaminergic neurodegenerative disease commonly recorded in domestic horses. PPID causes hirsutism, chronic and recurrent laminitis, hyperhidrosis, lethargy, weight loss, polyuria, polydipsia, an increased susceptibility to worms due to a compromised immune system. PPID is almost exclusively attributed to hyperplasia or adenoma formation in the pars intermedia (PI) of the pituitary gland, caused by reduced inhibitory dopamine (DA) innervations [Schott, 2002; Johnson *et al.,* 2004] of this structure. Research with rodents and primates have shown that DA antagonists lead to suppression of eye blink rate, whilst agonists bring elevations in EBR. Therefore, a simple behavioural measure such as EBR has the potential to reveal central fluctuations in DA release which are notoriously difficult to monitor in peripheral plasma. The aims of this study were to determine if there was a relationship between EBR and PPID in horses and if horses with PPID showed altered behaviour pre and post PPID diagnosis.

Material and methods A sample of 40 horses, 20 with clinically diagnosed PPID all of whom were being treated with Pergolide and 20 normal horses, were randomly selected from livery yards and charities across the UK. The mean age of the control group was 7.6 years (range; 4-15), while that in the PPID group was 19 years (range; 9-32). The spontaneous EBR of all the horses was counted over 3 x 10-minute periods, during quite times in each yard, totalling 120 observations. A blink was counted as one full shut of the left eyelid. A previously validated questionnaire profiling temperament and related behavioural changes in horses was given to the owners of the PPID horses [Momozawa *et al*., 2005]. Questions addressed features of aggression, depression and stereotypic behaviour in the horse prior to and after the diagnosis of PPID. Student T-test for two independent groups was used to analyse EBR data, while Chi squared test was used for analyses of questionnaire data. Both tests were performed using Genstat 15 (Laws Agricultural Trust, 2013) and $P<0.05$ was taken as the level of significance.

Results The average EBR determined for 20 normal horses were 179 and for 20 PPID horses, 115. The normal horses made significantly more blinks (65) in 10 minutes than the PPID horses. In all 20 PPID horses the owners stated that their horses had exhibited changes in behaviour notably they had become more aggressive ($P = 0.003$), more depressed ($P= 0.014$) and had an increase in performance of stereotypic behaviour ($P=0.04$). These findings were not affected by the age difference between normal and PPID horses.

Conclusion Although the dopamine agonist Pergolide which was administered to PPID horses works to increase DA levels, the measured EBRs in this study showed that the PPID horses had significantly lower EBR than those without PPID, indicating lower DA production. This might be a consequence of the irreversible dopaminergic neurodegeneration and subsequent DA depletion [Singh *et al.,* 2012]. Consequently, horses with PPID will always present lower DA levels than control horses regardless of any pharmacological intervention. As such EBR and behavioural screening have roles as indicators of PPID and could be cheap non-invasive compliments to current diagnostic techniques.

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073

Is there an association between hindlimb lameness and saddle slip in the horse?

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Implications The most common cause of saddle slip is hindlimb lameness. Saddle slip may highlight the presence of subclinical lameness.

Introduction We have observed saddle slip consistently to one side because of a crooked rider, an ill-fitting saddle, asymmetry in a horse's thoracolumbar shape and hindlimb lameness. Prior to the current studies there were no objective data assessing the relative importance of each factor. We have performed a two part study.

Part 1: An investigation of the relationship between hindlimb lameness and saddle slip (Greve, and Dyson 2013[a])
Our objectives were to document the frequency of occurrence of saddle slip in horses with hindlimb lameness compared with other horses; to describe the effect of lameness characteristics and grade, the abolition of lameness by diagnostic analgesia, breed, size, thoracolumbar shape and symmetry and the rider's weight.

One hundred and twenty-eight horses were assessed prospectively and lameness and saddle slip were assigned a grade before and after diagnostic analgesia. The thoracolumbar shape and symmetry were measured objectively using a Flexible Curve Ruler at four predetermined sites. In three horses the force distribution and magnitude underneath the saddle were measured before and after diagnostic analgesia.

The saddle consistently slipped to one side in 38/71 (54%) of horses with hindlimb lameness, compared with 1/26 (4%) horses with forelimb lameness, 0/20 (0%) with back pain and/or sacroiliac joint region pain and 0/11 (0%) non-lame horses. The association between saddle slip and hindlimb lameness was significant (rs 0.548, $p<0.001$). Diagnostic analgesia abolishing the hindlimb lameness eliminated the saddle slip in 37/38 horses (97%). In two horses the saddle continued to slip after resolution of lameness; one horse had bilateral forelimb lameness and the other horse had concurrent hindlimb and forelimb lameness. The saddle of both horses was asymmetrically flocked. The saddle slipped to the side of the lamest hindlimb in most horses (32/37 [86%]). No horse with saddle slip had significant left–right asymmetry of the back at the four predetermined sites.

Part 2: The interrelationship of lameness, saddle slip and back shape in the general sports horse population (Greve, and Dyson 2013[b])
There are no studies of the frequency of occurrence of saddle slip and risk factors within a tested sample population of the general sports horse population. Our objectives to quantify the frequency of saddle slip and to describe the association with lameness, thoracolumbar shape/symmetry, crooked riders and ill-fitting saddles. The study design was a non-random, cross-sectional survey using convenience sampling.

Five hundred and six sports horses in normal work were assessed prospectively. Thoracolumbar shape/symmetry were measured at predetermined sites; the presence of lameness (in-hand and/or ridden) and saddle slip was recorded. Descriptive statistics, univariable and multiple logistic regression were performed to assess the relationship between horse-saddle-rider factors and saddle slip.

The frequency of lameness, quadrilaterally reduced cranial phase of the stride or stiff, stilted canter was 45.7%, saddle slip 12.3%, left-right thoracolumbar shape asymmetries ≥ coefficient of variance of 8% (1.2cm) 0.6%; 103/276 riders (37.3%) sat crookedly. The saddle consistently slipped to one side in 30.3% of horses with hindlimb lameness, compared with 5.4% with forelimb lameness, 17.4% with stiff, stilted canter, 20% with quadrilaterally reduced cranial phase of stride and 5.6% non-lame horses. Nineteen horses (30.6%) with saddle slip had no detectable hindlimb lameness, however, 14 had a gait abnormality, particularly in canter. Multivariable analysis revealed that saddle slip was significantly associated with hindlimb lameness and gait abnormalities (OR=52.62), saddle fitted with even contact and uniform flocking (OR=15.49), riders sitting crookedly (OR=6.32), a well-balanced saddle (OR=3.05), and large back shape ratio at T18 (OR=1.2).

Conclusions In the first study we demonstrated a causal relationship between hindlimb lameness and saddle slip. In the second study we demonstrated that many horses with hindlimb and/or forelimb lameness go unrecognised. Saddle slip can be associated with a crooked rider, or an ill-fitting saddle, but hindlimb lameness appears to be the most important cause. Education of the equestrian population to identify lameness and saddle slip is required.

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074

An investigation of exercise-induced changes in equine back shape

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Implications The thoracolumbar muscles of horses working correctly enlarge transiently with work. If a saddle does not fit properly before work, this increase in size does not occur, which may impair back function. Saddle fit should be assessed both before and after exercise to ensure correct fit.

Introduction It is well established in the human literature that exercise induces an acute increase in cross-sectional area of the muscles during work (Mitchell *et al*., 2013) There has been no investigation of changes in back shape that occur subsequent to ridden exercise in sports horses. Recommendations concerning saddle fit are empirical and based on anecdotal information. We know that the saddle needs to fit the horse in motion, but there has been no investigation of whether the thoracolumbar region changes in shape in association with exercise or how improper saddle fit may influence potential changes. In man, mean muscle fiber area increases post exercise depending on type and intensity of exercise , trunk versus limb muscles and whether or not blood supply is restricted (Mitchell *et al*., 2013). The objectives were to quantify acute exercise-induced back shape changes in horses and to describe the association with work quality, saddle fit and rider skill level.

Material and methods A convenience sample of 63 sports horses, varying in age, work discipline and level of athletic activity was selected, based on proximity to the authors. The thoracolumbar shape was measured at the eighteenth thoracic vertebra (T18), T13, T8 and the shoulder-region using a Flexible Curve Ruler, using a previously validated technique (Greve and Dawson, 2013) both before and immediately after a 30 minute exercise period. All measurements were made by one skilled observer. Saddle type and fit (Harman, 1999) were assessed. Horse (including presence of lameness), saddle and rider data were recorded. Rider skill level was graded using a 1 to 3 scale (good, moderate and poor). Horses were worked on the flat by their usual rider at their normal work level. Video recordings were obtained from the side and from behind on both left and right reins, going large and in 20m and 10m diameter circles on both reins, in both trot and canter. The work quality was graded by consensus of both authors using a 0-10 scoring scale (as for FEI dressage scoring), for trot and canter independently and on left and right reins. The sum of the scores for trot and canter was calculated. The horses were divided into two categories based on the total score, Group 1, ≥ 11 and Group 2, ≤ 10. The back shape widths 3 cm and 15 cm ventral to the dorsal midline before and after exercise were measured and ratios calculated (Greve and Dawson, 2013) . The relationships between horse, saddle and rider factors and back shape changes were assessed using a two-sample t-test, one-way ANOVA if ≥ 3 groups, or linear regression to predict the value of one numerical variable (response) from that of another (predictor). All statistical analyses were performed using SPSS Statistics 20, with significance set at $P<0.05$.

Results The mean back shape ratio immediately after ridden exercise was greater compared with before work for all measured sites [mean difference in ratio before and after exercise at the shoulder 0.89; T8 0.48, T13 1.33 and T18 1.95]. The total work quality score could significantly predict the quantitative changes in ratios using linear regression at T18 (R^2 0.268; $P<0.001$) and T13 (R^2 0.085; $P=0.020$), but not at T8 or the shoulder-region (R^2 0.024, $P=0.118$). Mean changes in back shape were greater in Group 1 compared with Group 2 at each site (e.g., mean width at 3cm, 15cm and ratio [shoulder 0.6cm P=0.06; 0.85cm P=0.09; 0.77 P=0.46]; [T8 0.37cm P=0.30; 1.28cm P=0.06; -0.12 P=0.88]; [T13 1.21cm P=0.01; 0.93cm P=0.12; 1.75 P=0.02] and [T18 1.67cm P=0.01; 1.15cm P=0.05; 2.36 P<0.001]). Mean changes in back shape at each site were greater in horses with correctly-fitting saddles compared with ill-fitting saddles. The difference was significant at 3 cm ventral to the dorsal midline at T8 [mean 1.23cm P<0.001], T13 [mean 1.72cm P<0.001] and T18 [mean 1.38cm P<0.001] and also significant at T8 15 cm ventral to the dorsal midline [mean 2.25cm P=0.001]. Mean changes were greater in horses ridden by good > moderately > poorly skilled riders, with significant difference 3 cm ventral to the dorsal midline for T18 [poor -0.14cm; moderate 1.43cm; good 2.02cm, P=0.02] and T8 [poor -0.29cm; moderate 1.71cm; good 0.43cm, P=0.02]. For T8 horses with moderately-skilled riders had greater changes compared with good riders. However, all horses ridden by moderate riders had well-fitting saddles.

Conclusions Exercise induced acute changes in the back shape at each site, which were influenced by the horse's work quality, saddle fitting and rider ability. Ill-fitting saddles restricted acute post-exercise expansion of the 'top-line' muscles underneath the saddle.

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Assessment of variation in movement symmetry measures in horses between trials, days and weeks based on inertial measurement units

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Implications This study provides guidelines for evaluating changes in movement symmetry, such as before and after diagnostic analgesia, or when evaluating the long-term effect of treatment or rehabilitation regimes in horses with orthopaedic disease.

Introduction Inertial Measurement Units (IMUs) are increasingly utilised as a tool for objective gait analysis in horses allowing unobtrusive instrumentation and quantification of clinically relevant gait parameters such as movement symmetry that are crucial for assessment of lameness. IMU systems have been shown to agree well with gold standard motion capture (Pfau *et al.*, 2005) and force platform measurements (Keegan *et al*., 2012). IMUs agree moderately well with subjective assessment by Veterinary surgeons (Thomsen *et al*., 2010). While repeatability of repeat assessments, conducted in short (5 minute) succession has been assessed and good agreement been reported (Keegan *et al*., 2010), there is to date no data about biological variation of IMU derived symmetry measures days or weeks apart. This however is essential in order to establish reliable thresholds for monitoring the success of treatment or rehabilitation regimes. Hence, this study aimed at quantifying variation in kinematic symmetry parameters for serial measurements over time, attributable to intra-individual variation.

Material and methods IMUs were attached to head (poll), sacrum and left and right tuber coxae of six horses trotting on a motorized equine treadmill and vertical displacement was calculated during trot. Established movement symmetry measures were derived from these data over a five week period. These measures assess overall amount of asymmetry present (symmetry index; SI), differences in weight bearing between contralateral limbs (minimum difference; MinDiff) and differences in propulsion between contra-lateral limbs (maximum difference; MaxDiff) for front limbs (poll sensor) and hind limbs (sacrum sensor). Between-trial (differences between multiple measurements on a given day), between-day and between-week limits of agreement (LoA) were calculated following the method described by Bland and Altman (1986) as mean difference +/- 2 standard deviations of the difference.

Results LoAs were narrower for the sacrum compared to the poll. LoA for the sacrum SI increased from [-0.095 to +0.115] between trials to [-0.156 to +0.145] between days and to [-0.256 to +0.268] between subsequent weeks. Poll SI also increased from [-0.169 to +0.21] (between-trial) to [-0.331 to 0.353] (between days) to [-0.450 to 0.458] between weeks. Sacral MinDiff (MaxDiff) increased from [-4.9 to +4.5] ([-2.8 to +5.1]) mm (trials) to [-7 to +7.9] ([-5.1 to +5.1]) mm (days) and [-9.1 to +9.5] ([-16.6 to +17.7]) mm (weeks). Poll MinDiff (MaxDiff) showed increase from [-10.2 to +8.6] ([-10.9 to +12.3]) mm, [-12.7 to +13.3] ([-13.1 to 13.8]) mm to [-31.5 to 34.7] ([-17.3 to 21.2]) mm.

Conclusion In this study we have quantified variation in head and pelvic movement symmetry measures from IMUs by establishing limits of agreement on a trial-to-trial, day-to-day and week-to-week basis. This has demonstrated an increase in variation in particular on a week-to-week basis. Trial-to-trial and day-to-day LoA generally show values smaller or at least similar to our currently established thresholds for discriminating between sound and mildly lame horses and hence confirm these. In particular trial-to-trial LoA values should be considered when evaluating the effect of diagnostic analgesia comparing movement symmetry values before and after a nerve or joint block and day-to-day LoA values when investigating the effect of treatment or rehabilitation regimes.

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The effects of chiropractic treatment on the range of motion of the carpus and tarsus of horses

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Implications Chiropractic treatment of horses may improve symmetry of joint range of motion (ROM) of the carpus and tarsus. This may be important when assessing movement symmetry of the horse.

Introduction The use of complementary therapies has grown over the past decade for both humans and animals. Equine back problems are more frequently reported as a major contributing factor to poor performance, and back dysfunction can affect limb kinematics. Clinical signs of back pain and spinal dysfunction can include asymmetric or restricted joint motion (Haussler, 2009). Joint range of motion (ROM) is the degrees difference between joint flexion and extension. McTimoney treatment uses short lever, high velocity, low amplitude thrusts to induce a therapeutic response in joint structures, muscle function and nerve reflexes (Faber *et al*, 2003). The aim of this chiropractic technique is the resolution of musculoskeletal disorders that are induced by biomechanical factors. Symmetry in movement is also important to the balance and performance of the horse (Kuhnke *et al*, 2010). This study aims to determine if (McTimoney) chiropractic treatment has an effect on the range of motion (ROM) of the carpus and tarsus joints.

Material and methods Hemispherical 35mm markers were applied to 7 anatomical landmarks of both forelimbs and both hind limbs of 10 sound, healthy horses from the same riding school with similar workload. The treatment group (n=5) received (McTimoney) chiropractic treatment for the neck, back, pelvis and front feet. 2-D Kinematic data at walk and trot, 1 day before treatment, day of treatment and 7 days post treatment, was collected for all horses using two digital video recorders, filming both sides concurrently. Data was analysed using Kinovea software, minimum and maximum joint angles were measured and ROM calculated. Statistical analyses included Two-way ANOVA, Students t-test and symmetry indices.

Results Post treatment, significant increases in joint ROM occurred for the control and treatment groups on left side at walk and trot. However, only the treatment group significantly increased ROM on the right side for the carpus at walk ($p=0.04$) and trot ($p=0.02$). For the treatment group, there was a significant change in carpus ROM asymmetry from left towards neutral at walk ($p=0.004$) and trot ($p=0.04$). Also tarsus ROM asymmetry change from left towards neutral was significant ($p=0.02$) at trot. There were no such significant effects for the control group.

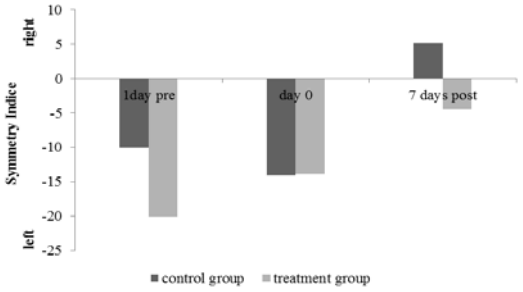

Figure 1 Mean symmetry indices for carpus in walk.

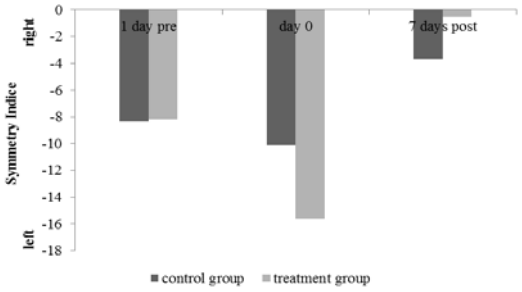

Figure 2 Mean symmetry indices for carpus in trot

Conclusion These results are promising and support the hypothesis that (McTimoney) chiropractic treatment may help to improve the symmetry of tarsus and carpus ROM of horses. Further research is recommended to elucidate measurable effects.

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Evaluation of horse fitness for exercise: the use of a logit-log function to model horse post-exercise heart rate recovery

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Implications The logit-log model shows high potential to be used as model of choice in simple portable equipment allowing quick, *in situ*, evaluation of horse fitness.

Introduction Post-exercise heart rate (HR) recovery has been used as an indicator of fitness, due to the positive correlation between recovery and overall physical performance of individuals (e.g. Bitshnau *et al*., 2010). Several studies have demonstrated the relationship between quicker recovery and better performance (e.g. Hagberg *et al*., 1980).
The mono and bi-exponential models have been the functions of choice to model HR recovery (Thompson *et al*., 1983), with the bi-exponential often proving to be the best fit (Rugh *et al*., 1992) due to the fast and slow components of HR recovery. This is supported by the theory of coordinated interaction of parasympathetic reactivation and sympathetic withdrawal during exercise recovery, as explained by Bitshnau *et al*. (2010) which therefore, allows a biological interpretation of the parameters of the model. But several authors have studied the subject inconclusively (e.g. Hada *et al*., 2006) and this interaction is still unclear in both man and horse. The logit-log function is used to model decay in other field of knowledge (e.g. in radioimmunoassay). In this study, horse HR recovery is fitted by this model to investigate its adjustment and test its application as a measure for horse fitness. The model is tested in polo ponies playing matches of 4 parts, known as chukkers, each lasting for 7.5 minutes. Each rider can either play 1 "full chukker" (FC) or 2 "half chukker" (HC) ponies in each chukker. Any pony can only be reused with a FC in between.

Material and methods Data was collected in 32 ponies (16 FC and 16 HC) in the SW of England, using a Polar® HR monitor. HR at rest was measured prior to play, immediately after, and 2, 4, 6, 10 and 20 minutes after play. The variables time and HR were transformed respectively in log(time) and in logit(HR) using (1), where HR_t is HR at time t and HR_m is the maximum HR obtained immediately after exercise. Both these values are net values, therefore obtained after subtracting HR at rest. All the transformation were made using $\log_{10}$. After the transformations, the means logit(HR) for each log(time) were calculated to create time series for both the FC and HC. Linear regressions were adjusted between log(time), as independent variable, and mean logit(HR), as dependent variable. The fitness of the models were evaluated with r^2. The normality of residual distribution was assessed via normal PP plot and the analysis was performed in SPSS.

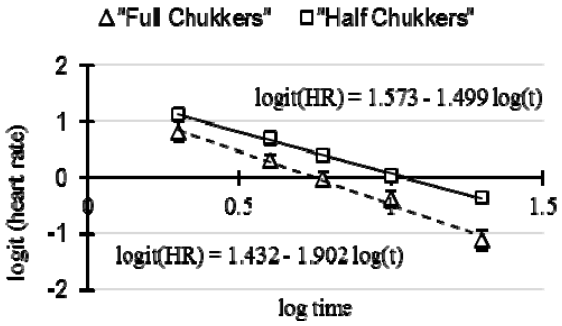
$$\text{logit}(HR) = \log\left(\frac{HRt}{HR_m - HR_t}\right) \quad (1)$$

$$\log\left(\frac{0.5HR_m}{HR_m - 0.5HR_m}\right) = \log 1 = 0 \quad (2)$$

$$\alpha + \beta\left(\log\left(\frac{t}{2}\right)\right) = 0 \quad (3)$$

$$\log\left(\frac{t}{2}\right) = \frac{-\alpha}{\beta} \Leftrightarrow \frac{t}{2} = 10^{-\alpha/\beta} \quad (4)$$

Results The linear regressions r^2=0.989 and r^2=0.998 respectively for FC and HC. In both regressions, both the intercepts and slopes are significant (P<0.001). Analysis of the PP plots didn't suggest deviance from normality.



The log half-life of the decay can be identified in the graph by the point in time where logit(HR)=0. The log half-life of the decay is the point in time where half of HR_m is achieved, therefore from (1) and replacing HR_t by $0.5HR_m$ we arrive to (2), from where we conclude that the log half-life of the decay is achieved when logit(HR)=0, and so from (3) we arrive to (4). Using the regression parameters, we can now compute log half-lives or half recovery times, for the FC as 1.432/1.902=0.753 and for the HC as 1.573/1.499=1.049, which is in agreement with the observations taken from the plot. From here, and by taking the log out, we can compute the real recovery times as $10^{1.499} \approx$ 5.7 minutes for the FC and $10^{0.753} \approx$ 11.2 minutes for the HC. FC has faster recovery time and shorter half recovery time than HC, and therefore fitness is the reason behind the choice to play H/F C.

Conclusion. The logit-log model adjust HR decay data very well, with the log half recovery time being a good candidate for comparition of performance capabilities of sport horses. The Logit-log model is parsimonious, easy to compute and can potentially be the choice in the development of software to be included in expeditious equipment. The logit-log model shows, therefore, potential to be used in simple portable equipment allowing quick evaluation of horse fitness.

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Assessment of the effects of fifteen compounds from essential oil extracts on the metabolism of polyunsaturated fatty acids by rumen microorganisms *in vitro*

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Implications The compounds: 4-Allylanisole, *trans*-anethole, menthol, *p*-cymene and 3-carene inhibit biohydrogenation of both linoleic and α-linolenic acid; if replicated *in vivo*, they could enhance beneficial fatty acids in ruminant food products.

Introduction Recently, there has been a surge in interest in the use of essential oil components (EOCs) to modulate rumen fermentation (Benchaar *et al.* 2008, Calsamiglia *et al*. 2007). The majority of studies on EOCs have tended to focus mainly on effects on rumen protein breakdown and methane production, whilst their effects on fatty acid biohydrogenation have largely been ignored. The benefits of *n*-3 polyunsaturated fatty acids (PUFA) such as α-linolenic acid (LNA, C18:3*n*-3), eicosapentaenoic acid (EPA, C20:5*n*-3) and docosahexaenoic acid (DHA, C22:6*n*-3) in human diets are widely recognised. Recent work (Sgwane *et al*. 2013) which screened 20 EOCs for their effects on rumen metabolism of PUFA showed that some EOCs such as pinene, linalyl acetate, L-methone and pulegone have the potential to inhibit biohydrogenation. The aim of study is to widen the range of EOCs tested by screening a further 15 EOCs for their effects on biohydrogenation.

Material and methods A basal feedstock of a 70:30 mixture of grass hay (*Lolium perenne)* and concentrate was formulated and milled (1 mm screen). The basal substrate was supplemented with 32.5 g oil/kg (60% fish oil and 40% ground linseed oil). Serum bottles were incubated for 48 h in each run and repeated twice. In each run 432 serum bottles were incubated, each bottle contained 80 ml buffer, 20 ml inoculum, and 1 g substrate and then treated with 300 mg/l of EOC. Rumen fluid was collected from 6 Hartline × Texel cross cull ewes on the same basal diet. In total, there were 16 treatments, with 6 replicates per EOC as follows: control (CON), eucalyptol (EUC), 3-carene(CAR), *trans*-anethole (ANE), (-)-α-bisabolol (BIS), (-)-borneole (BOR), (-)-*trans*-caryophyllene (CPY), 4-Allylanisole (ALA), *trans*-cinnamaldehyde (CIN), (S)-(-)-β-citronellol (CIT), (R)-(+)-limonene (LIM), menthol (MEN), myrtenol (MYT), *P*-cymene (CYM), (-)-α-thujone (THU) and vanillin (VAN). Samples were frozen at -20^{0}C and analysed for fatty acid (FA) methyl esters by gas chromatography. Data were analysed using one-way ANOVA with experimental runs as a blocking factor using GenStat 15th Edition.

Results Effects of the 15 EOCs investigated are summarised in Table 1(NB. to improve clarity of the table, compounds with minor effects such as VAN, BIS and CPY were not included). Linoleic acid (LA) was significantly different (P<0.001) across treatments; ALA, ANE, MEN, CYM and CAR were the most effective with concentrations 149%, 131%, 117%, 113% and 84% higher than the control, respectively. In a similar pattern, the levels of LNA were also maintained at significantly *(P<0.001)* higher concentrations by ALA (188%), ANE (170%), MEN (147%), CYM (146%) and CAR (102%) compared with the control. BOR, THU and MYT significantly *(P<0.001)* sustained higher the concentrations of both EPA (119%, 105% and 104%) and DHA (96%, 82% and 80%), respectively, than the control. Except for LIM, CIN and CAR, all compounds reduced *(P<0.001)* the build-up of stearic acid (SA). The biohydrogenation intermediate, *trans*-vaccenic acid (TVA) was elevated *(P<0.001*) by all compounds relative to the control, except for CIN and CYM. Only MEN increased the proportion of CLA *(cis-9, trans-11*) compared to the control. All the compounds in this study reduced the total volatile fatty acids concentrations (data not presented), MEN was most inhibitory causing a 24% reduction.

Table 1 Effects of EOCs on concentration (g/100g total FA) of PUFA and biohydrogenation intermediates

	CON	EUC	CAR	ANE	BOR	ALA	CIN	CIT	LIM	MEN	MYT	CYM	THU	*sed*	*P*
LA	0.9^{a}	1.2^{b}	1.7^{c}	2.1^{de}	1.6^{c}	2.3^{d}	1.3^{bf}	1.4^{bc}	1.6^{c}	2.0^{e}	1.5^{cf}	1.9^{eg}	1.6^{c}	0.14	***
LNA	1.1^{a}	1.4^{abf}	2.2^{c}	3.0^{de}	2.1^{c}	3.2^{d}	1.6^{bf}	2.0^{c}	2.2^{c}	2.7^{e}	1.9^{bc}	2.7^{e}	2.2^{c}	0.19	***
EPA	1.0^{a}	2.0^{dg}	1.4^{b}	1.9^{de}	2.3^{c}	2.0^{dg}	1.3^{b}	1.3^{b}	1.4^{b}	2.0^{dg}	2.1^{g}	1.6^{f}	2.1^{g}	0.07	***
DHA	1.0^{ag}	1.8^{ef}	1.1^{a}	1.6^{d}	2.0^{c}	1.7^{de}	1.0^{ag}	0.9^{g}	1.1^{a}	1.7^{de}	1.9^{cf}	1.2^{b}	1.9^{cf}	0.08	***
SA	13.6^{ac}	11.6^{b}	14.1^{a}	13.2^{ac}	11.4^{b}	12.3^{bc}	14.1^{a}	11.7^{b}	14.6^{a}	11.5^{b}	11.4^{b}	13.3^{ac}	11.4^{b}	0.65	***
TVA	1.0^{a}	1.1^{c}	1.1^{c}	1.1^{c}	1.2^{d}	1.1^{c}	0.9^{b}	1.1^{c}	1.1^{c}	1.3^{e}	1.2^{d}	1.0^{a}	1.1^{c}	0.05	***
CLA	0.08^{a}	0.10^{a}	0.07^{a}	0.10^{a}	0.11^{a}	0.10^{a}	0.08^{a}	0.08^{a}	0.07^{a}	0.14^{b}	0.10^{a}	0.08^{a}	0.10^{a}	0.016	***

Means within a row with different superscripts are different (P<0.05) NS = not significant

Conclusion These results clearly demonstrate that out of the 15 EOCs compared in this study, ALA, ANE, MEN, CYM and CAR have the greatest potential to reduce biohydrogenation of linoleic acid and α-linolenic acid, the two most abundant PUFA in ruminant feedstuffs. It is worthwhile to investigate further whether these effects are sustained *in vivo*. The use of EOCs to protect PUFA from biohydrogenation needs to be balanced against their inhibition of VFA production.

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Effect of silage additive (tannin or inoculate) on protein degradability of legume and grass silages

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Implications Undegradable protein supply from forages can be increased by incorporation of tannin at ensiling.

Introduction Tannin has the ability to bind with different compounds such as protein and carbohydrate to form complex undegradable molecules. Tannin protein complexes formed in the rumen pH (6-7) can disassociate post-ruminally in the abomasum (pH< 3.5) facilitating digestion in the small intestine. As a consequence, rumen degradable protein supply is reduced and undegradable protein supply is increased (Muller-Harvey, 2006). Salawu *et al.* (1999) have reported an improvement of silage quality (low pH and NH3-N) as a result of incorporating tannin at the point of ensiling. McMahon *et al.* (2000) reported that the absorption of dietary amino acid in the small intestine was increased when the diet contained tannin. The aims of this study is to evaluate, using an *in situ* (nylon bag) technique, the potential hydrolysable tannin to reversibly bind with plant protein during ensiling in order to protect dietary protein from ruminal degradation.

Material and methods Whole crop grass, pea and bean forages were harvested on 14th July 2011 and ensiled in 25 kg experimental silos at Harper Adams University. Prior to ensiling each forage was treated with one of 4 additives: 40 g/kg fresh weight (FW) tannin (HT), 20 g/kg FW tannin (LT), an inoculant (*L. plantarum*) 10^6 colony-forming units/g FW (In.), or untreated (C). A standard volume of water (1 ml. /kg FW) was applied to all treatments. Silos were opened after 100 days and subsamples stored at -20 °C prior to analysis for: pH, NH3-N, DM, NDF and CP. Rumen degradability (*in situ*) characteristics were determined in 4 mature, wethers fitted with permanent rumen cannulae (AFRC, 1992). The experiment was analysed by ANOVA as a 3 x 4 factorial design using Genstat 15 (VSN International, Oxford, UK).

Results Grass silage had highest pH, DM, CP and NDF and lowest NH3-N compared to pea and bean silage (Table 1).

Table 1 Effect of forages types and additives on proximate analysis of silage samples

F.	Bean silage				Pea silage				Grass silage				s.e.d	*P*		
A.	C	In.	LT	HT	C	In.	LT	HT	C	In.	LT	HT	FxA	A.	F.	Inte
pH	3.8	3.9	3.9	3.9	4.0	4.0	4.0	3.9	4.3	4.2	4.2	4.4	0.01	0.69	0.001	0.23
NH3[1]	6.5	6.1	5.1	4.8	6.6	6.2	5.5	3.9	4.8	4.2	4.0	3.7	0.34	0.01	0.001	0.02
DM[2]	221	206	205	203	257	255	256	253	302	303	302	303	6.98	0.38	0.001	0.54
CP[2]	150	153	154	155	149	147	147	141	175	177	169	173	4.93	0.72	0.001	0.50
NDF[3]	391	397	399	392	369	369	368	362	459	475	489	486	13.8	0.52	0.001	0.66

1:NH3-N g/kg total nitrogen, 2, g/kg, 3: g/kg DM. F: forage types, A: additives. Inte: interaction, FXA: forage x additives.

Additives significantly reduced NH3-N compared to control silages. Treating fresh forage with tannin or inoculate at ensiling reduced (P<0.05) the immediately soluble fraction of degradation curve compared to untreated silages samples (0.39, 0.41, 0.46 and 0.48, SED =0.006; P<0.05) for (HT, LT, In. and C respectively). The insoluble but potentially degradable fraction was reduced by additional tannin or inoculum as compared to control (0.35, 0.36, 0.33 and 0.38, SED =0.012, P<0.05) for (HT, LT, In. and C respectively). However the rate of degradation for CP was not found to be significantly affected by additive. The total potentially degradable 'a + b' fraction (TPD) were significantly (P<0.05) reduced by additives (15, 11 and 7%) for (HT, LT and In. respectively) comparing to C. HT had the lowest (TPD) value in all forage types especially in PS compared to other additives. Treating silage with inoculate reduced the (TPD) value in BS and GS but not in PS when compared to C. Both tannin levels reduced significantly the effective degradability (ED) at an outflow rate 0.05 compared to In. and C, especially in GS and PS. Rumen undegradable protein supply to the small intestine increased by 17 and 10 % for HT and LT compared to C.

Conclusion Additional tannin at ensiling reduced significantly the ED of dietary protein and increasing rumen undegradable protein.

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Evaluation of faecal NIRS for describing differences in digestibility resulting from feed restriction and realimentation

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Implications NIRS was able to detect chemical differences in cattle faeces due to differences in feeding level, and shows promise as a high-throughput method for characterising digestibility for breeding studies.

Introduction This study used near infrared spectroscopy (NIRS) as a rapid technique to investigate between-animal differences in the chemical composition of faeces. Feed restriction and subsequent realimentation were used to generate differences in digestion whilst animals remained on the same feed. The study was part of a broader investigation of mechanisms for compensatory growth (Keogh *et al.* 2012). NIRS uses the near infrared region of the electromagnetic spectrum to evaluate chemical composition; faecal NIRS has been used to predict differences in digestibility and other attributes such as crude protein and fibre (Dixon and Coates 2009). Principle component analysis (PCA) is a multivariate chemometric technique used to compare NIR spectra.

Material and methods Holstein Friesian bulls with a mean (s.e.m.) age of 497 (15) days and mean bodyweight 370 (35) kg were assigned to either restricted feeding (REST, n=15), fed to grow at 0.6 kg/day for 125 days (Period 1), followed by a realimentation period (Period 2) of *ad libitum* feeding for 55 days, or a high feeding rate (AD LIB, n=15), fed *ad libitum* during both periods. Diets were based on a total mixed ration consisting of 70% concentrate and 30% grass silage. Two faecal grab samples were taken on days 70 and 71 of Period 1 and days 43 and 44 of Period 2. Faecal samples were dried at 65^{o}C for 80 h and ground through a 0.75 mm sieve. Samples were dried for a further 16 h at 80^{o}C before being scanned between 1100-2500 nm at 2 mm intervals using a NIRSystems 5000 monochromator (FOSS, Warrington, UK). The four spectra per animal were averaged to give one spectrum per bull within each period. Mean spectra were transformed using standard normal variate and detrend using the second polynomial. Transformed data were then analysed by PCA (The Unscrambler® X; Camo Software). Within each period, the effect of feeding level on the first (PC-1) and second (PC-2) principal component scores was assessed by one-way ANOVA (Genstat®).

Results Compared with the AD LIB group, intake (relative to body weight) by REST animals was approximately 61 and 124 per cent in periods 1 and 2 respectively. Feeding level had a significant effect on PC1 in Period 1 ($P<0.001$) but no residual effect during Period 2 ($P=0.679$). Feeding level had no effect on PC2 during Period 1 ($P=0.087$) or during Period 2 ($P=0.191$). The score plot for PC-1 and PC-2 (Figure 1a) shows two contiguous groups relating to feeding level during Period 1 (+, ADLIB; ■, REST). The circled AD LIB animal showed lower intake than others in the AD LIB group. There was no separation of the REST and AD LIB groups during Period 2 (Figure 1b). Wavelength regions associated with changes in digestibility showed highest correlations with factors (not presented), particularly in Period 1.

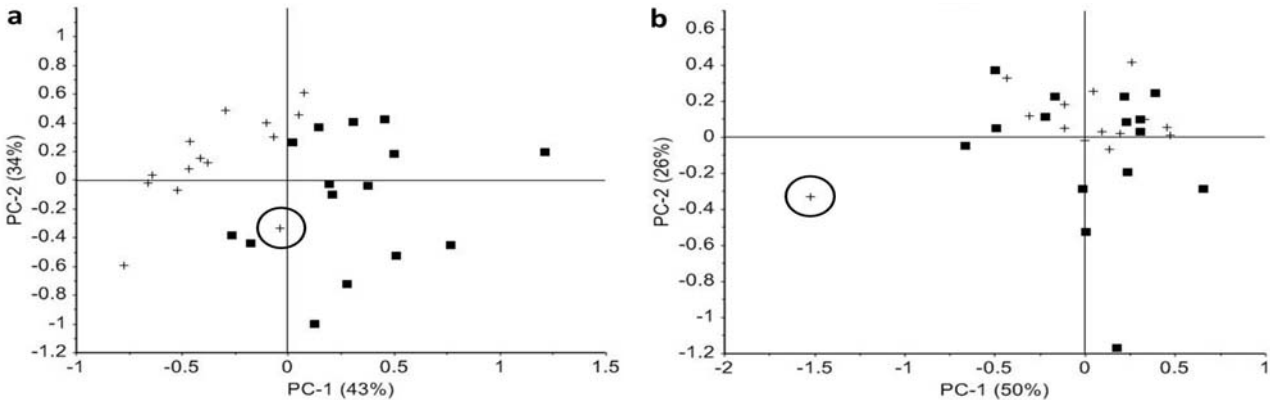

Figure 1 Principal component scores for faecal samples from individual bulls in (a) Period 1 and (b) Period 2

Conclusion NIRS was able to detect differences in faecal composition resulting from different intake levels during Period 1. There was no apparent residual effect of restricted feeding during the realimentation period.

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081

A review of the impact of increased dietary starch concentration or addition of oils, fats, tannins or saponins in the diet on enteric methane emissions

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Implication Formulation of diets with the addition of oils/fats, tannins/saponins or increased starch concentration can reduce methane (CH_4) emissions per kg DMI within different livestock sectors.

Introduction Agriculture is a significant source of GHG emission accounting for 9.6% of the UK's total GHG emissions based on 2011 estimates (NAEI, 2013). The industry therefore has a responsibility to explore potential GHG mitigation strategies. Improvements in production efficiency, animal breeding, nutrient management and farming systems are all possible means of reducing emissions per unit of agricultural product but direct dietary manipulation has also been shown to impact on enteric methane emissions. The objectives of this review were to investigate, by means of meta analysis of published scientific papers, the impact of dietary inclusion of lipid/oil and tannins/saponins, and increasing the starch concentration of the diet on enteric CH_4 emissions.

Materials and methods Literature searches were performed using Web of Science and Google Scholar using the terms most likely to identify research and review papers from which enteric emission factors might be derived for all the required ruminant livestock groups. Data extracted from the identified literature included: animal types (dairy cows, beef cattle adn sheep); physiological state; CH_4 emissions (g/d and MJ/d); intake (g/d and MJ/d); and management regimes (e.g. grazed vs housed). A meta analysis was performed on data whereby all variables were analysed, independently for each mitigation type, as a linear mixed model with "Study" fitted as a random effect. In addition each analysis was weighted by the square root of the number of animals on each treatment in each study due to not having individual animal values per treatment, a technique also used by Eugène *et al*. (2008). Regression analysis only included treatments were total diet lipid or starch concentrations were clearly specified. Pseudo R-squared values were calculated by saving the fitted values from each model, correlating these with the response variable in each case, and then squaring this to produce a pseudo R-squared value. All analyses were carried out using the REML algorithm in GenStat version 15.1.

Results Significant linear relationships ($P<0.001$) were found between CH_4 g/kg DMI and the fat concentration in the diet. On average a 10 g/kg increase in dietary fat decreased CH_4 emissions by 0.8 g/kg DMI. When data was restricted to diets containing ≤ 8% fat an average a 10 g/kg increase in dietary fat decreased CH_4 emissions by 0.9 g/kg DMI (Figure 1). Significant linear relationships were found between CH_4 per unit of DMI ($P<0.001$) and CH_4-E per unit of GEI ($P<0.05$) and the starch concentration of the diet. No significant reduction in daily CH_4 emissions were found for diets with added tannins or saponins, however CH_4 emissions per unit of DMI ($P<0.001$) and per unit of GEI ($P<0.05$) were significantly lower.

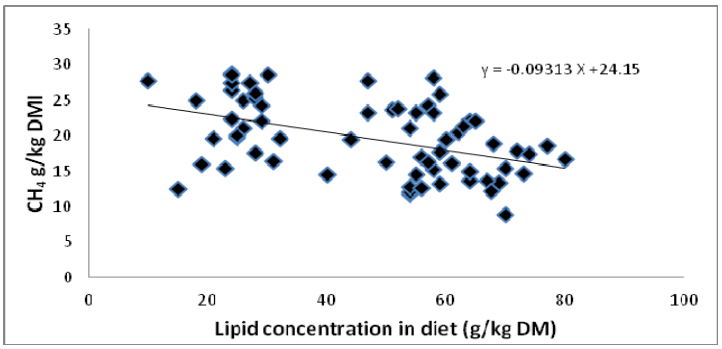

Figure 1 Relationship between methane emissions per unit of DM intake and the concentration of lipid (≤8% lipid DM) within the diet of dairy and beef cattle and sheep

Conclusions The results of this review and meta analysis demonstrate that the addition of lipid/oil, tannin/saponin and increased dietary starch concentrations are all potential mitigation strategies resulting in reduced enteric CH_4 emissions from ruminant livestock when expressed on a per unit of intake basis. However as with all potential GHG mitigation strategies it would be prudent to complete a lifecycle assessment ensuring reductions in CH_4 emissions weren't overshadowed by increased GHG emissions within the complete production system. Many studies identified in this review imposed dietary treatments and measured emissions over a short period of time. With a number of authors reporting concerns on the persistency of CH_4 reductions as a result of many dietary strategies, longer term studies are required.

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Soluble lipopolysaccharide concentrations in caecal digesta but not ruminal digesta correlate with inflammation of rumen wall tissue at slaughter in commercial beef cattle at risk of SARA

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Implications Caecal soluble lipopolysaccharide (LPS) concentrations were found to be much higher than those found in the rumen. Caecal LPS and not ruminal LPS concentrations correlated with visible inflammation of the rumen wall. Thus, some of the inflammation associated with sub-acute ruminal acidosis (SARA) may actually originate in the lower gut.

Introduction SARA primarily occurs in ruminants reared in intensive farming conditions. LPS, a component of gram-negative bacteria, has been shown to increase in concentration in extracellular ruminal fluid of SARA-induced animals (Li *et al.*, 2012). Once soluble LPS interacts with the rumen wall and is translocated into the bloodstream, the inflammatory nature of LPS is thought to cause the inflammation typically associated with SARA, including rumen wall damage and laminitis. However, little is known about the role of LPS originating in the hindgut. Furthermore, studies have so far been conducted mainly on SARA-induced animals, and not in "real farm" situations. Therefore, the aim of the present experiment was to determine LPS concentration in the rumen and caecum of commercially reared cattle, and to compare to other SARA-associated indicators such as rumen damage scores and volatile fatty acid (VFA) concentrations.

Material and methods Twelve beef farms situated in Aberdeenshire provided dietary and farm management information by completing a questionnaire. The chemical composition and particle size of the total mixed ration from each farm was also determined. From this information, 3 farms were selected as being at high risk for SARA incidence, and 3 categorised as low risk, mainly based on the starch content of the diet. In total, 98 continental crossbreed steers and heifers of an average deadweight of 374 kg were assessed for rumen damage. At slaughter, the rumen wall was inspected and photographed both pre- and post-cooking at the abattoir (80-90°C, 10 min). Scoring systems were used to assess the condition of the rumen wall. Pre-cooking scores were taken for: pinkness/inflammation, bare/necrotic areas and papillae shape. A score was also taken for any blackness of the rumen wall which is evident post-cooking. Ruminal fluid and caecal content were collected, processed and analysed for soluble LPS concentration according to the *Limulus*-based method of Li *et al*. (2012). VFAs in ruminal and caecal digesta fluid were measured by GC. ANOVA analyses (grouped by rumen scores and farm risk ratings) were performed on the LPS results by using Genstat (13th Edition). Results were considered significant if $P < 0.05$.

Results At least 10-fold higher concentrations of soluble LPS occurred in caecal compared to ruminal digesta. There were significant differences between farms in VFA and soluble LPS concentrations, but results did not follow the 'high' or 'low risk' ratings. Across all results there was high variation, even within farms, which may reflect differences in the susceptibility of individual cattle to SARA. ANOVA analysis of LPS and rumen damage scores revealed a relationship between caecal LPS and rumen pre-cooking colour (Table 1), and ruminal LPS and rumen pre-cooking damage ($P < 0.001$). There were no other significant results for the other rumen damage scores.

Table 1 Visible inflammation of ruminal wall and soluble LPS concentrations in ruminal and caecal digesta

	Rumen colour score					s.e.d.	Sig.
	0 (n = 33)	1 (n = 22)	2 (n = 19)	3 (n = 17)	4 (n = 7)		
Rumen LPS (10^6 EU/mL)	0.097	0.066	0.091	0.101	0.101	0.042	0.611
Caecum LPS (10^6 EU/mL)	0.559	0.725	1.010	1.087	1.524	0.463	0.042

0 = Black/brown, 1 = grey/brown, 2 = grey/brown small areas with pink tips, 3 = grey/brown large areas with pink tips, 4 = pink.

Conclusion The high concentration of soluble LPS in the caecum and its relationship with pinkness of the rumen wall suggests that LPS originating from the caecum may contribute to the pathological signs of SARA in the rumen wall. Future studies should therefore investigate further the role of the hindgut on the pathogenesis of SARA.

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Potential use of tea leaves to reduce rumen ammonia and methane productions

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Implications Green tea leaves (GTL) have the potential to reduce rumen NH_3 and CH_4 productions without any detrimental effect on rumen function *in-vitro* and perhaps ruminant productive efficiency.

Introduction Tea leaves are good sources of protein, minerals and secondary metabolites such as tannins and saponins which have the potential to reduce rumen solubility and degradability of feed proteins. This can result in reduced rumen NH_3 and urinary N loss but increased rumen by-pass protein for its absorption in small intestine (Ramdani, *et al.* 2013a). This study tested the hypothesis that tea leaf inclusions into ruminant diets can not only reduce NH_3 but also CH_4 productions without any detrimental effects on the rumen function.

Material and methods A 5 x 2 factorial design, with 4 replicates, tested the effect of 5 different tea leaf inclusions at 0% (T0), 5% (GTL5) and 10% (GTL10) of GTL and 5% (BTL5) and 10% (BTL10) of black tea leaves (BTL) into 2 different total mixed diets (168 ± 20 g CP and 11.1 ± 0.54 MJ ME/kg DM) containing either rice straw (RS) or ryegrass hay (RH) on *in-vitro* Organic Matter Degradability (IVOMD, g/kg), pH, NH_3 (mg/L), total Volatile Fatty Acid (tVFA, mmol/L), total Gas Production (tGP, L/kg OM), CH_4 and CO_2 productions (L/kg OM) during their anaerobic incubation in glass syringes at 39^{o}C over 24h as reported by Ramdani *et al.* (2013b and c). tVFA were calculated as the sum of acetate, propionate, iso-butyrate, n-butyrate, iso-valerate and valerate following analysis by gas chromatography (GC). CH_4 and CO_2 were analysed by GC-MS after transferring the gas samples from syringes to evacuated gas tubes. The GLM on Minitab 16 was used to examine the statistical effects of different tea leaf inclusions, the diets and tea type x diet interaction on each parameter measured. Differences were considered significant if $P<0.05$.

Results Table 1 presents the means for only the main effects of tea leaf inclusions and the type of diets as these were mostly significant ($P<0.05$ to $P<0.001$) but not their interactions ($P>0.05$ in all cases). Across the diets, all GTL inclusions significantly decreased NH_3 and pH but only BTL10 significantly reduced NH_3 compared with T0. The tea leaf inclusions had no significant effect on IVOMD, tVFA, tGP and CO_2 but BTL10 had significantly lower IVOMD and tGP than GTL5 and GTL10. All tea leaf inclusions reduced CH_4 production compared with the T0 and it was significant for BTL10. The RS diet, across the tea leaf inclusions, had significantly higher NH_3 and pH but lower IVOMD and tGP than the RH diet.

Table 1 Means for the main effects of tea leaf inclusions and diets on *in-vitro* measurements

Measurements	Tea leaf inclusions (TLI)					Diets (D)		SEM and significances		
	T0	GTL5	GTL10	BTL5	BT10	RS	RH	TLI	D	TLI*D
IVOMD (g/kg)	766^{AB}	767^{A}	768^{A}	743^{AB}	727^{B}	719^{b}	790^{a}	10.1^{*}	6.29^{***}	15.3^{NS}
NH_3 (mg/L)	152^{A}	137^{B}	116^{C}	145^{AB}	136^{B}	142^{a}	132^{b}	2.93^{***}	1.77^{***}	3.83^{NS}
tVFA (mmol/L)	48.6	49.5	51.3	47.4	49.1	50.1	48.3	1.96^{NS}	1.18^{NS}	2.95^{NS}
pH	6.69^{A}	6.66^{B}	6.65^{B}	6.67^{A}	6.68^{A}	6.69^{a}	6.65^{b}	0.004^{***}	0.002^{***}	0.005^{NS}
tGP (L/kg OM)	243^{AB}	247^{A}	246^{A}	241^{AB}	239^{B}	236^{b}	251^{a}	2.00^{*}	1.21^{***}	3.03^{NS}
CH_4 (L/kg OM)	34.3^{A}	32.7^{AB}	31.9^{AB}	31.9^{AB}	31.6^{B}	32.0	33.0	0.69^{*}	0.41^{NS}	1.04^{NS}
CO_2 (L/kg OM)	165	162	170	162	158	159	167	6.56^{NS}	4.09^{NS}	9.92^{NS}

Mean values with different letters in the same row were significantly different at $P<0.05$ (*) or $P<0.01$ (**) or $P<0.001$ (***); NS, non-significant; SEM, standard error of mean.

Conclusion Tea leaf inclusions into ruminant diets can reduce NH_3 and CH_4 production without affecting the *in-vitro* degradability of forage based mixed diets. Generally, GTL inclusions gave higher IVOMD than those of BTL whereas RH diet had greater IVOMD than RS diet.

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The effects of whole clove bud oil or its three principal constituent components on the biohydrogenation of polyunsaturated fatty acids by rumen microorganisms *in vitro*

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Implications Clove bud oil and its three principal constituent essential oil compounds have the potential to protect some dietary polyunsaturated fatty acids from biohydrogenation by rumen microorganisms.

Introduction Ruminant food products contain high levels of saturated fatty acids (SFA) which are formed by the biohydrogenation of ingested polyunsaturated fatty acids (PUFA) by rumen microorganisms. High intakes of SFA are associated with numerous negative effects on human health. There has been an increase in research in recent years on the use of whole essential oils (EOs) or their individual components (EOCs) to alter rumen fermentation. These studies have focussed mainly on ammonia, volatile fatty acids and methane production in the rumen (Hart *et al.* 2008, Calsamiglia *et al*., 2007). Limited research has been published on the potential of EOs or EOCs to modify fatty acid metabolism; currently there are less than a dozen papers in this area. The aim of this study was to investigate the effects of either whole clove bud oil or its three major constituent compounds; eugenol, eugenyl acetate and (-) *trans*-caryophyllene on the biohydrogenation of linoleic acid (LA, C18:2*n*-6), α-linolenic acid (LNA, C18:3*n*-3), eicosapentaenoic acid (EPA, C20:5*n*-3), docosahexaenoic acid (DHA, C22:6*n*-3) in rumen contents *in vitro*.

Material and methods A basal feedstock made up of a 70:30 mixture of grass hay (*Lolium perenne)* and lamb grower concentrate mixture was milled through a 1 mm screen. The basal substrate was then supplemented with an additional 32.5 g oil/kg DM (of a 60:40 mixture of fish oil and linseed oil) to give a final lipid content of 50 g/kg DM. The basal diet (1 g) was then weighed into serum bottles to which 80ml of buffer and 20ml of strained rumen fluid were added and incubated (Theodorou *et al.,* 1994). To each serum bottle, 300mg/l of either whole clove bud oil or its three key constituent compounds were added to create five treatments as follows: control (no EO), clove bud oil, eugenol, eugenyl acetate and (-) *trans*-caryophyllene. Eight replicates of each treatment were incubated per treatment for measuring gas production at 3, 6, 9, 12, 24 and 48 hours post incubation. Three sets of replicates, treated as above, were incubated and stopped after 12, 24 and 48 hours and their contents analysed for linoleic acid, α-linolenic acid, *trans*-vaccenic acid (TVA), conjugated linoleic acid (CLA), EPA and DHA. The effects of each treatment was analysed using a one-way analysis of variance using Genstat Release, 16th Edition.

Results The proportions of PUFA and key biohydrogenation intermediates after 24 hours of incubation *in vitro* are shown in Table 1. In comparison to the control, the treatments caused significant (P<0.001) changes in the concentrations of TVA, with eugenyl acetate increasing the concentration, whilst *trans*-caryphyllene reduced its concentration by 8.3%. The proportions of CLA and LA were not altered by the treatments relative to the control. All treatments maintained higher concentrations LNA compared to the control (P<0.001), with both whole clove oil and eugenol having the greatest effect. The levels of LNA were over 50% higher than the control in clove and eugenol treated samples. All serum bottles in which either clove oil or its three constituent compounds were added had higher levels of both EPA and DHA than the control (P<0.001) after incubation in rumen contents for 24 hrs.

Table 1 Effects of whole clove oil or its major constituent compounds on PUFA levels (g/100g) after a 24 h incubation *in vitro*

	Treatments						
	Control	Clove oil	Eugenol	Eugenyl acetate	*trans*-Caryophyllene	sed	P-value
C18:1 trans-11	11.44^{a}	11.27^{a}	11.50^{ad}	11.72^{bd}	10.49^{c}	0.120	P<0.001
CLA	0.09	0.10	0.10	0.55	0.10	0.168	NS
C18:2*n*-6	1.47	1.60	1.59	1.54	1.50	0.117	NS
C18:3*n*-3	1.36^{a}	2.07^{b}	2.05^{b}	1.94^{ab}	1.80^{ab}	0.034	P<0.001
C20:5*n*-3	1.29^{a}	1.64^{b}	1.57^{b}	1.60^{b}	1.59^{b}	0.057	P<0.001
C22:6*n*-3	0.78^{a}	1.02^{b}	1.02^{b}	1.01^{b}	0.99^{b}	0.039	P<0.001

Conclusion The results from this study show that both whole clove bud oil and its individual constituent components inhibit disappearance of *n*-3 PUFA; α-linolenic acid, EPA and DHA, but have no effect on the biohydrogenation of linoleic acid. Biohydrogenation tended to be most inhibited by whole clove oil and eugenol, its principal constituent.

Acknowledgements We are most grateful to Mr Andrew Thompson for assistance with fatty acid analyses.

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Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) digestibility coefficient Of WAD ewe fed selected Nigeria indigenous forage species

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Implications The study was carried out to evaluate the effects of feeding tree forage species indigenous to Nigeria to growing WAD ewes on the apparent nutrient digestibility of NDF,ADF and ADL

Introduction The dry season in Nigeria results in a rapid decline in the quantity and quality of forages leading to low forage intake which results in poor animal performance. Adegbola (2002) reported that poor quality roughages fed to ruminants without supplementation during the dry season caused considerable weight losses and general poor performances. The conventional feed resources available during this period are very expensive because of the competition with man for availability (Akinmutimi, 2004) and this has necessitated the search for cheap alternative feed materials that can meet the nutritional requirements of farm animals. The alternative feed materials should not be in high demand by humans and should be very cheap and readily available (Akinmutimi, 2007). Therefore, the use of alternative feed resources such as multipurpose trees and shrubs (legumes and non-legumes) should be intensified.

Material and methods This experiment was conducted at the Sheep unit of the Directorate of University farms, Federal University of Agriculture, Abeokuta, Nigeria. Indigenous tree forage species (*B.macaranthi, Albizia saman, Gmelina arborea* and *Ficus sur*) were collected within the premises of the University while *P.maximum* was harvested from established pasture of the institution. Each tree forage plant was fed as a supplement amounting to 40% of the diet while *P.maximum* was offered at 60%, except for those on the control diet, which was 100% *P.maximum.* Thirty growing WAD ewe, 5-7 months (11.20-14.50kg) were randomly assigned to five dietary treatments having six replicate per diets in a completely randomly design. The pens were thoroughly washed and disinfected. Animals were quarantined before the feeding trial, voluntary feed intake was determined as the difference between feed offered and feed refused. The experiment lasted for 84 days. Digestibility studies were carried out by the total feacal and urine collection (Mc Donald *et al* 1987) from all the animals during the last 7days. Faeces voided by each animal were collected, weighed and 10% was kept for analysis. Urine was collected into bottles with few drops of H_2SO_4 to avoid ammonia losses. The weight of the ewes was measured at the beginning and at the end of the collection period. Faecal samples collected were analysed for ADF, NDF and ADL according to the procedure of Van soest (1995).

Results The diets had no significant ($P > 0.05$) effect on the NDF and ADF digestibilities of the ewes. Animals fed *A.saman+P.maximum* had significantly ($P < 0.05$) higher ADL digestibilities (77.58%) while the lowest ADL value was observed in animals fed *B.macaranthi+P.maximum* with intermediate value (68.65%) recorded in animals fed the control diet (*P.maximum* only). No significant ($P > 0.05$) difference was recorded in animals fed P.maximum only and those offered *G.arborea+P.maximum* in spite of the numerical variation.

Table 1 NDF, ADF and ADL digestibility coefficient of ewe fed selected Nigeria indigenous forage species

Parameters	*P.maximum* only(Control)	*B.micranthi +P.maximum*	*F.sur +P.maximum*	*A.saman+ P.maximum*	*G.arborea +P.maximum*	Standard Error of Mean
NDF	60.61	56.69	54.55	57.85	55.72	1.15
ADF	61.94	58.46	55.63	63.81	60.25	1.47
ADL	68.65^{ab}	65.25^{b}	65.80^{b}	77.58^{a}	71.96^{ab}	1.63

^{abc} Means along the same row with different superscripts are significantly different ($P < 0.05$)
NDF=Neutral detergent fiber, ADF=Acid detergent fiber, ADL= Acid detergent lignin

Conclusion It was concluded that acid detergent lignin (ADL) digestibility coefficient of ewes fed selected forage species was significantly influenced and that in spite of the numerical variation in the ADF and NDF values, no statistical difference was noted in the latter fiber fractions.

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Effects of bacterial and enzyme inoculation on the fermentation and aerobic stability of potato hash silage

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Implications Silage can be produced from potato hash with the aid of wheat bran as absorbent, and silage additives (enzyme and bacterial inoculants) to improve the fermentation of silage.

Introduction Potato hash (PH) a by-product from food production industry in South Africa, contains 188.4 g dry matter (DM)/kg, 34 g water-soluble carbohydrate (WSC)/kg DM, 85 g crude protein (CP)/kg DM and 14 MJ/kg DM GE (Nkosi and Meeske, 2010), can be a valuable feed source for ruminants. However, its low DM and WSC contents make it difficult to ensile. Therefore, dry materials and enzymes are used to improve both the DM and WSC contents for ensiling (McDonald *et al*., 2002). The present study was therefore conducted to evaluate the effects of bacterial and enzyme inoculation on the fermentation and aerobic stability of ensiled PH.

Material and methods Batches of PH were collected from Simba, South Africa for chemical analyses and ensiling. PH was mixed with wheat bran at 80:20 ratio and 500kg mixtures were treated with: T1. no additive (control), T2. Celluclast (enzyme), T3. Emsilage (bacterial inoculant), and T4. Silosolve (bacterial inoculant. The treatments were ensiled in 1.5 L anaerobic jars and 3 jars per treatment were opened on day 90 for the determination of nutritive values and fermentation characteristics. Aerobic stability was done by exposing silage samples to air for 5 days (Ashbell *et al*., 1991). Data were analysed in a completely randomized design for ANOVA using Genstat (2005).

Results The addition of enzyme (T2) reduced ($P<0.05$) silage pH, fibre fractions (NDF and ADF) while increasing ($P<0.05$) residual WSC and lactic acid (LA) production, compared to other treatments (Table 1). Silage had pH of < 4.0, indications of well-preserved silage. Inoculation (T3 and T4) improved ($P<0.05$) aerobic stability of silage, as indicated by increased number of hours and reduced CO_2 production, compared to other treatments.

Table 1 Chemical composition, fermentation characteristics and aerobic stability of ensiled potato hash treated with or without silage additives after 90 days of ensiling (n=3)

Parameters	Treatments				SEM	P
	1	2	3	4		
Chemical composition						
DM, g/kg	320.6^{bc}	317.1^{c}	326.5^{b}	355.9^{a}	2.52	0.001
CP, g/kg DM	140.5^{c}	148.2^{a}	142.5^{b}	123.0^{d}	0.565	0.001
GE MJ/kg DM	16.9^{b}	16.99^{b}	17.60^{a}	14.98^{c}	0.072	0.001
NDF, g/kg DM	442.8^{ab}	356.3^{c}	418.6^{b}	454.1^{a}	7.60	0.001
ADF, g/kg DM	130.6^{c}	120.4^{d}	148.4^{b}	151.4^{a}	0.796	0.001
Fermentation characteristics						
pH	3.51^{a}	3.36^{c}	3.42^{b}	3.35^{b}	0.0109	0.001
WSC, g/kg DM	9.00^{c}	16.74^{a}	12.74^{b}	12.13^{b}	0.427	0.001
LA, g/kg DM	66.39^{b}	86.65^{a}	61.45^{c}	61.85^{c}	1.167	0.001
Aerobic stability (after 5 days aerobic exposure)						
hrs	63.53^{b}	57.9^{c}	88.20^{a}	88.7^{a}	0.671	0.001
CO_2 g/kg DM	26.47^{b}	29.33^{a}	11.00^{c}	8.43^{d}	0.552	0.001

$^{a-d}$ Means in the same row with different superscripts differ significantly ($P<0.05$)
Treatments: 1, control; 2, celluclast; 3, emsilage; 4, silosolve

Conclusions Although enzyme addition improved the nutritive value of silage, it impaired the aerobic stability of silage. Further work to elucidate these effects on nutrient digestion and growth performance of ruminants fed the silage is needed.

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Effect of Thapra Stylo silage treated with dried mao pomace (DMP) on feed intake and nutrient digestibility in goats

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Implications Dried mao pomace (DMP) improved Thapra Stylo (*Stylosanthes guianensis* CIAT 184) silages and did not alter the feed intake and nutrient digestibility of Thapra Stylo silage in goats.

Introduction Mao luang is edible fruit tree (*Antidesma thwaitesianum*) divers in Northeast of Thailand. Mao juice and mao wine have become increasingly popular in Thailand and waste products such as mao pomaces from the process are plentiful. Several researches revealed that mao pomace contains high antioxidants, organic acid, amino acid and sugar. Thus, dried mao pomaces (DMP) may have potential as a silage additive by stimulating the lactic acid bacteria (LAB) growth on the ensiling process. Thapra Stylo is a promising legume available for ruminant production in tropical areas. It has a high protein content and grows on a variety of soil types. It has been difficult to make good quality Thapra Stylo silage without any additives, with a high pH value and high NH_3-N content. The aim of this study was to investigate the effect of applying DMP alone and combined with LAB on the fermentative quality and nutritive value of Thapra Stylo legume silages.

Material and methods Fermented juice of lactic acid bacteria (FJLB) were prepared from fresh Thrapra Stylo legumes by the method of Bureenok *et al.* (2011). Thapra Stylo legumes were chopped into 2-3 cm lengths and mixed with the silage additives. Silages were untreated (control), or prepared with DMP, or DMP plus FJLB (DMP+FJLB), or DMP plus *Lactobacillus plantarum* ST1 (DMP+Lp). DMP was applied at 100 g/kg of fresh matter (FM). FJLB and Lp were applied at log 6.03 and 5.58 cfu/g FM, respectively. Four male ruminally fistulated crossbred Boer x Saanen goats (~ 16 kg body weight) were randomly assigned to one of the four dietary treatment silages in a 4 x 4 Latin square design. The 28-d experimental period consisted of a 21-d feed intake and 7 d of sampling. All goats received concentrate at 1.5% of body weight (BW) and *ad libitum* silage. Feed and silages were sampled once a week and kept for analysis. Faeces samples were collected at the end of each period and analysed for DM, CP, EE, ash, ADF and NDF. The data were analysed using the General Linear Model procedure by SAS. Silage profiles were compared by Duncan's multiple range test. Data from the feeding trial were analysed using the procedures of SAS for a 4 × 4 Latin square design.

Results Dry matter content of silage was increased with addition of DMP portion. Without any additives, the silage showed the highest pH value. The NH_3-N contents of DMP silages were lower ($P<0.01$) compared with the control silages, but did not appear to be significantly different from the combined silages. Silage intake tended to be higher in goats fed with DMP silages. However, no significant differences ($P>0.05$) in nutrient digestibility were observed in goats fed silages (Table 1).

Table 1 Silage quality, feed intake and nutrient digestibility of Thapra Stylo legume silages in goats

Items	Control	DMP	DMP+FJLB	DMP+Lp	s.e.m.	P-value
Silage profiles						
DM (g/kg)	305^{d}	405^{a}	333^{c}	367^{b}	5.1	<0.001
pH	4.49^{a}	4.01^{c}	4.31^{b}	3.98^{c}	0.027	<0.001
NH_3-N (g/kgTN)	89.3^{a}	52.4^{b}	80.3^{ab}	69.0^{ab}	9.85	0.124
Silage intake						
%BW	2.5	3.1	2.4	2.6	0.11	0.314
g/kg $BW^{0.75}$	51.8	64.9	50.8	55.0	2.25	0.309
Nutrient digestibility (%)						
DM	67.1	63.2	60.1	71.5	2.17	0.446
CP	66.9	68.6	61.6	75.6	1.77	0.224
NDF	62.0	53.7	52.0	65.4	3.12	0.511
ADF	64.8	59.6	55.7	67.7	2.46	0.446

Conclusion Ensilage of Thapra Stylo legume with DMP showed a significant reduction in pH value and NH_3-N contents of silages. However, addition of 100 g/kg of DMP did not affect on silage intake and nutrient digestibility of goats.

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Accounting for herd movement characteristics in carcase trait genetic evaluations of commercial beef cattle

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Implications Accounting for herd movement by fitting variables as random effects was more appropriate considering the unstructured nature of the commercially derived beef carcase trait dataset.

Introduction Carcase traits, measured on commercial animals processed in abattoirs, have the potential to be useful for genetic improvement programs. The data have the characteristics of being measured late in life and collected in high volume at a low cost. However, unlike the pedigree performance recording system, animals are not treated alike as commercial animals can frequently be moved between different herd locations. Previous genetic evaluation studies have recognised the unstructured nature of commercially derived data, but lacked a unified approach as to how to account for herd movement (Hickey *et al*. 2007; Pabiou *et al*. 2011). The aim of this study was to determine an appropriate method to account for herd movement for the purposes of genetic evaluation of abattoir carcase data.

Material and methods This study used carcase data for prime slaughter heifers and steers (n = 243,819) from commercial abattoirs. Traits recorded were carcase weight, EUROP conformation (CONF) and fat class (FAT), with the latter two traits recorded on an 8-point and 7-point scale, respectively, and both converted to a numeric scale. Herd movement records for the commercial animals were obtained from the British Cattle Movement Service (BCMS), which is part of the Rural Payments Agency. Herd variables derived included birth herd (BH), finishing herd (FH), birth and finishing herd as a concatenated variable (BH_FH), birth and finishing herds as independent terms (BH + FH). Further data edits required animals to have sire and dam identities, records for the 3 traits, be slaughtered in 2008 and 2009, from 7 beef or 1 dairy breeds, had ≥3 observations for the birth or finishing herd and be ≤900 days of age at slaughter. Animals with an unknown slaughter category that fell within the 99.4% confidence interval of mean age at slaughter of prime slaughter heifers and steers were assigned to the relevant category. The final dataset consisted of 44,957 animals. Variance components were estimated in a single trait model in ASReml (Gilmour *et al*., 2006). For all 3 traits a base model included birth-year-season, date and place of slaughter, breed and sex with animal as a random effect. Carcase weight (linear and quadratic) was included as a covariate, except where it was the dependent variable. The impact of herd movements was examined by fitting various combinations of BH, FH, BH/FH and also as either fixed and random effects. Where sires had at least 10 progeny, the correlation between sire estimated breeding values (EBV) was estimated for the different models.

Results The average carcase weight was 340kg. Average conformation was 24 on the numeric scale, which corresponds to 'R' on the EUROP grid ('Good'). Average fat class was 28 on the numeric scale, which falls in between the '3' and '4L' classes ('Average' and 'High', respectively). The average number of observations per level was 10.8, 20.8 and 3.8 for the BH, FH and BH_FH, respectively. Table 1 lists the herd variables and the heritabilities for 2 of the carcase traits. Heritabilities were moderate to high and tended to be higher when herd variables were fixed versus random effects. When either BH or BH + FH were fitted as random effects heritabilities were similar to those reported in other studies (Hickey *et al*. 2007; Pabiou *et al*. 2011). Sire EBVs were highly correlated when the variables were fitted as random effects indicating that EBVs were similar regardless of the variable (results not shown). Many herd variables had levels with few observations making it more appropriate to fit them as random effects.

Table 1 Heritability (standard errors) estimates for 2 carcase traits

	Fixed		Random	
	CONF	FAT	CONF	FAT
BH	0.30 (0.023)	0.41 (0.025)	0.34 (0.020)	0.33 (0.020)
FH	0.61 (0.020)	0.48 (0.020)	0.55 (0.018)	0.38 (0.016)
BH_FH	0.30 (0.027)	0.41 (0.028)	0.49 (0.020)	0.39 (0.020)
BH + FH	0.29 (0.024)	0.40 (0.026)	0.29 (0.020)	0.28 (0.018)

Conclusion Given the structure of the herd variables (i.e. few observations per level) in this commercial dataset, it is more appropriate to fit herd variables as random effects. The high correlations were favourable for the practicalities of genetic evaluations as the primary information available is usually the birth herd with a potential lag in obtaining finishing herd information due to delays in updating and receiving data from BCMS.

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Effect of feed restriction and subsequent compensatory growth on the transcriptional profile of hepatic tissue in Holstein Friesian bulls

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Implications This study provides an insight into the molecular control of compensatory growth in cattle. This data will contribute to identifying DNA based biomarkers to select for cattle with a greater ability to undergo compensatory growth following a period of dietary restriction.

Introduction Compensatory growth is defined as a physiological process whereby an animal has the potential following a period of restricted feed intake to undergo accelerated growth upon re-alimentation (Hornick *et al*., 2000). Previous studies including our own have identified reduced liver tissue growth in cattle during a period of restricted feed intake. However, this is quickly reversed during compensatory growth (Keogh *et al*., 2012; Yambayamba *et al*., 1996). Given the central role of the liver in regulating metabolism, the objective of this study was to conduct a global transcriptional examination of the biochemical pathways expressed in hepatic tissue of Holstein Friesian bulls, during a restricted feeding regime followed by re-alimentation and compensatory growth.

Material and methods This study utilised tissue collected as part of the study of Keogh *et al*. (2012). Briefly, 60 Holstein-Friesian bulls were assigned to one of two groups: (i) restricted feed allowance for 125 days (RES; n=30) followed by *ad libitum* access to feed for 55 days or (ii) *ad libitum* access to feed throughout the trial (ADLIB; n=30). The first 125 days was denoted as Period 1 and the subsequent 55 days, Period 2. At the end of each period, 15 animals from each group were slaughtered. During Period 1 RES were managed to achieve a target mean daily growth rate of 0.6 kg/day. Hepatic tissue was collected from all animals at each slaughter time point. Total RNA was isolated and purified using the Qiagen RNeasy clean up kit. Total RNA was verified for yield and quantity. Messenger RNA was purified from total RNA, with the mRNA then fragmented and reverse transcribed into cDNA. Adaptors were ligated to the cDNA which was then enriched by PCR. Single end RNA sequencing was carried out using the Illumina Hiseq. Raw sequence reads were first checked for quality. Input reads were then aligned to the bovine reference genome (UMD3.1) using TopHat. The number of mapped reads was counted and differential gene expression was determined using EdgeR. Significantly differentially expressed genes ($P<0.05$), with a false discovery rate of 10% and a fold change of 1.5 or over were then subjected to pathway analysis. Analysis of physiological pathways over-represented in the set of statistically significantly differentially expressed genes was conducted using KEGG pathway analysis and GOseq.

Results Average daily gain (ADG) for Period 1 was 0.6 kg/d for RES and 1.9 kg/d for ADLIB. During re-alimentation an ADG of 2.5 and 1.4 kg/d was observed for the RES and the ADLIB groups, respectively. A total of 1995 and 116 genes were identified as differentially expressed between RES and ADLIB in periods 1 and 2 respectively. These were manifested as 995 genes with increased expression and 1000 genes with decreased expression in RES relative to ADLIB in Period 1. During compensatory growth 48 and 67 genes had increased and decreased expression respectively in RES compared to ADLIB animals. In Period 1, a total of 33 over represented pathways were identified. During the initial stages of compensatory growth in Period 2, 20 over represented pathways were evident. The top five pathways for each period are presented in Table 1.

Table 1 Over represented pathways during restricted and compensatory growth

Period 1		Period 2	
Pathway	Over represented p value	Pathway	Over represented p value
Metabolic pathways	<0.001	Focal adhesion	<0.001
Citrate cycle	<0.01	ECM receptor interaction	<0.001
PPAR signalling	<0.01	Protein processing	<0.001
Protein processing	<0.01	Biosynthesis of fatty acids	<0.05
Tryptophan metabolism	<0.05	Protein export	<0.05

Conclusion Over represented pathways in both periods included those involved in metabolism, protein processing and cell proliferation. As the liver is a highly metabolic organ, the reduction in hepatic volume previously observed (Keogh *et al*., 2012) coupled with differentially expressed genes in pathways involved in metabolism and cell growth imply changes in the RES animals metabolic rate as well as hepatic cell turnover during feed restriction and compensatory growth. This study provides an insight into the molecular mechanisms regulating the compensatory growth phenomenon in cattle.

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The effect of feed restriction and realimentation on the transcriptome of bovine jejunal epithelium

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Implications This study provides an insight into the molecular mechanisms underlying compensatory growth in cattle, with particular focus on the role of nutrient digestion and absorption in the small intestine.

Introduction Compensatory growth (CG) refers to accelerated growth following a period of undernutrition, to facilitate an animal reach its genetically pre-determined growth potential. We have recently shown that restricted feeding and subsequent CG affect the transcriptional profile of ruminal epithelial tissue (O' Shea *et al*., 2014). The small intestine plays a central role in post ruminal digestion and nutrient absorption and has been shown to adapt to different planes of nutrition by altering its form and function. In this study, we quantified the response of the jejunal epithelial transcriptome in cattle to nutrient restriction and CG, using RNA-seq technology and bioinformatics tools.

Material and methods This study utilised tissue harvested by Keogh *et al*. (2012). Briefly, in that study, 60 Holstein Friesian bulls were assigned to one of two groups: restricted feed allowance (RES; n=30) for 125 days (Period 1) followed by *ad libitum* access to feed for 55 days (Period 2) or (ii) *ad libitum* access to feed throughout (ADLIB; n=30). At the end of both periods, a subset of 15 animals from each treatment group was slaughtered and jejunal tissue was harvested and epithelial tissue carefully removed from the underlying musculature. Total RNA was extracted from jejunal tissue of 10 animals per treatment and processed using an Illumina TruSeq RNA sample prep kit v2. Prepared libraries were pooled based on their respective sample-specific 6b adaptors and sequenced across 4 flowcell lanes at 100bp/sequence read using an Illumina HiSeq 2000 Sequencer by BGI Europe. Approximately 11.3 million sequences per sample (Mean±SD = 11,361,109 ± 1,215,419) were generated. Preliminary quality control analysis was carried out using FASTQC software (version 0.10.0). FASTX-Toolkit (v0.0.13) was then used to trim 3' adaptor sequences. Input reads were then aligned to the UMD3.1 *Bos Taurus* genome assembly using Bowtie2 ultra-fast short read alignment software (v2.1.0). The R (v2.14.1) Bioconductor package EdgeR (v3.4.1), which uses a negative binomial distribution model to account for both biological and technical variability was applied to identify statistically significant differentially expressed genes (DEGs). A gene was deemed to be expressed if the number of reads per gene per animal was ≥4. The analysis was undertaken using moderated tagwise dispersions. DEGs were defined as having a Benjamini and Hochberg corrected P value of < 0.05. Ingenuity Pathway Analysis (IPA) was employed and identified canonical pathways that were most statistically significant. The significance of the association between the DEGs and the pathway was measured using two approaches: (1) as the ratio of the number of DEG that map to the pathway and (2) using Fisher's exact test, which calculates a P-value to determine the probability that the association between the DEG and the pathway is explained by chance alone.

Results An average of 10,491 genes were observed to be expressed (≥4 reads per gene per animal) across both periods. A total of 271 and 220 genes were identified as differentially expressed in Periods 1 and 2, respectively. In Period 1, a total of 27 over-represented pathways were identified. Upon realimentation in Period 2, 37 over-represented pathways were evident. Pathways of interest in each period are presented in Table 1.

Table 1 Over represented pathways (P<0.01) among DEG in RES (compared with ADLIB) during Periods 1 and 2

Period 1		Period 2	
Pathway	P-value	Pathway	P-value
Protein digestion and absorption	<0.001	Steroid hormone biosynthesis	<0.001
PPAR signalling	<0.001	Retinol metabolism	<0.001
Vitamin digestion and absorption	<0.001	Linoleic acid metabolism	<0.001
Nitrogen metabolism	<0.01	Ascorbate and aldarate metabolism	<0.001
Pancreatic secretion	<0.01	Starch and sucrose metabolism	<0.001

Conclusion Over represented pathways in both periods included those involved in metabolism, cell growth and immune response. This is the first study to examine jejunal epithelium transcriptome response to feed restriction and subsequent compensatory growth in cattle.

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Differential gene expression of LIM domain proteins in two divergent growing muscles of broiler chicken (Ross 308 genotype)

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Implications Selection for growth is associated with differential expression of LIM domain protein genes between broilers and layers. We have shown these genes are also differentially expressed between fast and slow growing muscles within broilers.

Introduction Selective breeding has led to broiler chicken phenotype which is characterised by a rapid growth rate and a higher proportion of breast muscle relative to body weight at the expense of the leg muscle (Al Musiwa *et* al., 2011). In a comparative study between broiler and layer chickens, Zheng *et al.,* (2009) identified 543 differentially expressed genes in the *Pectoralis major* (PM) across five developmental stages after hatching. The genes with the greatest differential expression were those encoding LIM domain proteins, Cysteine and Glycine Rich Protein 3 LIM protein (*CSRP3*) and four and a half LIM domain protein (*FHL2*); low expression being associated with muscle cell proliferation and hypertrophy seen in broilers. The objective of the current study was to determine the expression of these genes in a fast (PM) or slow *Peroneous tertius* (PT) growing muscle from within the same growing broiler chickens in relation to muscle protein and DNA contents.

Materials and methods Six male broiler chickens raised on a standard broiler diet were euthanized using CO_2 in a gas chamber followed by cervical dislocation at 14, 36 and 43 days post-hatch. A sample of the PM and PT were immediately dissected and snap frozen in liquid nitrogen and stored at -80^{o}C prior to analyses, whilst whole contra lateral muscles were weighed. Total muscle protein and DNA content was assayed as previously described (Lowry *et al*., 1951; Rago *et al*. 1990). RNA was extracted using QIAGEN RNeasy fibrous tissue mini kit, then quantified and normalised to the same concentration. First strand cDNA generated using random primers and a Roche transcriptor kit. The relative level of mRNA (primers reported by Zheng *et al.*, 2009) to cyclophillin reference gene expression (no treatment effect) was determined by quantitative RT-PCR analysis (Roche 480 lightcycler). Data were analysed by two way ANOVA (Genstat). Significance was accepted at P<0.05.

Results The PM weight was significantly heavier than the PT at all ages (P<0.001, Table 1). There was a significant muscle x age interaction for the DNA content (p=0.003) with the PT having a higher value at all ages, while the protein to DNA ratio was significantly higher in the PM at all ages (P<0.001). There was a significant muscle x age interaction in *CSRP3* and *FHL 2* expression (P<0.001 and P=0.02 respectively) with the PT having the highest level of expression in both genes seen at day 43.

Table 1 Effects age on muscle weight, muscle protein deposition and gene expression in two muscle types

Measurement	Muscle	Day			SED[1]	Effect (P value)		
		14	36	43		Muscle	Age	Muscle x age
Tissue weight (g)	PM	21	149	170	4.3	< 0.001	< 0.001	< 0.001
	PT	1	14	12				
DNA content/tissue(μg/g)	PM	29	40	37	14.8	< 0.001	0.054	0.003
	PT	235	172	244				
Protein/unit DNA (mg/μg)	PM	6.7	5.1	4.7	1.9	< 0.001	0.497	0.729
	PT	0.3	0.4	0.1				
CSRP3 gene expression	PM	0.09	0.04	0.07	1.0	< 0.001	0.002	< 0.001
	PT	2.0	0.9	7.5				
FHL2 gene expression	PM	3	1	2	3.6	0.110	0.085	0.018
	PT	2	2	15				

[1]SED = standard error of the differences of the means.

Conclusion As described by Zheng *et al.,* (2009) our study identified that *CSRP3* expression appears to display a similar relationship within broilers, *CSRP3* expression in the fast growing PM always had a lower expression than slower growing PT. The d36 transient drop in *CSRP3* expression may reflect muscle adjustments to gene expression associated with the starting period of relatively slower growth. *FHL2* expression increased with age in the PT but not in PM. More work is needed to investigate the specific roles of these genes in muscle hypertrophy and cell proliferation at specific points of broiler growth.

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Environmental sensitivity of Texel sheep estimated using reaction norms

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Implications Identifying genotypes best suited to certain environments, or that perform consistently across environments, will allow farmers to select animals appropriate to their farm environment, thus reducing inefficiency in performance.

Introduction The presence of genotype by environment interactions (GxE) can form a potential source of inefficiency in animal breeding if selection decisions are made without acknowledging their effects. As well as identifying the presence of GxE, the degree by which genotypes vary across environments is also of interest, which is often referred to as *phenotypic plasticity* or *environmental sensitivity*. Genotypes are considered "plastic" if they demonstrate highly variable phenotypes across environments or "robust" if they remain relatively constant, both of which can be calculated by reaction norm analyses. The objective of this study was therefore to quantify the effect of farm environment on the performance of Texel sire offspring.

Material and methods A continuous farm environment (FE) scale was developed previously by combining survey data, collected from a sample of 40 Texel flocks, and information available at the national level, for Texel flocks across the UK. Farm survey data included information on flock size and concentrate feed use. National data included adjusted flock averages for 21 week old weight (21WT), ultrasound back-fat (UFD) and muscle (UMD) depths, as well as regional climatic data. The FE scale was then combined with 181,555 (21WT), 175,399 (UMD) and 175,279 (UFD) records from Texel lambs, born between 1990 and 2011, on 494 different flocks. The data were analysed using ASReml (Gilmour *et al.*, 2002), using a sire model and a sire pedigree file containing 9,775 records. Phenotypic observations of lamb performance were regressed, within sire, on the FE scale, allowing a linear reaction norm to be estimated for each sire. Heritabilities were also estimated along the FE scale.

Results A range of positive and negative sensitivities (slopes) were estimated for the 5,938 rams represented in the data, indicating re-ranking of sires across the FE scale. A number of rams with sensitivities close to, or equal to, zero were also observed. Estimated reaction norms for a sample of six sires, for 21WT, are shown in Figure 1. Variations in heritability estimates across the FE scale were also observed (Figure 2). UMD and UFD results were similar to those for 21WT.

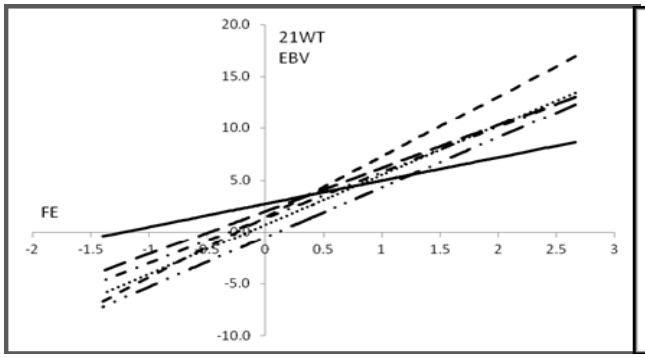

Figure 1 Sire reaction norms estimated across FE scale

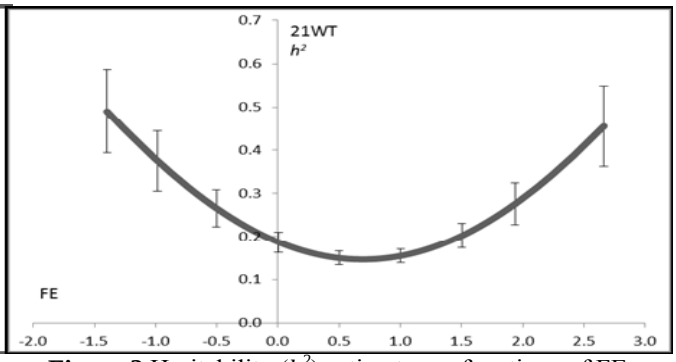

Figure 2 Heritability (h^2) estimates as functions of FE

Conclusion Evidence of GxE was observed for all lamb performance traits investigated. The range of sire sensitivities estimated across the environment scale confirmed the presence of genetic variability, with both "plastic" and "robust" genotypes present in the population. The variation in heritability estimates also suggests that the rate of genetic progress will vary depending on the environment. Future genetic evaluations would benefit by accounting for GxE observed.

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Testing new options for selecting sheep that are more resistant to internal parasites

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Implications Selection for host resistance to Strongyle worms will also confer resistance to *Nematodirus battus* (NEM). Low repeatability for these traits means more than one recording occasion is required for breeding programmes.

Introduction Nematode infection threatens the health and welfare of livestock, compromises the efficiency of production and is the most economically damaging endemic disease for the sheep sector in the UK with an estimated cost of £84M p.a. Faecal soiling from parasitised lambs poses additional labour costs to the producer prior to lambs going for slaughter. Exploiting host genetic variation in resistance to internal parasites in sheep is a long-term, sustainable solution to the problem of increasing resistance of internal parasites to anthelmintics. This will also improve the efficiency and productivity of sheep production, through healthier offspring. The current method of using faecal egg count as an indicator of worm burden is accepted internationally although its use in the UK's *Sheepbreeder* breed improvement programme is low. The aims of this study were to estimate genetic properties and test if two additional indicators of internal parasitism could be used in sheep breeding programmes that may in turn, lead to more farmers engaging in breeding for host resistance to parasites: (i) faecal soiling ('DAGG') scoring and (ii) the CARLA® test, that measures antibodies (IgA) against worm larvae in sheep saliva. Animals with high levels of such antibodies are better at preventing worms establishing in the gut, although the test has not yet been validated in the UK.

Material and methods About 3400 records of egg counts for Strongyles (FEC) and *Nematodirus battus* were measured on 1197 Scottish Blackface lambs reared on one SRUC farm near Edinburgh, on 6 occasions under natural challenge between July and October in 2011 and 2012. Immediately prior to FEC sampling, each lamb was dagg-scored (DAGG) on a visually assessed 5-point scale whereby 0 = no dagginess and 4 = extensive soiling and daggs to hocks (Bisset *et al*.,1994). At the same time, the lambs were weighed (LWT) and a saliva sample was taken that was analysed using the CARLA® test. Univariate heritabilities and bivariate genetic and phenotypic correlations were estimated using ASREML animal models accounting for key fixed effects including sex, year, grazing code, age, lab, dam age, birth/rearing rank and interactions of recording occasion x year, lab x year and grazing code x year.

Results

Table 1 Univariate results: σ^2_p phenotypic variance; σ^2_a genetic variance; σ^2_{pe} permanent environmental variance; h^2_a additive heritability; s.e. standard error of estimate; re repeatability

	σ^2_p	σ^2_a	σ^2_{pe}	h^2_a	s.e.	re
LWT	21.56	3.79	-	0.18	0.033	-
FEC	592060	78514	0	0.13	0.021	0.13
NEM	119885	24522	2486	0.20	0.043	0.22
DAGG	1.57	0.36	0.54	0.23	0.057	0.57
CARLA	0.64	0.16	0.08	0.25	0.08	0.37

Table 2 Bivariate results: genetic correlations above and phenotypic below diagonal (se)

	LWT	FEC	NEM	DAGG	CARLA
LWT		0.15(0.17)	0.32(0.14)	-0.09(0.09)	0.42(0.09)
FEC	-0.19(0.03)		0.46(0.14)	-0.24(0.13)	-0.16(0.09)
NEM	-0.19(0.03)	0.19(0.02)		0.20(0.07)	-0.09(0.03)
DAGG	-0.10(0.03)	0.12(0.02)	0.04(0.02)		-0.09(0.06)
CARLA	0.19(0.04)	-0.05(0.02)	-0.05(0.02)	-0.05(0.03)	

Conclusions All traits have low to moderate heritabilities and are within the range of other published estimates. The phenotypic relationships between DAGG and CARLA with NEM and FEC are low. Selection to reduce FEC will lead to a reduction in NEM. Further investigation is required to understand the inconsistent genetic relationship of DAGG with FEC and NEM. Breeding programmes designed to increase live weight should incorporate faecal egg counts to avoid increasing host genetic susceptibility. Selecting lambs with lower dagg scores will improve host resistance to NEM.

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Effect of residual feed intake phenotype on the expression of genes regulating oxidative phosphorylation in the liver and muscle of beef cattle

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Implications This study provides an insight into the molecular mechanisms underlying feed efficiency in beef cattle

Introduction Feed is the single greatest variable cost in beef production systems, accounting for approximately 80% of total costs of production. Additionally, up to 70% of dietary energy is used for body maintenance and is thus unavailable for productive proposes thus improvements in the efficiency of which feed is utilised by beef cattle is central to the economic viability of the enterprise. Residual feed intake (RFI) is a measure of feed efficiency and is defined as the difference between an animal's actual and its predicted feed intake, based on body size and rate of gain. Mitochondrial function has been identified as a likely contributor to inter-animal variation in energetic efficiency in livestock due to its central role in cellular ATP generation through oxidative phosphorylation (Bottje and Carstens, 2009; Kelly *et al*. 2010). The aim of this study was to examine mRNA expression of genes involved in oxidative phosphorylation in hepatic and muscle tissue of cattle divergent for residual feed intake (RFI).

Material and methods Simmental bulls (n = 67 over three years), offered a high concentrate barley-based diet, *ad libitum,* during a 105 day finishing period and the end of which they were ranked on the basis of RFI. Following slaughter, muscle and hepatic tissue was collected from all animals with samples from those ranking highest (n=15) and lowest (n=15) on RFI used for the current study. Mean bodyweight at slaughter (604 v 606 kg; SEM 16.4kg; P =0.91) and ADG (1.68 v 1.63 kg/day; SEM 0.099; P = 0.67) was similar for these animals while dry matter intake (DMI) was 11% (9.23 v 10.23 kg DM; SEM 0.245; P<0.01) lower for low compared with high RFI animals. Expression of 31 genes regulating oxidative phosphorylation was measured in both tissues using qRT-PCR. These genes coded for enzymes, electron carriers, proteins, uncoupling proteins, transporters and sensors and transcription factors. Stable reference genes for each tissue were chosen by analysis of a number of previously published reference genes using GeNorm. Data were analysed using mixed models ANOVA (PROC MIXED, SAS). Statistical models included effects for RFI group and year and their interactions as appropriate. Spearman correlation analysis was conducted using the CORR procedure of SAS. Statistical significance was deemed at $P < 0.05$.

Results Of the 31 genes examined, 9 and 5 were undetectable in liver and muscle tissue, respectively. There were no RFI x year of study interactions ($P > 0.05$) for either tissue studied. Genes differentially expressed in liver tissue included PPARα (P=0.001), UQCRC2 (P=0.01), and NDUFA5 (P=0.05), with expression of PPARα and NDUFA5 higher in high compared to low RFI bulls, whereas the opposite occurred for UQCRC2. Muscle expression of TFAM (P=0.004), COQ4 (P=0.04), PRPS2 (P=0.02) and LPIN1 (P=0.05), were all increased in low compared to high RFI bulls. These alterations in gene expression may allow efficient animals to exhibit greater protection against cell death, and enhanced electron transport compared to that of their inefficient counterparts. There were many statistically significant associations between individual genes as well as between gene expression and RFI co-efficients, across both tissues. Within liver tissue, PPARα had positive correlations with RFI (r=0.53, P< 0.01), AMPK (r=0.63, P<0.001) and TFAM (r=0.71, P<0.001). Correlations between expression of GSTZ1 (r = 0.47, P<0.01), NAMPT (r=0.66, P< 0.001), and PCK2 (r = 0.65, P<0.001) were positively associated with UQCRC2 expression. Positive correlations were observed between NDUFA5 gene expression and ANT1 (r = 0.47, P< 0.01), and NRF1 (r = 0.91, P< 0.001) gene expression. Within muscle tissue a negative correlation was detected between RFI and muscle TFAM (r =-0.42, P < 0.05). COQ4 expression was positively correlated with ATP6D (r = 0.44, P<0.01), UQCRC2 (r=0.6, P<0.001), PRPS2 (r =0.51, P<0.01), COXII (r =0.66, P< 0.001), PPARα (r=0.63, P<0.001), and ANT1 (r=0.46, P<0.01), and negatively correlated with NAMPT (r=-0.47, P<0.01). PRPS2 expression was positively correlated with UQCRC2 (r =0.43, P< 0.05), COXII (r =0.45, P< 0.01), and PPARα (r = 0.43, P< 0.05) expression. TFAM expression was positively associated with ATP6D (r =0.45, P< 0.01), LPIN1 (r =0.37, P< 0.05), PRPS2 (r =0.55, P< 0.01) and COXII (r =0.46, P< 0.01) expression. Expression of PPARγ was positively correlated with expression of LPIN1 (r = 0.48, P < 0.01).

Conclusion The study provides further insights into the influence of genes regulating the oxidative phosphorylation pathway in cattle divergent for RFI. These data will be useful to the identification of key candidate genes accounting for inter-animal variation in RFI and the subsequent detection of DNA polymorphisms that could be used in a genomically assisted selection programme.

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Gaseous emissions, rumen fermentation and animal performance of dairy cows supplemented with linseed oil or nitrate

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Implications Dairy cows supplemented with 2% nitrate had lower methane emissions per head per day and per kg DMI whereas linseed oil supplementation did not affect methane emissions.

Introduction Linseed oil and nitrate have the potential to decrease methane production in ruminants. Therefore the aim of this experiment was to investigate the effect of dietary linseed oil and nitrate supplementation on methane production, animal performance and rumen fermentation.

Material and methods Six rumen fistulated lactating dairy cows (21.5±4.5 kg milk yield, 578±52 kg BW) were used in a replicated 3 x 3 Latin square experiment. Each period lasted 33 days, during which animals were housed in tie-stalls and fed a TMR diet *ad libitum* once a day at 8:00am and milked twice a day. The TMR contained grass and maize silages (30% DM each), concentrate (33.3% DM) and supplements (including treatment additives). Dietary treatments consisted of a control, linseed oil (4% DM) or nitrate (2% DM) supplementation. Diets were formulated to be equal in nitrogen, fat and calcium through the addition of urea, a rumen inert fat source and limestone, respectively. Rumen samples were taken on day 25 and 26 at 0, 0.5, 1, 2, 4, 6 and 8 hours post feeding. Gaseous emissions were measured in open circuit respiration chambers on day 28 for a 120h period. Chambers were a larger version of the small ruminant chambers described by Hart *et al.* (2012). Data was analysed using the PROC MIXED procedure of SAS with treatment and time post feeding (VFA only; repeated measure) as fixed effects. Block, period and cow within block were included as random effects.

Results Nitrate supplementation tended to decrease DMI ($P<0.085$), but did not affect fat and protein corrected milk (FPCM) yield. Nitrate supplementation, but not linseed oil, decreased methane emissions per head per day and per kg DMI ($P<0.04$) compared to the control diet, but not per kg FPCM ($P>0.3$). Cows receiving nitrate had increased hydrogen emissions compared to the control and linseed supplemented cows. Neither linseed oil nor nitrate effected total VFA concentration ($P>0.25$), but nitrate treatment increased the acetate to propionate ratio ($P<0.001$).

Table 1 Effect of nitrate and linseed oil supplementation on DMI, milk yield, gaseous emissions and rumen fermentation parameters in dairy cows fed a grass and maize silage TMR.

	Control	Linseed oil	Nitrate	SEM	P
DMI (kg/day)	16.5	16.0	15.1	0.59	0.085
Milk yield (kg FPCM/day)	22.9	21.5	22.0	2.16	0.799
Methane (g/d)	421[a]	407[a]	332[b]	16.3	0.015
Methane (g/kg DMI)	25.6[a]	25.6[a]	22.2[b]	0.82	0.040
Methane (g/kg FPCM)	18.6	20.4	16.1	2.33	0.302
Hydrogen (g/d)	0.9[b]	1.0[b]	3.2[a]	0.27	0.011
Total VFA (mM)	128.6	138.4	127.7	6.63	0.259
Acetate:Proprionate ratio	2.66[b]	2.48[b]	3.40[a]	1.14	<0.001

Conclusion
Feeding nitrate significantly reduced methane emissions per head per day and per kg DMI, but not per kg FPCM. Linseed oil did not affect methane emissions. Total VFA concentrations did not differ between control and nitrate treatments. However, the increase in acetate to propionate ratio and the increased hydrogen production suggests changes in the distribution of metabolic hydrogen between methanogenesis, nitrate reduction and the production of individual VFAs when feeding nitrate.

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Influence of time of access to pasture and provision of a total mixed ration on the intake, milk fatty acid profile and methane production of high yielding dairy cows

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Implications Compared to continuous housing, grazing high yielding cows will decrease methane output and improve the fatty acid profile of milk but decrease performance unless grazed during the daytime with access to a TMR when at grass.

Introduction High-yielding dairy cows are unable to achieve their nutritional requirements from grazing alone, leading some higher output dairy farms to house their cows over the summer period as they can feed a more consistent diet. Reducing access to pasture is, however, often perceived as being less welfare friendly. Additionally, grazed grass is high in polyunsaturated fatty acids such as C18:3*n*-3 which can improve milk quality and reduce methane production (Martin *et al.,* 2008). The objectives of the current study were to investigate the effect of time of access to pasture and the provision of a total mixed ration at pasture on the intake, performance, milk fatty acid profile and methane production of dairy cows.

Material and methods Sixty dairy cows that were 71 (s.e. ±9.2) days into lactation and yielding 39.3 (s.e. ±0.72) kg were used. Thirty cows were allocated to one of five groups of six cows in each of two periods of 35 d duration. Based on their milk yield and live weight, cows were allocated to one of five treatments: C: continuously housed and fed a total mixed ration (TMR); DGT: housed at night and grazed during the day with access to TMR at grass; DG: housed at night and grazed during the day with no access to TMR at grass; NGT: housed during the day and grazed at night with access to TMR at grass or NG: housed during the day and grazed at night with no access to TMR at grass. The TMR was the same for housed and grazed cows and contained (kg/kg DM basis) 0.409 maize silage, 0.112 grass silage, 0.103 wheat, 0.076 soyabean meal, 0.076 rapeseed meal, 0.076 wheat distillers dark grains, 0.067 soya hulls, 0.035 molasses, 0.021 chopped straw, 0.013 protected fat and 0.012 mins/vits. Cows were milked twice daily at 0530 and 1530 h and were weighed at the beginning and end of each period. Milk samples were taken during the final 7-d of each period for subsequent analysis and methane output measured during the final 5-d of each period using the SF_6 technique (Johnson *et al.,* 1995). Grass intake was estimated using the *n*-alkane technique. Data were analysed as a 2 x 2 factorial with a control, using Genstat (v14.1).

Results Total DM intake was highest in DGT and lowest in NG ($P<0.01$). When at pasture, access to a TMR increased intake by 3.9 kg DM/d whereas grazing during the day compared to night increased intake by 1.8 kg DM/d. Intake of grass averaged 1.3 kgDM/d and was highest in NG ($P<0.05$). Milk yield was similar in C and DGT, but was lower in all other grazed treatments ($P<0.05$). There was no effect ($P>0.05$) of treatment on milk fat content, but live weight change was lower ($P<0.01$) in cows that did not receive access to the TMR at pasture (DG and NG). Methane output (g/kg milk) was lower ($P<0.05$) and milk fat 18:3*n*-3 higher ($P<0.05$) in cows that had access to pasture, whereas milk 18:2*n*-6 was higher in milk in cows grazing at night than during the day ($P<0.001$).

Table 1 Effect of grazing time and access to a TMR compared to continuous housing on the intake, performance, selected milk fatty acids and methane production of dairy cows.

	Treatments						P-value			
	C	DGT	DG	NGT	NG	s.e.d.	In vs. Out[1]	Time[2]	TMR[3]	Int[4]
Total intake, kg DM/d	26.2	26.9	22.8	25.0	21.1	0.94	0.004	0.011	<0.001	0.817
Grass intake, kg DM/d	---	1.1	0.8	0.7	2.6	0.45	---	0.044	0.015	0.002
Milk yield, kg/d	38.6	38.0	35.3	35.9	33.6	1.21	0.003	0.033	0.005	0.838
Milk fat, g/kg	37.0	37.9	35.4	35.8	37.6	2.68	0.876	0.973	0.866	0.264
Lwt change, kg/d[5]	1.1	0.45	-0.11	1.1	-0.10	0.437	0.041	0.311	0.006	0.296
Methane, g/kg milk	14.6	12.8	12.7	13.0	13.0	0.906	0.023	0.732	0.963	0.977
Milk 18:2*n*-6, g/100g	2.45	2.25	2.37	2.72	2.50	0.119	0.912	<0.001	0.538	0.049
Milk 18:3*n*-3, g/100g	0.40	0.42	0.51	0.52	0.51	0.056	0.042	0.223	0.334	0.255

[1]Inside cows vs. all cows at grass; [2]Grazing at night time vs. day time; [3]Grazing with vs. without access to TMR; [4]Interaction between grazing time and TMR provision at grass; [5]Lwt = live weight

Conclusion Grazing during the day and with access to a TMR at pasture results in a similar level of performance to continuously housed cows but with a reduced methane output when expressed per kg of milk.

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A comparison of enteric methane emissions by Holstein-Friesian dairy cows and crossbred/Norwegian dairy cows

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Implications At similar levels of feed intake, Holstein-Friesian (HF) dairy cows have the potential to produce less enteric methane (CH_4) than either HF crossbred cows or Norwegian dairy cows.

Introduction In the UK, HF is the most commonly used breed in dairy production systems, while HF crossbred cows and Norwegian cows have increasingly been adopted because of their better performance in some functional traits (e.g., health and fertility). The present study used a large calorimetric dataset to examine if there was a difference in enteric CH_4 emissions between these different genotypes of dairy cows.

Material and methods In the current study, 935 lactating dairy cow data were collected from 32 calorimetric chamber experiments, with 823 observations taken for HF cows and 112 observations for other genotypes of dairy cows including Norwegian (n=50), Jersey × HF (n=46) and Norwegian × HF (n =16). The animals were offered either forage only diets (n = 66), or a mixture of forage and concentrates (n = 869). Energy metabolism data, including enteric CH_4 emissions used in the present study, were measured in indirect open-circuit respiration calorimeter chambers (Yan *et al.*, 2010). All data were analysed using one-way ANOVA with the effect of experimental variation removed. The statistical analysis was conducted using GenStat 14.2 (Lawes Agricultural Trust, Rothamsted, UK).

Results Diets offered to HF cows were similar to those given to other types of dairy cows in terms of forage proportions (555 *vs.* 552 g/kg DM), NDF concentrations (412 *vs.* 421 g/kg DM) or CP concentrations (179 *vs.* 180 g/kg DM). The effects of cow genotypes on animal performance, feed intake and CH_4 emissions are presented in Table 1. There was no significant difference in body weight, DM intake or energy corrected milk yield between the two groups. Genotype also had no significant effect on CH_4 emission per kg energy corrected milk yield or on CH_4 energy (CH_4-E) over ME intake. However, HF crossbred and Norwegian cows produced significantly more CH_4 per day, per kg DM intake and CH_4-E over GE intake, when compared with HF dairy cows.

Table 1 Effects of cow genotypes on animal performance and methane emissions [1]

	Holstein-Friesian	Other genotype	s.e.m.	P value
Body weight (kg)	559 (5.7)	547 (9.5)	7.6	0.142
Body condition score	2.54 (0.047)	2.76 (0.073)	0.06	<0.001
DM intake (kg/d)	16.5 (0.45)	16.8 (0.58)	0.51	0.449
Energy-corrected milk yield (kg/d)	21.8 (1.02)	21.4 (1.27)	1.15	0.631
CH_4 output (g/d)	361 (10.5)	388 (13.5)	12	0.003
CH_4/DM intake (g/kg)	22.3 (0.57)	23.3 (0.71)	0.64	0.02
CH_4/energy-corrected milk yield (g/kg)	18.5 (0.83)	20.1 (1.41)	1.12	0.191
CH_4-E/GE intake	0.067 (0.0017)	0.070 (0.0021)	0.0019	0.036
CH_4-E/ME intake	0.107 (0.0025)	0.111 (0.0034)	0.0029	0.154

[1] Data in brackets are s.d. values

Conclusion The present study demonstrated that, at similar levels of feed intake, HF cows had lower ratios of CH_4/DM intake and CH_4-E/GE intake than HF crossbred and Norwegian dairy cows. The results suggest that rumen methanogenesis potential may be influenced by cow genotype.

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The effect of concentrate feed rate on the performance of grazing dairy cows

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Implications Increasing feed rate from 0.25 to 0.45 kg concentrate/litre milk, resulted in an improvement in milk yield, while a further increase to 0.65 kg concentrate/litre milk did not increase the milk production of mid lactation cows.

Introduction 'Feed-to-yield' supplementation strategies, whereby concentrates are allocated to individual dairy cows according to their milk yield potential, may offer an opportunity to increase concentrate use efficiency within grazing systems. Concentrate allocation within these systems is influenced by the level of milk production assumed from grazed grass, and the quantity of concentrates offered per litre of milk produced. This study was designed to examine the impact of concentrate feed rate, when offered on a 'feed-to-yield' basis, on dairy cow performance.

Material and methods This study involved 138 Holstein-Friesian dairy cows. Multiparous cows (n= 84; mean lactation 3.3) had a mean calving date of 20 December (s.d. 74 days), while primiparous cows (n=54) had a mean calving date of 26 December (s.d. 65 days). Animals commenced grazing on 6 April (fulltime grazing achieved on 16 May) and were allocated to one of three treatment groups on the 29 May. During the period from 29 May through to the 8 June (study start date), concentrate feed levels for each individual cow were adjusted to the feed levels associated with each of the experimental treatments. Treatments comprised three concentrate feed rates, namely 0.25 (L), 0.45 (M) or 0.65 (H) kg concentrate (FW)/litre of milk produced above the assumed milk production potential of grazed grass (when offered as the sole feed). Daily concentrate allocations were split between two equal feeds, offered at each milking. Concentrate levels were adjusted every 2 – 3 weeks, with the assumed milk production potential of grazed grass for multiparous cows being 21, 16, 18, 16, 15, 15 and 12 kg/cow/day at 8 June, 22 June, 10 July, 24 July, 7 August, 20 August and 10 September, respectively (derived from Ferris *et al.* 2007). The values for primparous cows were assumed to be 20% lower. Fresh herbage was offered daily after evening milking, with herbage allocated to each group to achieve a common target post-grazing sward height. The area required for each daily herbage allocation was defined by temporary fences, with target grass allowances of 16, 14 and 12 kg DM/cow/day for L, M and H, respectively. These values were increased to 17, 15 and 13 kg DM/cow/day for L, M and H on 25 July, and remained at this level until the end of the study. Cows remained on the experiment until 3 October (117 days). Animal live weight and body condition score were assessed during the final week of the study. Data for average daily concentrate intake, milk yield, fat plus protein yield, milk composition, and for live weight and body condition score recorded at the end of the study, were analysed by ANOVA in a 3 (treatment) x 2 (parity) factorial design, including covariates as fixed factors within the model (Genstat, 14th Edition).

Results Concentrate intake, milk yield, milk protein content, milk fat plus protein yield and final live weight were higher with multiparous than with primiparous cows, while the reverse was true for body final condition score. However, there were no treatment x parity interactions (P>0.05) and as such only the main effects of treatment are reported (Table 1). Daily milk yield and fat plus protein yield increased as concentrate feed rate increased from L to M, with these variables similar between M and H (P>0.05). Concentrate feed rate had no effect on milk fat or protein composition, or on live weight at the end of the study (P>0.05). Increasing concentrate feed rate from L to H had no impact on body condition score at the end of the study, although body condition was lower within M compared to L (P<0.05).

Table 1 Effect of concentrate feed rate on dairy cow performance

	Concentrate feed rate (kg/litre milk)			SEM	P-value
	0.25	0.45	0.65		
Average concentrate intake (kg/cow/day)	1.6^{a}	3.0^{b}	4.5^{c}	0.24	***
Milk yield (kg/cow/day)	17.3^{a}	19.8^{b}	20.4^{b}	0.36	***
Milk fat (g/kg)	41.5	40.3	40.4	0.50	NS
Milk protein (g/kg)	33.3	32.9	33.0	0.22	NS
Milk fat + protein (kg/cow/day)	1.27^{a}	1.43^{b}	1.48^{b}	0.027	***
Live weight at end of study	568	560	573	3.6	NS
Body condition score at end of the study	2.29^{a}	2.18^{b}	2.25^{ab}	0.03	*

NS, not significant; ***, $P < 0.001$; *, P<0.05, Means with different superscripts are significantly different (P<0.05)

Conclusion When concentrates are allocated to grazing dairy cows on a feed to yield basis, a significant milk yield response was achieved when concentrate feed rate was increased from 0.25 to 0.45 kg/litre of milk. However, increasing the feed rate to 0.65 kg concentrate per litre milk had no further impact on cow performance.

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Does the 305 day lactation total reflect dairy cow production potential?

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Implications Yield comparators are vital in the assessment of dairy breeding stock at individual, herd, and breed level, they are also a major component of estimated breeding values across the world. The evidence provided in this study suggests that the currently used 305d milk yield may discriminate against shorter lactations which, by inference, is a bias against animals which encompass both high production and good fertility.

Introduction Whilst the focus of estimated breeding values has moved from purely production to wider phenotypic evaluation, the liquid element of dairy cow production remains one of the key comparators within the industry. 305 day lactation totals are the current international metric for yield evaluation, however, with the increasing use of short voluntary waiting periods, many cows do not complete the full 305 days. Consequently, these animals record significantly lower yields than equivalent animals completing longer lactations. This study presents results which suggest that a review of yield comparators may be required to ensure that individual animal records truly reflect their production potential.

Material and methods Lactation records of all Holstein Friesian animals calving in the year ending May 2013 were obtained from National Milk Records PLC. The data set was discriminated to include parities 2-8, age at first calving 20-40 months and animals completing more than 5 test day weighings. Due to the unreliable nature of service data, conception days post partum (pp) were calculated by counting back from the subsequent calving (Kadamideen *et al*, 2000). Calving intervals of less than 310 days were removed to reduce the influence of abortion or abnormal occurences and intervals of greater than 450 days were removed as they were not applicable to this study. Regression analysis was applied to establish a test day weighing window that would provide a suitable prediction of lactation total from daily yield (Table 1). This was found to be most representative between days 50 and 60 so the data was subdivided to reflect these results (n=45604) and interval analysis applied to investigate both 305 day lactation totals and milk per day of inter-calving interval (Table 2).

Results

Table 1

Conception cohort	Regression of total lactation against test day yield (50-60 days pp)	R - Sq	N
40-50 days pp	Lactation total (kg) = 998.2 + 193.2kg per kg test day yield (days 50-60pp)	73.20%	4831
70-80 days pp	Lactation total (kg) = 1575 + 193.1kg per kg of test day yield (days 50-60pp)	66.90%	4833
100-110 days pp	Lactation total (kg) = 1785 + 204.0kg per kg of test day yield (days 50-60pp)	65.10%	4430

Table 2

Test day yield cohort	30-35kg			35-40kg			40-45kg		
Conception cohort (days pp)	Mean 305 day lactation	Mean milk / day intercalving interval	N	Mean 305 day lactation	Mean milk / day intercalving interval	N	Mean 305 day lactation	Mean milk / day intercalving interval	N
30-49	7204	22.42	604	8192	25.44	654	9095	28.24	553
50-69	7564	22.31	1492	8582	25.3	1703	9470	27.9	1533
70-89	7814	21.99	1744	8832	24.83	1928	9754	27.44	1633
90-109	7986	21.81	1625	8986	24.53	1801	9987	27.3	1555
110-129	8072	21.52	1249	9088	24.2	1521	10043	26.77	1522
130-149	8190	21.42	1023	9183	23.99	1195	10142	26.53	1116
150-169	8298	21.22	800	9191	23.51	1026	10216	26.2	878

Conclusion The results indicate that animals which conceive very early in lactation show the highest levels of production when measured on milk per day of intercalving interval, as conception days pp increase there is a continuing decay in this measurement (range -5 to -7% across the yield cohorts). Conversely, as conception days pp increase there is a marked increase in the 305 day lactation total (range +12 to +15% across the yield cohorts). As conception days pp approach 85, this is driven by a continued increase of days in milk recorded until eventual completion of the full 305 days, after that point it is assumed that the continuing increase is due to the effect on lactation of later conception but this remains to be established. The results indicate a bias against those animals which are best suited to high yielding, intensive systems where strong production combined with early resumption of oestrus and subsequent conception are key to maximising output.

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Hepatic copper status and performance of dairy cows fed copper sulphate or a copper containing rumen bolus either without or with added sulphur and molybdenum

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Implications Antagonists to Cu absorption and metabolism decrease intake in diets containing 0.5 of the forage DM as grass silage and rapidly deplete liver Cu reserves, although provision of a Cu bolus may reduce the rate of decline.

Introduction Copper (Cu) is one of the most important trace elements for normal health and performance in dairy cattle (Suttle, 2010). Clinical symptoms in dairy cows are rarely caused by a dietary deficiency of Cu but more often related to interactions with antagonists such as sulphur (S) and molybdenum (Mo) which form thiomolybdates in the rumen and adversely affect absorption and normal Cu metabolism (Suttle, 2010). Previous research has identified that these antagonists have a more pronounced effect in diets based on grass than maize silage (Sinclair *et al*., 2013), although the effect of form of Cu is unclear. The objectives of the current study were to determine the effect of Cu source ($CuSO_4$ vs. bolus) fed either without or with added S and Mo on indicators of Cu status and performance in dairy cows.

Material and methods Fifty six Holstein-Friesian dairy cows that were 97 d (s.e. ± 5.7) post calving were used. Cows were fed a total mixed ration based on soya hulls, cereals, soyabean meal, rapeseed meal and malt distillers dark grains, 2nd cut grass silage and maize silage (in the ratio of 0.5:0.5 DM basis), and received Cu supplied either in the form of $CuSO_4$ (C) or from two slow release ruminal boluses (CoSeCure, Telsol Ltd., Leeds, UK; B) to supply 18 mg Cu/kg total diet DM. Basal Cu, S and Mo levels were 9.7 mg/kg DM, 2.5 g/kg DM and 3.1 mg/kg DM respectively. Each of the diets were either unsupplemented (-) or supplemented (+) with an additional 1.3 g S/kg DM and 7 mg Mo/kg DM. To account for the supply of Co and Se from the two boluses, animals being fed C- or C+ received an equivalent amount as Co carbonate and Se selenite respectively. Cows were milked twice daily at 06:00 and 16:00 h with milk yield recorded at each milking and samples collected fortnightly for subsequent analysis. Liver biopsy samples were taken from all cows during wk 0 and 14 of the study with blood samples collected via jugular venepuncture during wks 0, 2, 4, 8 and 14. Cows remained on study for 14 wks. Liver and blood plasma samples were analysed for Cu and Mo by ICP-MS. Data was analysed as repeated measures ANOVA using Genstat (v14.1) with main effects of Cu source (S), antagonists (A) or their interaction (I). Performance parameters recorded in the week prior to allocation were used as a covariate where appropriate.

Results Cows receiving added S and Mo consumed 1.3 kg DM less than those receiving the unsupplemented diets, although milk yield and fat concentration were not affected. Liver Cu levels declined on all treatments, but the rate of decline was greater with added S and Mo, and tended (P=0.09) to be less in cows receiving the rumen boluses. Hepatic Mo concentrations were higher (P<0.01) at the end of the study in cows receiving added S and Mo, although this increase tended (P=0.09) to be less in cows receiving the rumen bolus. Plasma Cu levels were unaffected (P≥0.80) by treatment.

Table 1 Intake, performance and indicators of Cu status of dairy cows supplemented with $CuSO_4$ or a Cu containing rumen bolus either without (-) or with (+) added S and Mo.

	Diets					P-values		
	C-	C+	B-	B+	s.e.d.	S	A	I
Intake, kg DM/d	22.8	21.1	23.0	22.2	0.84	0.324	0.041	0.438
Milk yield, kg/d	33.9	33.9	34.3	34.0	0.94	0.651	0.856	0.822
Milk fat, g/kg	42.2	44.0	43.8	43.0	2.61	0.866	0.778	0.494
Liver minerals								
Final Cu, mg/kg DM	550	432	586	476	31.6	0.087	<0.001	0.878
Cu change, mg/kg DM/d	-0.75	-1.95	-0.37	-1.50	0.323	0.087	<0.001	0.878
Final Mo, mg/kg DM	3.35	4.04	3.56	3.78	0.201	0.807	0.004	0.093
Plasma Cu, µmol/l	14.2	14.1	14.1	13.9	0.86	0.800	0.821	0.969

Conclusion The reduced intake of cows fed a diet containing 0.5 of the forage DM as grass silage with additional S and Mo confirms previous findings. The use of a Cu containing bolus may improve liver Cu levels compared to $CuSO_4$, and plasma Cu levels are not a useful indicator of Cu status.

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The performance of grazing dairy cows offered concentrates on a 'feed-to-yield' basis, when grazed grass is assumed to sustain either a 'high', 'medium' or 'low' milk yield

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Implications This study suggests that increasing the proportion of grazed grass in the diet of dairy cows will reduce daily milk yields and increase the amount of concentrate feed required for milk production in a 'feed-to-yield' dairy system.

Introduction Concentrate feed is frequently offered on a 'feed-to-yield' basis in dairy-cow grazing systems, with allowances being determined based on the difference between an individual cow's actual milk yield and the milk yield assumed to be sustained from grazed grass. However, there is little information available on the impact of the value adopted for the assumed milk yield sustained from grazed grass on concentrate requirements and cow performance. Thus, this study examined the effects of assumed milk yield sustained from grazed grass (MFG) on the performance of grazing dairy cows allocated concentrates on a feed-to-yield basis throughout the grazing season.

Material and methods Seventy-two (48 multiparous, 24 primiparous; mean stage of lactation of 154 (s.d. 55) days at the start of the experiment) Holstein-Friesian dairy cows were allocated to one of three treatment groups (16 multiparous and eight primiparous cows per treatment). The treatments comprised three MFG, namely high (HIGH), medium (MED), or low (LOW). For HIGH, grazed grass as the sole feed was assumed to be able to sustain the cows' maintenance requirements plus 25.2 kg of milk/cow/day on 24 May (start of experiment), with this decreasing to 13.6 kg of milk/cow/day by 30 September (end of experiment). The latter values for the HIGH treatment were based on milk-yield data presented by Ferris (2007). The MFG for the MED and LOW treatments were assigned as 0.85 and 0.70, respectively, of that of the HIGH treatment throughout the experiment, and the MFG for primiparous cows was assigned as 0.80 of that of the multiparous cows. Throughout the experiment, daily herbage allocation to each treatment group was determined based on the sum of the group's total metabolisable energy (ME) requirement for maintenance plus the sum of the group's total ME requirement to support the MFG, divided by an assumed herbage ME content. The concentrate requirement (offered in-parlour) for each cow was adjusted every 14 days (approximately) by calculating the difference between the cow's actual milk yield during the previous 14 days, and it's MFG (i.e. treatment) at that time. Concentrates were then allocated to each cow at a rate of 0.45 kg/kg of milk produced above the MFG. Cows were milked twice daily. Data were analysed by GenStat as a 3 (MFG) × 2 (Parity; primiparous or multiparous) factorial design, using analysis-of-variance. Appropriate pre-experimental variables were included as co-variates in the model when analysing corresponding dependant experimental variables.

Results There were no MFG by Parity interactions ($P > 0.05$) for any of the variables presented. Cows on HIGH had lower ($P < 0.05$) daily concentrate allocations, and lower ($P < 0.05$) daily milk yields, than those on MED or LOW, while MED was greater ($P < 0.05$) than LOW for both variables (Table 1). Cows on HIGH had lower ($P < 0.05$) total fat-plus-protein yields than those on LOW, whereas MED did not differ ($P > 0.05$) from LOW or HIGH. The final body condition scores (BCS) and final liveweights (LW) of the cows were greater ($P < 0.05$) for cows on LOW than for those on MED or HIGH, which did not differ ($P > 0.05$) from each other for either variable. The MFG had no effect ($P > 0.05$) on milk fat or protein concentrations. All concentrate and milk-yield variables, and final cow LW, were lower ($P < 0.001$) for primiparous than for multiparous cows. Parity had no effect ($P > 0.05$) on milk fat or protein concentrations.

Table 1 Effect of MFG on concentrate allocation, milk yield and composition, and final BCS and LW of cows

Assumed milk yield sustained from grazed grass	LOW	MED	HIGH	SEM	P-value
Daily concentrate allocation (kg/cow)	4.90[a]	3.17[b]	1.79[c]	0.216	<0.001
Daily milk yield (kg/cow)	23.8[a]	21.8[b]	19.0[c]	0.66	<0.001
Total fat plus protein yield (kg/cow; 129 days)	226[a]	202[ab]	186[b]	10.9	0.037
Milk fat concentration (g/kg)	43.6	44.1	44.9	0.55	0.253
Milk protein concentration (g/kg)	34.2	34.1	35.0	0.36	0.139
Final cow liveweight (kg)	579[a]	556[b]	547[b]	5.3	<0.001
Final body condition score	2.28[a]	2.15[b]	2.14[b]	0.350	0.017

Conclusion Increasing the yield of milk assumed to be sustained from grazed grass decreased the required daily concentrate allocation and daily milk yields of grazing dairy cows, but had no effect on milk composition.

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The effect of concentrate feed level on the rumen pH of grazing dairy cows

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Implications Increasing concentrates fed from 2.0 to 8.0 kg/cow/day had no effect on the rumen pH of grazing dairy cows.

Introduction Rumen acidosis (RA) occurs when rumen pH is <5.8, while acute RA occurs when rumen pH is <5.2 (Penner *et al*., 2007). While RA has been historically associated with indoor feeding regimes, this study was undertaken to examine the effect of concentrate feed level on the rumen pH of grazing dairy cows.

Material and methods Five rumen-fistulated Holstein-Friesian dairy cows were used in a three-treatment, three-period (each of 3-weeks duration) change-over design experiment, comprising two 3 x 3 Latin squares (one with a missing cow). Treatments comprised three concentrate feed levels (2.0, 5.0 and 8.0 kg/cow/day), with the daily concentrate allocation split equally during twice daily milking. Cows grazed as part of a much larger group of cows within a rotational paddock grazing system, with fresh herbage offered after evening milking. During the final four days of each experimental period, rumen pH was measured at 10 minute intervals using a pH meter placed in the cow's rumen, and which was weighted to ensure it remained below the fibre mat. Data was downloaded at the end of each period. For each of days 1 – 4, the average pH, minimum and maximum pH, hours when pH was <5.0, <5.6, <6.2, and mean hourly pH were determined, and mean values for the four-day period subsequently calculated. Data were analysed by GenStat using ANOVA taking account of the change-over design nature of the study, while hourly data were analysed by repeated measures ANOVA.

Table 1 Effect of concentrate feed level on dairy cow performance and rumen pH characteristics

	Concentrate level (kg/day)				
	2.0	5.0	8.0	SED	P-value
Milk yield (kg/day)	19.4	22.9	24.3	1.1	*
Live weight (kg)	579	586	595	3.1	**
pH data					
Average	6.1	6.2	6.4	0.18	NS
Maximum	7.0	7.0	7.3	0.17	NS
Minimum	5.4	5.3	5.5	0.15	NS
Hours <5.0	0.4	0.5	0.1	0.42	NS
Hours <5.6	4.2	3.4	3.2	2.15	NS
Hours <6.2	13.1	12.3	8.4	3.32	NS

Results Milk yield (P<0.05) and live weight (<0.01) increased with increasing concentrate feed level (Table 1). Neither average pH, maximum pH nor minimum pH were affected by concentrate feed level. The number of hours each day that pH was <5.0, <5.6 and <6.2 was unaffected by concentrate feed level (P>0.05). While hourly pH changed over the 24 hour sampling period (P<0.001), there were no effects of treatment, nor was there a treatment x time interaction on pH (P>0.05) (Figure 1). The main drop in rumen pH was observed after evening milking.

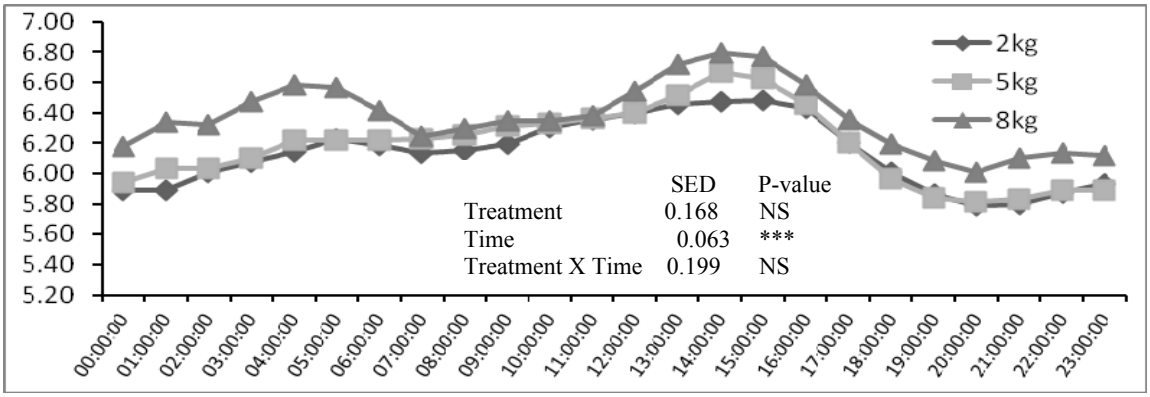

Figure 1 Effect of concentrate feed level on rumen pH over a 24 hour period.

Conclusion While a positive milk yield response to concentrate feed level was observed, concentrate feed level had no effect on rumen pH, with all measurements indicating low risk of RA.

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Effect of concentrate supplementation level on enteric methane emissions from grazing dairy cows

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Implications Offering concentrates to grazing dairy cows increased milk production per cow and decreased methane (CH_4) emissions per unit of milk. Increasing performance per cow by feeding concentrate can be used as a strategy by which to reduce CH_4 production per unit of milk produced.

Introduction Livestock farming, especially dairying, is a major contributor to atmospheric CH_4 accumulation. Having accurate information on CH_4 emissions from different livestock types, within different production system, is necessary to allow national greenhouse gas (GHG) inventories to be developed, and to identify more efficient and sustainable production systems. Factors influencing CH_4 emissions for dairy cows managed on confinement diets have been extensively researched, and robust prediction equations exist (Yan *et al.*, 2010). However, much less data is available describing CH_4 emissions from grazing dairy cattle. As dairy cattle graze for over six months of the year in many parts of the United Kingdom (UK), this represents a significant knowledge gap. The current study was designed to examine the effect of concentrate feed level on enteric CH_4 emissions from grazing dairy cows.

Material and methods Forty Holstein-Friesian dairy cows [12 primiparous and 28 multiparous (mean parity, 2.4 (±1.23 SD)) were blocked according to parity, days-in-milk, milk yield and live weight, and randomly allocated to one of four concentrate feed levels (2, 4, 6 and 8 kg/cow/day). The concentrate offered had a calculated metabolisable energy (ME) content of 13.0 MJ/kg DM and a crude protein content 206 g/kg DM. Cows grazed perennial ryegrass based swards, with full time grazing commencing 19 April. Each of the four treatment groups grazed separately. Enteric CH_4 production was measured on four occasions using the SF_6 technique (6 - 9 June, 26 - 30 June, 31 July - 4 August and 4 - 8 September), with cows a mean of 160, 182, 217 and 238 days in milk, respectively, at the start of each of these CH_4 measurement periods. A key grazing management target was that pre and post grazing sward heights were similar with all treatments (target 5.5 cm), with this relatively high post grazing height chosen so that herbage intakes would not be restricted within any treatment. Cows were offered their daily concentrate allocation in the milking parlour during milking, split between two equal feeds. Milk yield, live weight and milk composition for each cow was recorded daily during each CH_4 measurement period. Daily herbage DM intake was estimated by 'back calculation' for each cow, based on performance data. Total energy required for maintenance, production, tissue change, pregnancy and activity was determined using equations described in 'Feed into Milk' (Agnew *et al*., 2004), while herbage ME content was estimated using near infra-red reflectance spectroscopy. All data were analysed using REML with a repeated measurement mixed model in Genstat 14.2.

Results Energy corrected milk (ECM) yield increased with increasing concentrate level ($P<0.001$). Concentrate level had no effect ($P = 0.524$) on daily CH_4 emissions (g/d), while CH_4/DMI (g/kg) and CH_4/ECM (g/kg) decreased with increasing concentrate level ($P<0.01$ and $P<0.001$, respectively).

Table 1 Effect of concentrate level on performance, feed intake and CH_4 emissions

	Concentrate level (kg/d)				SED	*P*-value
	2	4	6	8		
LW (kg)	577	552	565	570	12.4	0.210
DM intake (kg/d)	14.5^{a}	14.2^{a}	15.5^{b}	15.4^{b}	0.42	0.007
ECM (kg/d)	21.1^{a}	22.8^{b}	24.8^{b}	25.1^{c}	0.75	<0.001
ECM/DMI (kg/kg)	1.46^{a}	1.59^{b}	1.59^{b}	1.63^{b}	0.035	<0.001
CH_4 (g/d)	287	273	272	277	11.1	0.524
CH_4/DMI (g/kg)	20.5^{a}	17.8^{ab}	18.7^{bc}	18.2^{c}	0.72	0.005
CH_4/ECM (g/kg)	14.1^{a}	12.5^{ab}	11.4^{b}	11.1^{c}	0.56	<0.001
CH_4-E/GEI (MJ/MJ)	0.059^{b}	0.057^{b}	0.053^{a}	0.054^{a}	0.0020	0.015

Conclusion Increasing concentrate feed level with grazing dairy cows increased ECM yield per cow, while decreasing CH_4 output per kg of ECM.

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Study of the plasma metabolome to describe rumen function and diet utilization in dairy cows fed differing in carbohydrate and protein concentrations.

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Implications Analysis of plasma using Fourier transform infrared spectroscopy (FTIR) and flow infusion electrospray Ionization mass spectroscopy (FIE-MS) are high-throughput techniques that could help nutritionists to predict the efficiency of N utilization in on-farm conditions and limit the use of cannulated animals.

Introduction Measurements of rumen fermentation, duodenal flow of nutrients and efficiency of diet utilization are important to optimize ruminant nutrition for efficient production. However, their determinations require rumen cannulated animals and laborious analytical procedures, making them unfeasible in on-farm conditions. Thus, there is a need for a simple and fast technique. The aim of this study was to investigate the potential use of blood plasma samples to provide an insight in ton the efficiency of diet utilization by dairy cows. Two high-throughput techniques, FTIR and FIE-MS, were used to investigate the plasma metabolome.

Material and methods To generate variability in the diet nutrient utilization, 4 Holstein cows (662±62kg BW), fitted with rumen and duodenum cannulas were fed with 4 different diets combining 2 protein levels (10.8% vs. 14.4% CP) and 2 energy sources (1.3 vs. 2.9 neutral detergent fiber (NDF)/starch ratio) in a 4x4 Latin square design. Detailed descriptions of rumen fermentation, microbial synthesis and N partitioning have been reported (Belanche *et al*., 2012, Fanchone *et al*., 2013). Blood samples (n=32) were taken just before and 2.5h after feeding. For FTIR analysis, plasma was deproteinized and infrared spectra were collected from 4000 to 600 cm^{-1} using an Equinox 55 FTIR spectrometer fitted with HTS-XT 96 well plate reader (Bruker Optik GmbH, Germany). For FIE-MS, deproteinized plasma was injected in a surveyor liquid chromatography system and data were acquired in altering positive and negative ionization profile modes on a LTQ linear ion trap (Thermo, Electron Corporation, US). Correlations between the plasma metabolic profiles and diet use parameters were determined by partial least square regressions (PLS) using Matlab (7.5.0, The MathWorks Inc, Cambridge). Prediction models were cross-validated and their fitting was assessed by determining the correlation coefficient (R^2), the root mean square error of the cross validation ($RMSE_{CV}$) and the ratio prediction:deviation (RPD).

Results Energy intake (in terms of DM, NDF and starch), rumen fermentation pattern (i.e. pH, NH_3 and volatile fatty acids (VFA) and duodenal DM flow were poorly correlated with the plasma metabolomic profile (R_{CV}^2 < 0.60). However, plasma profiles were well correlated with most of the parameters related to N metabolism such as N intake, duodenal N flow, milk N excretion, urinary N excretion, as well as with the efficiency of N utilization for milk production by the cow (R_{CV}^2 > 0.50). Duodenal flow of microbial N was poorly correlated with FTIR and FIE-MS spectra, possibly due to the limitations of these techniques to discern between microbial and non-microbial protein using plasma samples. Although both techniques generated similar relationships, R^2 values were slightly higher for FIE-MS than for FTIR.

	Range		FTIR spectroscopy			FIE-MS		
	Min	Max	$RMSE_{CV}$	R^2_{CV}	RPD	$RMSE_{CV}$	R^2_{CV}	RPD
Intake (kg DM/d)								
DM	18.6	21.8	0.96	0.25	1.01	0.85	0.27	1.15
NDF	6.76	10.9	1.78	0.16	0.83	1.59	0.04	0.93
Starch	2.82	6.8	1.74	0.01	0.93	1.58	0.10	1.03
Rumen fermentation								
pH	6.10	7.14	0.31	0.37	1.19	0.24	0.57	1.55
NH_3 (mg/l)	9.39	385	81.8	0.41	1.24	76.6	0.42	1.32
VFA(mM)	58.5	151	21.9	0.18	1.06	19.2	0.35	1.21
Acetate (%)	56.3	75.4	4.73	0.05	0.99	3.46	0.50	1.36
Propionate (%)	13.2	22.5	2.99	0.00	0.93	2.85	0.17	0.98
Butyrate (%)	7.89	21.1	1.97	0.18	1.41	2.53	0.21	1.10
Duodenal flow (g/d)								
DM	9894	15064	1553	0.04	0.97	1762	0.01	0.85
Non-ammonia N	213	467	47.0	0.61	1.57	45.8	0.61	1.61
Microbial N	211	493	78.3	0.02	0.84	58.9	0.25	1.11
N balance (g/d)								
N intake	0.30	0.46	0.03	0.65	1.68	0.03	0.73	1.94
Milk N	84.8	135	10.5	0.37	1.23	9.57	0.47	1.35
Faecal N	0.55	0.9	15.5	0.08	0.89	13.0	0.19	1.05
Urinary N	135	182	19.5	0.65	1.75	16.5	0.76	2.07
N use ratios								
Urinary N / N intake	0.05	0.10	0.04	0.59	1.68	0.03	0.70	1.85
Urinary N / Faecal N	1.71	2.84	0.01	0.79	2.20	0.01	0.75	2.01
Milk N / Urinary N	0.75	2.34	0.29	0.66	1.70	0.09	0.20	5.18
Milk N / N intake	45.9	152	1.77	0.71	1.83	2.03	0.62	1.60

*Grey cells indicate coefficients of determination above 0.5.

Conclusion FTIR spectroscopy and FIE-MS have potential to predict N partitioning by dairy cows based on the plasma metabolome profile. More research is needed to validate this observation using a greater number of samples and in different nutritional situations.

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Effect of cow genotype and concentrate feed level on enteric methane emissions from grazing dairy cows

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Implications Methane (CH_4) emissions per kg energy corrected milk was unaffected by cow genotype. This study provides no evidence of differences between cow genotypes in CH_4 emissions, at similar levels of milk output.

Introduction Globally, livestock systems represent a significant source of anthropogenic CH_4 emissions, with dairying making a significant contribution to this. While CH_4 production from dairy cows offered diets based on conserved forages have been extensively examined, much less information is available on factors influencing CH_4 emissions from grazing cows. In addition, while there is evidence of genetic differences between individual animals in terms of CH_4 production (Pickering *et al.*, 2013), there is relatively little information available on CH_4 production from cows of different genotypes, or from crossbred cows. With regards to the latter, interest in crossbreeding has increased in recent years as crossbred cows have been shown to have improved health, fertility and longevity, compared to pure bred Holstein-Friesian cows. Thus the current study was undertaken to compare CH_4 emissions from grazing Holstein-Friesian and crossbred dairy cows, when offered two levels of concentrate supplementation.

Materials and methods Forty dairy cows [12 primiparous and 28 multiparous (mean parity, 2.4 ±1.23 SD)] were managed on a 2 × 2 factorial design experiment. Factors examined comprised two dairy cow genotypes (Holstein-Friesian and a 'three-way' crossbred comprising Swedish Red × Jersey × Holstein-Friesian: 20 cows of each genotype) and two concentrate feed level (3.0 and 6.0 kg/cow/d). Cows grazed perennial ryegrass based swards, with part time grazing and full time grazing commencing on 16 May and 28 May, respectively. Cows grazed in two separate groups (10 Holstein and 10 crossbred cows) with all cows within each group managed on a single concentrate level. Within each genotype, cows within each group were balanced for pre-experimental milk yield (31.6 ± 6.53 kg SD), days in milk (112 ± 21.8 d), and lactation number. Cows grazed within a rotational paddock grazing system (24-hour paddocks), with paddock size being 0.22 ha and 0.20 ha, for the groups receiving 3.0 and 6.0 kg, respectively. Enteric CH_4 emissions were measured using the SF_6 technique during three separate measurement periods (18 – 21 June, 29 July – 2 August and 10 – 14 September, respectively). Cows were milked twice daily, with the daily concentrate allowance offered in the milking parlour during milking, split between two equal feeds. During each CH_4 measurement period, milk yield, milk fat, protein and lactose content, and cow live weight, were measured daily, while daily herbage DM intake was estimated by 'back calculation' for each cow, based on performance data. Within the latter calculation total energy required was determined using published equations, while herbage ME content was predicted using near infra-red reflectance spectroscopy. All data were analysed using REML with a repeated measurement mixed model in Genstat 14.2.

Results Energy corrected milk yield (ECM) increased with increasing concentrate feed level, but was unaffected by genotype. Increasing concentrate level had no significant effect on daily CH_4 emissions ($P = 0.177$), CH_4/DMI ($P = 0.058$) and CH_4/ECM ($P = 0.120$). Crossbred cows had higher daily CH_4 emissions than Holstein cows ($P < 0.05$), while CH_4/ECM (g/kg) was unaffected by genotype. There were no interactions ($P > 0.05$) for any of variables examined.

Table 1 Effect of concentrate level and genotype on animal performance, feed intake and CH_4 emissions

	Concentrate level (kg/d)			Genotype			*P*-value		
	3.0	6.0	SED	Holstein	Crossbred	SED	C	G	C×G
LW (kg)	505	522	18.0	534	492	18.0	0.363	0.016	0.725
DMI (kg/d)	15.4	17.1	0.67	16.5	16.0	0.68	0.018	0.439	0.650
ECM (kg/d)	20.3	23.2	1.27	21.6	21.9	1.28	0.023	0.940	0.284
ECM/DMI (kg/kg)	1.32	1.34	0.034	1.31	1.36	0.035	0.556	0.145	0.121
CH_4 (g/d)	242	255	9.6	238	259	9.8	0.177	0.037	0.761
CH_4/DMI (g/kg)	16.2	15.0	0.65	15.0	16.2	0.68	0.058	0.026	0.206
CH_4/ECM (g/kg)	12.5	11.4	0.70	11.7	12.1	0.72	0.120	0.350	0.074
CH_4-E/GEI (MJ/MJ)	0.047	0.045	0.001	0.043	0.048	0.001	0.053	<0.002	0.717

Conclusion Increasing concentrate feed level had no significant effect on CH_4/ECM. Although total CH_4 emissions were higher with crossbred cows, when expressed per kg ECM, CH_4 emissions were unaffected by genotype.

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Preparing an agri business strategy (plants) for the challenges of 2020

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Rising food demand and the challenges of fulfilling this demand in sustainable ways for a growing and increasingly prosperous global population within the limitations of our planet has been widely recognized as a major task for mankind. In particular after the perceived food crisis in 2008, progressing against this task has become a top item of the political agenda and of civil society.

Agri businesses will have to make an essential contribution to achieving this task, enabling growers across the world to deliver significant improvements in crop yield and quality by providing leading-edge technologies and appropriate education. Syngenta is a world-leader in this space with more than 27,000 people working on "bringing plant potential to life".

Syngenta has the ambition to achieve global food security in an environmentally sustainable way by creating a step-change in farm productivity. Every farmer has a role to play in this. For Syngenta, delivering on this ambition will also secure reaching revenues of 25 billion dollars by 2020.

Syngenta's strategy puts the farmer in the center by "thinking like a grower at scale", to *integrate*, *innovate*, and *outperform*. For about 3 years now, we have moved from a more traditional product technology-driven approach that helped to solve narrow grower problems towards a customer-needs focused strategy in which integrated solutions play a crucial role. These are more in sync with how growers think and work and deliver more value on the farm and beyond the farm.
Syngenta addresses the six key areas of grower needs, mainly in biotic and abiotic stresses and in terms of yield and quality. We have the broadest technology portfolio in the industry and deliver this against eight crop areas that represent the majority of farmed produce. We also have a consumer business targeting lawn and garden, bringing innovation into turf, golf courses and flowers.

Syngenta has developed a three-level approach to integrated solutions, ranging from cross-selling through combining technologies to provide yield, quality and convenience benefits to breakthrough innovation that transforms agriculture and the way crops are grown. R and D is focused particularly on the latter two levels and specific examples in crops such as wheat or rice illustrate the powerful impact of integrated solutions.

A lot of Syngenta's success relies on the passionate people we have and their collective capabilities that span the broadest palette of technologies and crops in the industry. In 2013, about 1.4 billion dollars of R and D spent have driven a company with close to 15 billion dollars of sales. The R and D organization bases its creativity and innovation not only on the in-house work but also engages broadly with academic institutions and industry partners.

For an agri business company like Syngenta, one of the biggest challenges resides in the increasingly unpredictable regulatory system, where the demands are becoming less about science and more about politics, accelerating over the past five years. The arguments are shifting from a benefit/risk assessment towards a "hazard" review, getting in the way of putting modern agricultural technologies into the hands of farmers.

Syngenta is fully committed to address the food security agenda and deliver it through sustainable agriculture. To that end, we have announced our Good Growth Plan in September 2013: we will deliver against six major food security and sustainability challenges by 2020, which include commitments for essential areas such as crop productivity, biodiversity, smallholder success, and labor safety. Our intention is to make a deep, lasting, and positive impact on farmers and rural communities to ensure the world's food security and one the long-term sustainability of our planet.

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Opportunities and challenges in animal nutrition

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Introduction Livestock production will increasingly be affected by external factors. These include surging demands for animal products and struggling supplies of feed raw materials, resulting from the competition for natural resources and trade barriers. Simultaneously, there is growing concern about food and its impact on health, and the impact of production systems on animal welfare and the environment.

Results Optimization of productivity and efficiency within such constraints are important objectives, as well as maximization of the profit for all stakeholders. Animal feed and nutrition are the essential link in the livestock production chain, i.e. between crop cultivation and animal protein production and processing. It is usually the biggest cost factor in livestock production. Several indicators demonstrate that further optimization of animal feed and nutrition is potentially still possible. The genetic potential is only partially utilized. The utilization of most nutrients appears to be low and there is a huge variation in performance among farms and, within farms, among animals. In addition, environmental performance can be improved significantly. New science and technologies seem to offer many opportunities for innovation in animal feed and nutrition. Key drivers for future innovation are basically (gen)omics, microsystem- and nanotechnology and information and communication technology (ICT).

Nutrigenomic technologies, such as genomics (DNA level), transcriptomics (gene activity at mRNA level), proteomics (protein level), metabolomics (metabolite level) and epigenetics (phenotype and gene expression level caused by mechanisms other than changes in the underlying DNA sequence) enable to refine measurements of performance indicators and also on cell and tissue level, in particular in combination with bioinformatics and systems biology. The end result will be a precise determination of the nutrient requirement of an animal under the specific conditions, e.g. production phase, health status, farm management, and environment and social interaction. Alternatively, nutrigenomics also studies how feeds (nutrients, additives or other compounds) affect genes and gene expression. In animal nutrition, the practical application of these scientific developments is already showing results on-farm. Studying the effect of functional ingredients on the intestinal microbiota for instance, has led to feed additives that can support the normal microbiota balance of weaned piglets. This application has showed improvements in piglet performance and can help farmers reduce their need for antibiotics. Another development in nutrigenomic science, called metabolic programming, has shown that young animals can be prepared to adapt to the environment to which they are expected to be exposed. This means in practise that, with optimal nutrition, young calves for example can be metabolically programmed to become high producing dairy cattle.
Concerning microsystems, there are many potential applications of for instance biosensors of various types in livestock production. In particular, the monitoring of macro- and micronutrients, additives, pathogens, contaminants and toxic metabolites in- and outside the animal may offer the possibility to fine tune performance in a well-controlled environment. Moreover, it is a powerful tool in research. Finally, for information and communication technology (ICT), farm automation and full system control and near-infrared spectroscopy are examples. ICT is also the basis for dynamic predictive livestock modelling programs. Such tools not only simulate nutrient requirements but also simulate responses to physical, social (health and feeding behaviour), economic and environmental changes. The new generation of predictive models focuses on nutritional optimization whereby solutions (e.g. requirements) are no longer static values solely based on biological responses (e.g. maximizing lean gain) but can also be expressed in terms of economic responses.

These three mainstream technologies are the foundation of many application technologies of relevance for animal feed and nutrition. Even so, acceptance by consumers and society is a critical success factor. Managing consumer and societal acceptance definitely needs openness and transparency. Communication with interested stakeholders, corporate reporting and building relationships with citizens and organizations will be as important as the implementation of new science and technologies.

Conclusion In conclusion, animal feed and nutrition are crucial in livestock production. Innovations have the potential to meet the challenges and to result in resource efficiency, healthy livestock and people, responsible production systems and optimal profit throughout the value chain.

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Social challenges facing animal industry and business in supporting global food provision

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Implications There is no societal agreement on the future of animal production systems, and their role in meeting the global food challenge. Research processes and innovation policies will have to be re-oriented in order to support socio-institutional innovation and arrive at future visions and options that can count on sufficient societal support.

Introduction For a long time, innovation in animal production systems has been regarded primarily as a technical affair. However, progressive insight in innovation studies has revealed that meaningful innovation is often a highly politicised process, in which technical innovation may or may not be effectively linked to innovation in the social-organisational and institutional sphere. In line with this way of thinking, this paper presents some of the social challenges that need to be considered. Subsequently, it reflects on the limited ability of agricultural innovation systems to address such challenges, and identifies possible ways forward in re-orienting the management of research and innovation trajectories.

Results Social challenges facing participants in animal-protein food chains can be found in different spheres and arenas, ranging from global debates on agricultural production systems, to national policy and politics, to consumer/citizen preferences and perceptions, and farmer-level considerations and possibilities.
First of all, there is by no means agreement on what 'the global food challenge' is, and how it needs to be addressed. Is it a problem of too limited production? Or of distribution and waste? Should we accept that diets in developing countries will evolve in the same way as in Western countries? Or direct ourselves to protein production systems that are much more efficient? At national level we see that countries have different agendas and that there is competition between different value systems. We witness the emergence of strong social movements around 'food sovereignty', 'animal welfare' or 'sustainability', and often these value systems clash with the models for intensive animal production that have come to dominate the European landscape. In the meantime, consumer preferences are highly diverse, and especially elite consumer-citizens increasingly demand guarantees that food is produced in line with their value systems. At the same time, there is a considerable level of distrust in agri-business companies and science, and consumer-citizens are well able to organise public pressure through conventional and social media. While farmers across the world operate in very different circumstances, a common denominator seems to be that their space for manoeuvre is rather limited. In the North, previous investments cause path dependencies that make it difficult for farmers to change direction. And in Southern countries, agricultural development is frequently prevented by failures in the institutional environment, for example in the sphere of land-tenure, pricing systems, credit provision, transport and marketing.

Conclusion Meeting global food challenges will not only require technical innovation, but also social innovations that address institutional constraints to meaningful change. Moreover, stakeholders and scientists involved in pursuing innovation will require abilities to handle and navigate disagreement, tension and competing value systems. Agricultural innovation systems are not necessarily well equipped to deal with the social and political dimensions of innovation. All too often science and research operate in relative isolation of the societal dynamic, and propose technical solutions without considering the social complexity. Subsequently, such solutions meet with resistance and contestation. As an alternative, it is proposed that meaningful innovation happens in society, and is unlikely to be steered by science and research. In order to have impact, science and research must somehow connect with the on-going dynamic, plug-in to the everyday conversations among citizens and in policy circles, and through this route enter societal decision-making. This may be operationalised through platforms for collaborative research and innovation. Such platforms can usefully engage in three types of research:
Characterising changing selection environments – This type of collaborative research is more diagnostic in nature, and serves to find promising entry-points for further investigation, vision development and action.
Creating variation: engaging in societal experimentation with multiple socio-technical options –A second type of research is geared to creating more variation in society. In order to be adaptive and create space, society needs to experiment in the field with multiple (combinations of) social and technical options, that may be located at different levels.
Studying existing diversity: understanding on-going space creation - In any context society is not waiting for science to come by and make its contribution. Change is already in-the-making. Thus, as a third strategy, science and research can usefully identify and study existing, and aim to understand what is special about cases that stand out positively. And subsequently underlying principles may be further tested, enriched and adapted into feasible social and technical options for others.

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Embracing Technology

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My background was in arable farm management and in particular vegetable production where technology, especially imported from mainland Europe was an everyday occurrence.

Following a change in career and the purchase of a very much traditional 1960's broiler farm it didn't take long before I was able to see the scope for technology in my new broiler business. Redeveloping the farm allowed the influx of a whole range of ideas, some born from existing developments in the industry (automatic scales, feed and water monitoring, *etc*) and some from other experiences that I felt had a place on the farm (underfloor heating, webcams, *etc*). As my experiences grew and my relationship with the technology manufacturers grew a new relationship was born where my site became a test bed for new ideas. This continued for a couple of years until we have arrived at the current situation where technology and computers are not only running my farm but also assisting in bird welfare monitoring on a daily basis.

I believe my lack of industry experience has helped me to evaluate what could be done without any restrictions on my thoughts/ ideas and once this was coupled with a company's desire to develop this type of technology (Fancom in Holland) we together have took significant steps. The projects continue to develop and the latest research is part of the European approach to asking how can technology improve efficiency and help food security going forward.

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Is there any link between herd size and welfare of pigs?

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Implications Sow and slaughter pig herds were visited and animal based welfare measurements were collected which were aggregated into welfare indices. The association between herd size and the welfare index score were investigated for sow and slaughter pig herds, respectively. No association was found.

Introduction Structural development in pig production over the last 30 years has led to increasing sow and slaughter pig herd size in Europe. Increasing herd size, is in the view of the citizens, expected to compromise animal welfare. The objective of this paper was to investigate the association between herd size and animal welfare based on aggregated animal welfare index scores at herd level.

Material and methods A total of 64 sow herds and 37 slaughter pig herds were visited once during 2011 and 2012. The sow herd size varied from 50 – 2500 sows with a mean of 494 sows. The herd size of the herds producing slaughter pigs varied from 650-34094 produced pigs per year with a mean of 7824 produced pigs per year. On each farm, an on-farm welfare assessment (inspired by Welfare Quality®) based on a clinical examination and animal behavioural observations as well as a farmer interview were conducted. Using weights derived from expert opinion panels, and the frequencies of the measures from the on-farm welfare assessment herd specific animal welfare indexes (AWI's) were calculated. Thus, AWI were based on simple summation of the weighted frequencies of measures. The herd specific animal welfare index scores were plotted against respective herd sizes, and a general linear regression analysis was conducted.

Results The herd specific AWIs were plotted against respective herd sizes and a general linear regression analysis was conducted. The results showed no significant associations between herd size and AWI score in neither sow (P=0.80) nor slaughter pig herds (P=0.39). The result indicates that the herd size is not associated with the herd welfare on sow and slaughter pig herds. Other risk factors of poor welfare on herd level as management routines and housing conditions maybe have a bigger impact on herd level welfare than herd size.

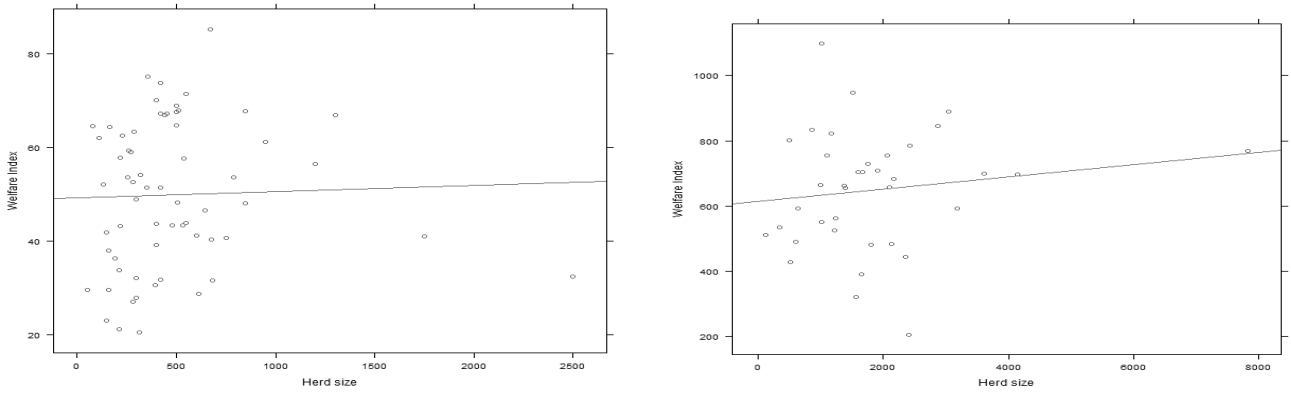

Figure 1 The plot to the left is sow herd herd size plotted against 'Sow welfare index' with a trend line and the plot to the right is slaughter pig herd size plotted against 'Slaughter pig welfare index' with a trend line

Conclusion There were no significant, simple relation between herd size and animal welfare index score in neither sow nor slaughter pig herds.

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Can the UK learn to love mega-farming? A study of economic, environmental, animal welfare and social outcomes, and their impact on planning, politics and PR

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Few issues have divided opinion more starkly in recent times than increasing scale and intensification of livestock production. Depending on which side of the divide one sits, it is either the logical and inevitable evolution of an industry, driven by unrelenting technological and socio-economic factors, or the elevation of corporate greed above concerns for the environment, animal welfare and the wider interests of society.

The highly partisan nature of this debate was brought into sharp focus in 2009 with the proposal to house up to 8,100 high-yielding dairy cows year-round on a dairy farm built on a greenfield site at Nocton in Lincolnshire. The proposal generated a level of resistance never before seen in the UK. At the same time a proposal by Midland Pig Producers to develop a 2,500 sow breeder-finisher pig unit at Foston in Derbyshire met a similar outcry. These applications exposed not only the strength of feeling in some quarters with regard to the acceptability of intensive livestock production but also the lack of awareness among the wider consuming public about how food is produced today and the challenges the agricultural industry faces with regard to delivering a sustainably-produced, affordable food supply tomorrow.

In 2012, the 'Can Big Be Beautiful' report written by David Alvis, Amy Jackson and John Allen and published by the Worshipful Company of Farmers, examined whether large scale and/or intensive livestock farming could be sustainable, using the dairy sector as a case study.

In 2013, Amy completed her Nuffield Farming scholarship asking 'Can we Learn to Love the Megadairy? – Planning, Politics and PR'. This latest report examines whether scale and intensification are necessary and why they are happening, whether the economic, environmental and animal welfare arguments stack up, and how the UK agriculture industry can improve the acceptability of larger scale farming among planners, communities, the public and other farmers.

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Precision sheep management – new approaches and future development

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Implications The development and implementation of precise sheep management show great promise and could provide farmers with exciting tools to tackle the future challenges of farming lying ahead.

Introduction Hill Sheep farming in north west Europe has not dramatically changed over the last two centuries. However, with the mandatory introduction of low frequency electronic identification (EID) in the European Union, there is an opportunity for hill sheep farmers to improve their management and to turn the burden of EID into an opportunity. At SRUC's Hill and Mountain Research Centre, there is a 5-year research programme implemented to investigate Precision Sheep farming (Umstatter, *et al*. 2013). Two main strands are being examined; one is focusing on breeding ewes and one on growing and finishing lambs. In addition, further future developments are coming up on the horizon and are briefly explained.

New approaches and future development The first strand is a more precise winter feeding programme for ewes based on individual weight changes throughout the winter. It also integrates the Ultrasound scanning results for pregnant ewes in order to aim to optimise feeding. The ewes are kept in fields or on fenced enclosed hill area supplementary feed is provided to batches grouped according depending on in the weight change category and pregnancy status. From pre-mating through to lambing, the ewes are brought in in a roughly 4-weekly interval to be weighed and then allocated to their optimal feeding group. The aim is to reduce the wide range in weight change experienced under conventional systems and to avoid overfeeding and underfeeding.

The second strand deals with Targeted Selective Treatment (TST) for worm control in the lamb. Traditional worming strategies can become ineffective, leading to severe problems with anthelmintic resistance and poor growth performance of lambs. The use of an anthelmintic wormer to treat against a mixed population of worms that are resistant and non-resistant to the drug, just leads to the resistant worms taking over and lambs being infected with greater and greater numbers of worms for which the treatment does not work. Therefore, the new strategy entails only worming the lambs that do not thrive and need treatment, thus retaining anthelmintic susceptible worms in the wider parasite population and slowing the development of resistance. In order to be able to identify the individuals that require worming, lambs need to be weighed regularly. Initial experimental studies on TST, run by Moredun Research Institute, were based on fortnightly weighings. As this would not be practical on a commercial hill sheep farm, monthly intervals are being investigated at the farm system scale, in terms of suitability for the TST approach. An algorithm calculates expected (targeted) growth rate for each individual lamb, which is then compared to their actual growth rate and underperforming lambs are selectively wormed.

The TST approach becomes workable due to the use of EID and an automatic weighing and drafting system. Additional economic and labour measurements are currently collected in order to be able to evaluate the viability of the precise sheep management within a farming system context.

There is also a new technology coming up in the EID area which could potentially lead to further improvements for sheep farmers. It is currently mainly discussed in conjunction with cattle and pigs in Europe and it is not part of the legislation but this might change in future. The main benefits of ultra-high frequency (UHF) EID are the reading speed, the ability to read several transponders simultaneously and the reading distance of up to 5 m for UHF ear tags. The capacity to write data, such as management information or even the movement history onto the UHF tags is suitable. There is a fast development of memory size currently ongoing and it will be improved substantially in the near future. The use of UHF technology in livestock farming could have a large impact on labour savings and improved ease of documentation and traceability.

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Applying precision technologies to the on-farm measurement and management of grazing

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Implications Although intensive livestock production systems have been the first to adopt precision technologies, there is potential to develop commercially viable monitoring and control technologies that can help farmers manage grazing.

Introduction Grazing provides a cheap source of feed for ruminant livestock, and it is perceived by the public as being both natural and welfare-friendly. However, the inability of farmers to measure how much forage is consumed by grazing livestock means that grazing is often seen as an "act of faith", and methods to measure grazing are needed to facilitate an improvement in the management of grazing. Researchers have developed a variety of techniques to measure grazing behaviour, and this paper reviews the potential of these technologies for use as commercial, on-farm decision-support tools.

Recording grazing behaviour Penning (1983) reviewed early attempts to automatically record grazing, including vibration sensors to detect *head motion*, tip switches to detect *head position* and submandibular pressure sensors to detect *jaw movements*. None were wholly satisfactory, so Penning (1983) devised an elastic noseband sensor that changed in electrical resistance as the jaws opened and closed, with the resultant signal being recorded on analogue cassette tapes. This approach was later updated to use digital recording (Rutter *et al.*, 1997), and sold around the world as a commercial grazing recorder for research scientists. However, this type of noseband sensor is too fragile for use on farms. More recently, Oudshoorn *et al.* (2012) showed that head mounted accelerometers can be used to estimate grazing time and intake with a precision between ±1.2 and ±1.4 kg DM/cow/day. An alternative approach to recording grazing is to use bioacoustics i.e. record and analyse the sounds ruminant livestock make when they graze (Laca *et al.*, 1992). Automatic algorithms are now available to discriminate between bites and chews in acoustic signals (Milone *et al*., 2012), and the energy density of chewing sounds is proportional to bite mass (Laca and WallisDeVries, 2000), giving the potential to measure herbage intake (in terms of kg DM). There is also evidence that bioacoustics can be used to determine plant species being eaten and quality of ingested herbage. One potential issue with the acoustic monitoring of grazing is that the sensor on one animal can inadvertently detect the sound of grazing from an adjacent animal (Unger and Rutter, 2006). One way to overcome this problem would be to use an accelerometer alongside the acoustic sensor, and any acoustic signals not accompanied by the appropriate head movements could be ignored. The feasibility of using bioacoustic sensors in on-farm livestock monitoring has already been demonstrated in a commercial rumination monitor (VocalTag, SCR Engineers Ltd, Netanya, Israel).

Complementary technologies to help manage grazing Although measures of grazing time and intake on their own should be of use to farmers, their integration with other technologies could add further benefits. Aerial imagery (either from satellites or unmanned aerial vehicles) is already being used by Australian rangeland famers to estimate herbage availability and help them decide where to graze their stock (Hill *et al.*, 2004). Automatic segregation gates, remote-release gates and robotic moving electric fences can be used to automatically control access to pasture, and these could be linked to grazing sensors to allocate fresh pasture only when appropriate. Measures of grazing could also be integrated with "virtual fencing" technology (Umsatter, 2011) to allow the dynamic control of grazing by a "virtual shepherd" (Rutter, 2012).

Conclusion A combination of bioacoustics and accelerometry appears to offer the greatest potential for the on-farm monitoring of grazing, giving measures of grazing time, ratios of bites to chews and an estimate of herbage intake and quality.

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EU Projects on Precision Livestock Farming

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Introduction The worldwide demand for meat and animal products might increase by 40% in the next 15 years. A question is how to achieve high-quality, sustainable and safe meat production that can meet this demand. At the same time, livestock production is currently facing serious problems such as animal health in relation to food safety and human health. Europe wants improved animal welfare and has made a significant investment in it. At the same time, the environmental impact of the livestock sector is far from being solved. Finally we must ask how the farmer, who is the central figure in this process, will make a living from more sustainable livestock production.

Material and methods One tool that might provide real opportunities is Precision Livestock Farming (PLF). PLF systems aim to offer a real time monitoring and managing system for the farmer. This is fundamentally different from all approaches that aim to offer a monitoring tool without improving the life of the animal under consideration. The idea of PLF is to provide a real-time warning when something goes wrong so that immediate action can be taken by the farmer. Continuous, fully automatic monitoring and improvement of animal health, welfare, yields and the environmental impact should become possible. This paper presents examples of systems that have already been developed in order to demonstrate the potential of this technology.

Results Several examples are given of PLF systems that are operational today in about 60 compartments for pigs and broilers all over Europe. The paper gives details of which variables these systems measure in real time in a fully automated way. Moreover we show how in the running EU-PLF project we analyse how these data can generate added value for the farmer.

Conclusion PLF systems can replace the ears and the eyes of the farmer and work 24 h a day and 7 days a week. The challenge now is to show how the farmer gets an advantage from these systems as we start to see in the EU-PLF project. Collaboration between “animal people” (physiologists, veterinarians, ethologists, *etc*.) and technical people is needed to make these systems become real support systems for farmers.

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Study on the feasibility of udder conformation measures in sows

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Implications The increase in sow prolificacy over the last decade, as a result of genetic selection strategy in specialised damlines, now poses challenges for piglet survival. Improved udder conformation in sows might reduce suckling latency and reduce neonatal mortality.

Introduction Morphological and genetic studies on the sow udder are few and are focussed on teat functional numbers and mammary gland characteristics in terms of milk production (Farmer and Sorensen, 2002; Chalkias *et al*., 2013). In contrast, udder conformation, in cows, sheep and goats has been well studied, since this determines the aptitude for machine milking. In sows there is only one study on udder morphology, aimed to determine genetic parameters of mammary gland firmness in relation to milk production (Aziz *et al*., 1995). To date, no study has been done on the associations between udder conformation and piglet survival and milk production. Therefore, the aim of this pilot project was to develop practical measures of udder conformation to use in studying the correlation between these traits and piglet survival, health and lifetime performance. For each measure, four questions were addressed: 1) Is the measure repeatable, 2) are measures from the left and right side closely related, 3) is there a typical conformation for each sow and significant variation between sows and, 4) does the measure differ in a systematic way over the days before and after farrowing.

Material and methods 24 sows were scored for 8 conformation traits on each side of the udder measured twice a day (repeated measures: morning and afternoon) every day from the sows' entrance into the farrowing crates (Monday) until farrowing (1-4 days later). To evaluate the effects of posture the measures were taken from both sides when the sow was lying down and when she was standing up. To evaluate teat position, 5 measures were taken: distance between the two rows of teats (SR; mm between the equivalent teat bases); distance from the base of the teats in the upper row to the abdominal mid-line (BLU); distance from the base of the teats in the upper row to the ground (FLU); distance from the teat bases of the lower row to the abdominal mid-line and distance from the teat bases of the lower row to the ground. When the sow was standing up, 3 distances were recorded: between teat tips and the ground (FL), between the tip of the pairs of teats from one row to the other (OR), and distance from the anterior leg - a measure of the fore udder abdominal attachment to the ligament of the leg on both sides. Teat size was evaluated through 2 measures: length (L) was measured from the tip to the base and diameter (D) was measured at the tip of the teat.

Results Measures of udder conformation were repeatable within sow and showed significant ($P<0.001$) variability between sowand did not differ significantly between sides, in either standing or lying posture. The absolute difference between the mean of the measurements taken from left and right side were: FL 3%, SR 6%, L 5% and D 4%. Regression analysis between the same traits taken in a standing and lying down position show that is possible measure the trait on one posture to predict the measurements in the other posture (SR $r^2=0.61$, $p<0.001$; OR $r^2=0.64$, $p<0.001$). The results did not change significantly in the days shortly prior to farrowing (effect of day in linear mixed model with sow as random factor, $P>0.1$ for all measures). Measures which used anatomical landmarks as the reference point were more reliable than those using the floor of the pen (Inter-class correlation BLU=0.83, FLU=0.79).

Conclusion Udder conformation measures can be used as a reliable phenotype for further study. They can be collected on any day shortly before farrowing, and only from one side and in one posture to save time. Further studies are necessary to define how these udder conformation traits influence piglet suckling behaviour, survival and performance, and whether these traits are heritable and correlated with other important production traits such as prolificacy and milk production.

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The effect of supplementing piglet creep feed with Propyl Thiosulfinate (PTS) and Propyl Thiosulfinate Oxide (PTSO) on piglet and sow performance

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Implications The addition of PTS and PTSO to piglet creep diets improved weaning weights which was related to a significant increase in feed intake in the week before weaning.

Introduction The efficiency of production on pig farms can be related to post-parturitional growth efficiency (Houde *et al*., 2008) and piglet pre-weaning performance (Panzardi *et al*., 2013), subsequently affecting post-weaning performance (Quiniou *et al*., 2002). Since antibacterial growth promotors (AGPs) were banned in 2006, searches for alternatives have lead to phytochemicals, or plant extracts. Multiple secondary metabolites present in garlic (*Allium sativum* L.;Tsai *et al*., 2012) have been shown to express antimicrobial properties. However, with no research having been carried out on the effect of PTS and PTSO on piglet performance and general litter health prior to weaning, the objectives of this study were to determine the effects of PTS and PTSO on piglet performance and health status.

Material and methods Twelve litters were used in the trial, six litters were used as control groups (CON), subject to a control diet, Easiwean PLT (BOCM Pols, England). The other six litters were subject to PTS and PTSO (GAR) in the form of XTRACT® Allium XL X60-7037 (Pancosma, Switzerland) mixed with wheat middlings and mixed into the control feed giving a final concentration of 20% of a garlic tincture standardized at 40% PTS + PTSO. Therefore, XTRACT® *Allium* contains 8% of PTS + PTSO in the piglets creep feed, as used by Ruiz *et al*. (2010). Individual piglets were weighed every seven days from birth to weaning, along with incidences of litter scours, creep feed disappearance and sow backfat depth changes.
Data were analysed using IBM®`SPSS® Statistics v21.0. The sows' backfat, piglets' weight and incidence of scours exhibited normal bayesian distribution, however, creep feed disappearance and piglet ADG did not even after log10 transformation. Piglet weight expressed heterogenous variance from processing to day 28 ($P < 0.05$) therefore a univariate analysis of covariance was carried out on the piglet weights. The effect of PTS and PTSO on piglet ADG was analysed using a Kruskall-Wallis test, as well as the within-group variation. Within group variation was determined by similar methods to those used by Magowan *et al*. (2005).

Results GAR piglets were weaned on average 1.5 kg heavier ($P < 0.001$) than CON piglets, however, 23.5% of GAR piglets were weaned at weights of more than 10kg, compared to 2% of CON piglets. Piglet ADGs were increased in GAR piglets from day 7 to day 14 and day 14 to day 21 ($P = 0.002$ and 0.007 respectively), however GAR piglet ADG dropped from day 21 to day 28 no longer different from the ADG of CON piglets ($P = 0.773$). GAR piglets only ate significantly more creep feed from day 21 to day 28 ($P = 0.002$), an average of 320 g more per litter. GAR piglets exhibited a lower scours incidence than CON ($P = 0.001$) by 7.66 (3.67 and 11.33 respectively).

Conclusion Supplementation of piglets with XTRACT® Allium XL caused an increased weaning weight in piglets and a reduction in the incidence of scour. The increased weaning weights of the piglets was projected to reduce production costs on the farm of £7.67 per pig finished at 105kg.

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Management strategies to improve the performance of low birth weight pigs to weaning and their long term consequences

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Implications Low birth weight piglets benefitted from being grouped with similar weight animals during lactation. Supplementary milk did not improve the growth performance of pigs but it did reduce body weight variation in mixed litters.

Introduction During the pre-weaning stage, piglets are reliant on the sow for nutrition, and sibling competition is likely to affect growth, in particular for low birth weight (LBiW) piglets. The objective of the experiment was to determine if LBiW piglets perform better to weaning, and subsequently, when grouped with other small piglets and given supplementary milk, in comparison to those grouped with normal birth weight (NBiW) piglets and given no supplementary milk

Material and methods The experiment was a 2 x 2 factorial using a total of 288 pigs. Treatments comprised 2 litter types (L, consisting of LBiW pigs only (≤ 1.25 kg), or Mixed (MX) consisting of both LBiW and NBiW pigs (1.6 to 2.0 kg)), and either milk supplementation or not from day 1 to 28. During the first 24 hours after birth all piglets selected for trial were cross fostered into a litter according to their treatment i.e. L or MX litters. Where possible, each experimental litter contained an equal number of piglets of each sex and from at least 3 different birth litters. Once the litters were set, they were randomly assigned within batch to the second treatment; half of the litters were given access to supplementary milk (S), and half were not (N). A feeder containing supplementary milk for the piglets was added to the pen of S litters from 24 hours after birth. The behaviour of litters given milk was recorded to identify milk consumption patterns. From day 1 to 28, pigs were individually weighed twice a week. From day 28 to 49, pigs were weighed once a week. Additional weights were then taken as they moved between buildings, once at 10 weeks and then at 16 weeks; a final weight was taken on the day before slaughter. Piglets were kept in their litters until d 70, and then subsequently housed in mixed groups until slaughter.

Results No difference was observed at any stage in the average daily gain (ADG) of LBiW pigs given access to supplementary milk or not ($P > 0.05$), nor was there any significant interaction between milk provision and litter type ($P > 0.05$) as shown in Table 1; however L litters drank significantly more supplementary milk than MX litters ($P < 0.001$). There was a significant effect of litter type on ADG to weaning, with LBiW pigs in L litters performing better than those in MX litters. The performance consequences of litter type were maintained throughout life, but were no longer significant at slaughter. Additionally, in MX litters there was a significant interaction between BiW category and supplementary milk on the CV of body weight (BW) (d 14 to 143) with a reduced CV in LBiW piglets in litters given milk.

Table 1 The effect of littermate weight and milk supplementation on the body weight (kg) of low birth weight pigs (LBiW) pigs from day 1 to 143

Litter type	L		MX					
Milk provision	S	N	S	N	SED	Littermate weight	Milk supplementation	LW x MS
Day 1	1.11	1.14	1.13	1.15	0.21	0.595	0.285	0.697
Day 14	3.84	3.78	3.64	3.89	0.151	0.752	0.544	0.308
Day 28	7.54	7.13	6.73	6.87	0.265	0.049	0.596	0.292
Day 49	15.5	14.5	14.2	14.3	0.594	0.109	0.487	0.362
Day 143	74.5	74.8	72.3	73.5	1.59	0.294	0.642	0.778

Conclusion Birth to weaning ADG of LBiW pigs can be increased by cross fostering LBiW piglets into litters with similar weight piglets, thus reducing the effect of indirect competition from significantly heavier littermates. Supplementary milk does not benefit their growth, but does reduce the variation in BW of pigs and an increase in the duration of supplementary milk intake was noted for LBiW pigs in mixed litters. Finally, whilst the advantages of the treatments applied during the lactation period are still present at slaughter, they are no longer significant

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High specification starter diets improve the performance of low birth weight pigs to 10 weeks of age

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Implications Feeding low birth weight pigs a more digestible, high specification starter diet improves nursery performance. When used in combination with an additional link diet it results in the same nursery exit weight as normal birth weight pigs.

Introduction Starter regimes are critical to maximise performance during the nursery phase as pigs transition from liquid to solid feed. Low birth weight (LBiW) pigs may exhibit poor post weaning weight gain as starter regimes may not meet their nutritional requirements. Some producers feed specialised regimes low body weight (BW) pigs at weaning, but it remains uncertain if these are successful in improving nursery exit weight and are economic. The objective was to determine if LBiW pigs can benefit from a high specification starter diet in comparison to a standard commercial regime targeting normal birth weight (NBiW) pigs. Also, the effect of a link feed post starter regime and the cost of each diet were evaluated.

Material and methods For LBiW pigs, the experiment was designed as a 2 x 2 factorial; pigs were in groups of 5 and there were 6 group replicates per treatment. Treatments comprised of one of two starter regimes (A = standard starter regime or B = high specification, more digestible regime), and provision (Y) or not (N) of a link feed; the amounts of each feed offered are given in Table 1. Additionally, the effect of starter regime A and B on NBiW pigs was determined (also n = 5 per treatment); these pigs did not receive any amounts of the link feed. Pigs were selected at birth according to birth weight (LBiW (≤ 1.25 kg), NBiW (1.6 to 2.0 kg)) and were randomly cross fostered into one of three litters. At weaning (day 28) pigs were randomly assigned to treatment groups according to birth weight. Starter regime and link feed) was fed on a kg/head basis for approximately 3 weeks. Pigs were fed according to the feeding regime in Table 1. Once pigs had finished the dietary treatments, they were fed a common diet to 70 days of age (14.8 MJ DE, 14 g/kg lysine). Pigs were individually weighed twice a week (day 1 to 70), and feed intake for the pen was measured (day 28 to 70). For LBiW pigs, data were analysed as a 2 x 2 factorial with starter regime and link feed as factors. To investigate the effects of birth weight and starter regime, a 2 x 2 factorial with birth weight (L or N) and starter regime (A or B) was also used.

Results Starter regime, link feed as well as an interaction between the two had a significant effect on the average daily gain (ADG) of LBiW pigs from d 28 to 49, with BY performing the best (Table 2). Whilst there was no effect of treatments on the average daily feed intake (ADFI) of LBiW pigs, an improvement in FCR was noted between day 28 to 49 for pigs fed the link diet ($P = 0.030$). In contrast, for NBiW pigs there was no significant effect of starter regime on ADG, ADFI or feed conversion ratio (FCR) from d 28 to 70. On day 49 and 70, LBiW pigs on regime BY weighed the same as NBiW pigs (day 70 BW; 30.0 versus 30.6 kg; P= 0.413). Despite regime BY feed costing the most at £7.90 per pig, the margin over feed was greatest (£14.5) due to the gains in body weight in comparison to all other treatments.

Table 1 Allocation (total amounts (kg) of a feed offered) and composition of dietary treatments for LBiW pigs

				Allocation (kg/pig)			
	Lysine, (g/kg)	Lactose, (g/kg)	DE, (MJ/DE)	AY	AN	BY	BN
Feed 1	1.75	200	17.3	-	-	2.50	2.50
Feed 2	1.60	150	16.0	2.00	2.00	2.00	2.00
Feed link	1.50	50.0	15.3	5.50	3.00	5.50	3.00

Table 2 The effect of starter regime and link feed on the performance of low birth weight pigs, from d 1 to d 70

	Treatment						P-value	
ADG, (kg/d)	AY	AN	BY	BN	s.e.d	Starter Regime	Link Feed	SR x LF
Day 1 to 28	0.230	0.226	0.213	0.224	0.0070	0.177	0.642	0.310
Day 28 to 49	0.365	0.358	0.432	0.361	0.0140	0.019	0.010	0.029
Day 49 to 70	0.619	0.586	0.688	0.640	0.0240	0.017	0.100	0.748

Conclusion A post weaning feeding regime specifically formulated for LBiW pigs improved the ADG and FCR of LBiW pigs to the end of the nursery phase and, LBiW pigs were able to achieve the same BW as NBiW pigs by day 70. In contrast, NBiW pigs did not benefit from a higher specification starter diet. Limiting the usage of the most expensive regime to LBiW pigs only, will ensure maximum growth performance as well as increased profitability.

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Spray-dried porcine plasma products improve performance of enterotoxigenic *Escherichia coli*-challenged weaner pigs

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Implications The resilience-promoting benefits of spray-dried porcine plasma on ETEC-challenged weaner pigs may arise due to the presence of immunoglobulins.

Introduction Proglobulin 80P, a spray-dried porcine plasma (SDPP), has been shown to improve the resilience of weaned pigs exposed to a repeated enterotoxigenic *Escherichia coli* (ETEC) challenge (Houdijk and Van Vuure, 2012). Its basis may be related to presence of immunoglobulins (IgG) and positive effects on feed intake (Torrallardona, 2010). Here, this role of IgG was challenged through testing the hypothesis that Proglobulin 80P and an experimental IgG-enriched plasma (IgG-Plus) in diets with similar total plasma IgG content would similarly improve performance of ETEC-challenged weaner pigs compared to a SDPP-free control diet.

Material and methods Ninety-six pigs, weaned at 26.7±0.7 day old and weighing 8.70±0.09 kg, were divided over 24 pens with two male and two female pigs (two rounds of 12 pens each). Pigs were fed one of three iso-energetic diets (16.9 MJ DE/kg), either with dry skimmed milk powder (DSMP) at 50 g/kg (control), or with 50 g/kg Proglobulin 80P or 33 g/kg IgG-Plus. Test products were included w/w at expense of DSMP, lactose levels kept constant by including whey, pure amino acids were used to balance at 16.7 g lysine/kg, and mass balance was achieved by slightly reducing micronized wheat. The test diets were calculated to include the same levels of IgG (12 g/kg). Diets were fed *ad libitum* for two weeks post weaning (P1, d_0-d_{14}), when all pigs were trickle infected with 10^8 CFUs Nal-resistant ETEC strains at 5 time points between d_4 and d_{13} by offering inoculated food as previously described (Athanasiadou *et al*., 2011). Pigs were fed commercial diets from d_{14} to d_{28} (P2) to assess carry-over effects. Feed refusals were taken daily during P1 and weekly during P2 to calculate average daily feed intake (ADFI). Pigs were weighed weekly from d_0 onwards to calculate average daily gain (ADG). Feed conversion ratio (FCR) was calculated as ADFI/ADG. Faeces scores were taken daily on a scale from 1 (solid) to 4 (severe diarrhoea). Fresh pen faecal samples were collected twice weekly until d_{21} for assessment of Nal-resistant ETEC via standard plating techniques with ETEC F4-specific confirmation PCR. Data was analysed using ANOVA, with pen as experimental unit and round as block. Faeces scores and ETEC counts were averaged over time within P1 and P2.

Results Pigs fed the Proglobulin 80P and IgG-Plus diets had similar ADFI, ADG and FCR during P1, which deviated from pigs fed the DSMP diets (Table 1); these effects did not carry over into P2 though effects on weight gain remained present over P1 and P2 combined. Pigs fed the Proglobulin 80P and IgG-Plus diets tended to have higher faeces scores, both in P1 and P2, though overall scores were very low. Feeding treatment did not affect faecal ETEC counts.

Table 1 Effect of SDPP source on performance, faeces score and faecal ETEC counts of weaned pigs

Parameter	Days post weaning	DSMP	Proglobulin 80P	IgG-Plus	s.e.d.	P-value
ADFI	0-14	351	375	389	17	0.098
(g/d)	14-28	953	984	975	31	0.610
	0-28	652	680	682	21	0.324
ADG	0-14	332	374	387	19	0.024
(g/d)	14-28	729	770	762	26	0.259
	0-28	530	572	575	19	0.052
FCR	0-14	1.06	1.01	1.01	0.02	0.053
	14-28	1.31	1.28	1.28	0.03	0.541
	0-28	1.23	1.19	1.19	0.02	0.135
Faeces score	0-14	1.14	1.24	1.24	0.05	0.108
	14-28	1.02	1.03	1.06	0.02	0.081
ETEC	0-14	4.04	4.77	4.48	0.36	0.145
(log CFUs/g)	14-28	0.51	0.46	0.52	0.43	0.988

Conclusion The data suggest that the improved performance on Proglobulin 80P over DSMP is likely due to its IgG level. Overall, these outcomes support the view that SDPP products may be alternative protein sources for newly weaned pigs, subject to authorisation, registration, permission and safety requirements under UK regulations for feedstuff use.

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Post weaning pig performance using different levels of starter 1 diet and impact of feed and house change on feed intakes

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Implications Pig performance is not always improved by offering higher levels of starter 1 diet. This research also highlights that feed 'disappearance' (feed lost by either consumption or spillage), can be negatively affected by changing diet but positively affected by changing house. As such the results suggest an opportunity exists to optimise pig performance through changing house and diet in a strategic manner.

Introduction Starter 1 (S1) diets are essential to optimise pig performance post weaning since they contain high levels of highly digestible proteins and carbohydrates. However, much of the research conducted on starter diets use dry feed systems whereas a growing proportion of pig producers are adopting liquid feeding systems (LFS) for post weaned pigs. The objective of this study was to investigate the performance effects (Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)) of offering post weaned pigs different levels of S1 diet within a LFS, on a commercial farm. A secondary aim was to examine the impact of changing feed type and house on daily feed intake.

Material and methods A total of 2,400 post weaned pigs were used over eight time period (replicates). Within each time period 300 pigs were allocated to one of six groups according to their gender and weight at weaning. Therefore each group of 50 pigs was balanced for gender and weight. However the design of the house was such that one feed trough offered liquid feed to two pens, so within each time period a 'pair' of pens was taken as one measurement unit. The liquid (water) to feed ratio was 3:1. All diets were offered ad libitium where a probe within the trough was used to control feed being dispensed. The LFS used was a flush system (Big Dutchman, HydroMix system). The dietary treatments varied in S1 diet allowance which was either 2, 3, or 4 kg per pig. The main ingredients in the S1 diet were (g/kg) 200 wheat, 175 Soya, 100 Barley and 25 Soya Oil, the remaining 500g/kg of the starter diets was made up of a concentrate feed (JMW IP94C) which mainly include fish meal, cooked cereals, minerals and vitamins. The S1 diet contained 226 g/kg crude protein (CP), 16g/kg total lysine and 10.3 MJ/kg net energy (NE). A starter 2 (S2) diet was then offered until 32 days post weaning. The S2 diet contained (g/kg) 275 wheat, 240 soya, 150 barley, 125 maize and 10 soya oil with the remaining 200g/kg being a concentrate feed (JMW IP93C) which mainly included cooked cereals and minerals and vitamins. The S2 diet contained 226g/kg CP, 16g/kg total lysine and 10.2 MJ/kg NE. Pigs were then offered a grower diet until 57 days post weaning. The main ingredients in the grower diet were (g/kg) 450 wheat, 280 soya, 150 barley, 50 maize, 40 soya oil, 15 minerals and vitamins; 11.5 lime and 3.5 salt. The grower diet contained 182 g/kg CP, 13g/kg total lysine and 15.2MJ/kg digestible energy. In all trials pigs were weaned at 24 days of age ± 6 days. Pigs moved from stage 1 to stage 2 housing at 26 days post weaning. Daily feed intake was recorded. ADG, ADFI and FCR were calculated on a per 'dual' pen basis.

Results Post-weaning pig performance (ADG, ADFI, FCR) was similar (P>0.05) during stage 1 and 2 when either 2, 3, or 4 g/kg of starter 1 diet was offered. Pig start weight averaged 6.7kg, day 57 (end) weight 35.6kg, ADG 502g/day, ADFI 857g/day and FCR 1.71. On average pigs consumed 2kg S1diet in 10 days, 3kg in 12 days and 4kg in 14 days. However, there was a significant effect of feed type change and moving house on daily feed intake patterns (Table 1). There was little increase in feed intake when diet changed from S1 to S2, yet this was still significantly increased (P<0.01). However, when pigs changed house (from stage 1 to stage 2) feed intake increased significantly. Seven days later pigs changed onto grower diet and after which feed intake decreased significantly (Table 1).

Table 1 Impact of feed and housing change on daily feed intake (g/pig/day)

	Day No. before change				Day No. after change					P Value		
	-4	-3	-2	-1	1	2	3	4	Sem	Treat.	Linear	Quadratic
S1 – S2 Diet	0.34^{a}	0.36^{ab}	0.39^{b}	0.39^{b}	0.43^{c}	0.46^{cd}	0.47^{d}	0.51^{e}	0.012	<.001	<.001	0.251
Change house	0.62^{a}	0.69^{ab}	0.76^{b}	0.74^{b}	1.05^{c}	1.08^{c}	1.05^{c}	1.08^{c}	0.034	<.001	<.001	0.061
S2 - grower	1.08^{a}	1.06^{a}	1.15^{a}	1.30^{b}	0.90^{c}	0.91^{c}	0.99^{cd}	1.01^{d}	0.035	<.001	<.001	0.195

Conclusion Offering pigs more than 2 kg/pig of starter 1 diet did not further improve their performance up to the end of stage 2. A change from S1 to S2 diet had little effect on the progressive increase in feed intake that would be expected and this is likely due to the S2 diet containing similar ingredients to the S1 diet. However, changing from S2 to grower diet significantly decreased feed intake, perhaps due to the change in ingredient profile. The increase in feed 'disappearance' when pigs changed house may have been due to an increased interest in the pigs' new environment albeit that pen structure and feeder type was similar. It would now be interesting to determine if changing diet and housing together may counteract the negative effect of changing to the grower diet.

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The standardised ileal digestible lysine requirement of 7 to 16 kg weaned pigs fed an antibiotic-free diet

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Implications Lysine requirement of weaned pigs was not affected by absence of in-feed antibiotics since it was similar to NRC (2012) recommendations whose lysine estimate was derived from many experiments with diets containing in-feed antibiotics.

Introduction A ban in the use of sub-therapeutic levels of antibiotics in pigs' diets as growth promoters may cause changes in their performance and could affect their amino acids (AA) requirement (Bikker *et al*., 2006). Most of the published studies that determined lysine (Lys) requirement and have been used to make recommendations had diets that contained antibiotics (NRC, 2012). Thus, there is a need to evaluate the dietary Lys levels that would maximize performance under antibiotic-free feeding regimen. The objective of this experiment was to determine standardised ileal digestible (SID) Lys requirement for weaned pigs fed antibiotic-free diets.

Material and methods Ninety [Duroc x (Yorkshire x Landrace)] piglets with an average initial body weight of 6.9 ± 0.5 kg (mean ± SD) were randomly assigned to 5 dietary treatments in a completely randomized design, with 3 pigs per pen and 6 replicates per treatment. Five corn-wheat-soybean meal-based diets were formulated to contain varied levels of SID Lys at 11, 12, 13, 14, and 15 g/kg diet and other AA were balanced to meet the ideal AA ratio (NRC, 2012). The analysed dietary Lys content was corrected using feed ingredients' SID coefficients to yield SID Lys content of 10.3 to 12.5, 13.1, 13.6, and 15.1 g/kg diet, respectively. All diets were balanced to contain 10.4 MJ/kg net energy. The experiment was conducted for 21 d during which piglets had free access to diet and water. Animals and feed were weighed weekly to determine average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). Blood samples were collected from one pig per pen via jugular vein-puncture on d 0 and 14 to determine plasma urea nitrogen (PUN) concentration. Data were subjected to analysis of variance using Proc mixed procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC) and the pen was an experimental unit. Orthogonal contrasts were used to determine linear and quadratic effects and $P < 0.05$ was considered significant. The estimates of SID Lys requirement were calculated using two straight-line and quadratic broken line analyses.

Results Increasing dietary Lys content linearly ($P < 0.01$) increased ADG and G:F, linearly decreased d-14 PUN, and quadratically ($P < 0.05$) increased ADFI. The SID Lys requirement determined using two straight-line and quadratic broken line analyses were 12.9 and 13.4 g/kg diet, respectively, hence an average value of 13.2 g/kg diet.

Table 1 Effect of dietary Lys on growth, feed intake, feed efficiency and plasma urea nitrogen of weaned pigs

	Dietary SID Lys level						*P*-value	
	10.3	12.5	13.1	13.6	15.1	SEM	Linear	Quadratic
Initial body weight, kg	6.86	6.85	6.85	7.09	6.90	0.27	n.s	n.s
Overall, d 0 to 21[1]								
Average daily gain, g	339	421	441	429	443	18	0.001	0.019
Average daily feed intake, g	501	550	560	543	545	22	0.335	0.034
Gain to feed, g/kg	670	749	740	802	813	32	0.004	0.600
Plasma urea nitrogen, mmol/L								
d 0	3.55	2.97	4.03	2.58	3.83	0.42	0.899	0.507
d 14	2.72	2.45	1.41	1.63	1.00	0.32	0.0002	0.500

[1]N=6, 3 pigs per pen.

Conclusion On average, optimal dietary SID Lys content for optimal growth of 7 to 16 kg weaned piglets was estimated to be 13.2 g/kg diet, and at this level the ADG and ADFI were 444 and 560 g, respectively. This represents the SID Lys requirement, expressed on daily intake basis, of 7.4 g Lys/d or 16.76 mg Lys/g gain.

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Unveiling the causes of poor performance in piglets during the nursery phase

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Implications Growth retardation in piglets leads to a high heterogeneity in body weight and a higher mortality in the pre- and post-weaning phase. Identifying potential routes, as feed intake regulation might shed light into alternatives to restore the growth check.

Introduction In intensive pig production there is an increase in the number of piglets born per litter. This has enlarged the within-litter variation in birth weight (Foxcroft *et al*., 2007). Based on an algorithm including birth, weaning and BW at 6 weeks of age, two populations of piglets originating from 35 litters, were selected at 6 weeks of age: a low (**LP**) and a high (**HP**) performing population. The LP piglets (n = 30) were selected from a subpopulation of piglets with a predicted 10 weeks BW lower than the mean minus the s.d. of the population. The HP piglets (n = 30) were selected from a subpopulation of piglets with a predicted 10 weeks BW higher than the mean plus the s.d. of the population. Their development was monitored until the end of the nursery phase (10 weeks of age). The objectives of the current study were to quantify the differences in BW gain, voluntary feed intake in the defined groups and to determine whether apparent ileal and total tract nutrient digestibility and animal morphology differences might explain the lower growth performance observed in the LP group.

Material and methods Piglets remained with their littermates until 6 weeks of age when they were selected, housed individually and were fed a highly digestible diet. At selection, the BW of LP and HP piglets were 6.8 ± 0.1 and 12.2 ± 0.1 kg, respectively. For the overall 4-week period differences between groups were evaluated including treatment group, sex and their interaction as independent variables using the MIXED procedure in SAS.

Results Compared to the LP piglets the HP piglets grew faster (637 vs. 434 g/d), ate more (908 vs. 633 g/d) from 6 to 10 weeks of age and were heavier at 10 weeks (30.0 vs. 18.8 kg, all $P < 0.001$). ADG and ADFI did not differ between groups when expressed relative to $BW^{0.75}$. Utilization of the diet (feed to gain ratio) corrected for the requirements for maintenance per kg $BW^{0.75}$ tended to be poorer for the LP piglets (1.0 and 1.1 g/g respectively, $P = 0.094$). However, overall gain:feed ratio was similar for both groups. The LP piglets had a higher body length and head circumference relative to BW ($P < 0.010$). Relative to BW, LP had a 21% higher weight of the small intestine weight, a 36% longer length, and relative to total FI from 6 to 10 weeks of age, the small intestinal weight was 4 g/kg higher (P = 0.004; 0.001 and <0.001, respectively). Apparent ileal and total tract digestibility of DM, N and GE were similar between groups (P > 0.100).

Conclusion

- Despite differences in the length and weight of the small intestine, ileal and faecal N and GE digestibility were similar between high performing (HP) and low performing (LP) piglets.
- The lower growth performance of LP piglets from 6 to 10 weeks of age is caused by an inability to engage any compensatory gain and compensatory feed intake.
- The gain:feed ratio was unaffected, but utilization of ingested feed for body weight gain above maintenance tended to be higher in HP piglets, illustrating the inability of LP piglets to compensate.
- Morphological comparisons, e.g. increased body length and head circumference relative to BW at 10 weeks of age in LP piglets, indicate that these piglets have an increased priority for skeletal growth compared with HP piglets.

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Effect of dietary inclusion of *Saccharomyces cerevisiae* (CNCM I-1079) at 2x10^9 cfu/kg feed on post weaned pig performance and gut morphology

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Implications *Saccharomyces cerevisiae* (CNCM I-1079), an active dry yeast for use as a probiotic, improves pig performance post weaning when offered in the diet at inclusion rate of 2x10^9 cfu (colony forming unit)/kg feed.

Introduction *Saccharomyces cerevisiae* (CNCM I-1079) is an active dry yeast and is currently authorised within EU as a animal feed additive. *Saccharomyces cerevisiae* (CNCM I-1079) can be used in piglet and sow feed and could be useful in enhancing pig performance. The aim of this study was to evaluate the effect of probiotic *Saccharomyces cerevisiae* (CNCM I-1079) on post weaned pig performance, gut structure and faecal microbiology. This study was part of a series of studies to investigate the inclusion of yeast across a range of pig farming conditions.

Material and methods A total of 288 piglets were used ((Landrace x Large White) x Pietrain) over eight time periods (replicates). At weaning (28 ± 2 days) piglets were weighed, tagged and randomised onto treatment according to weight. Pigs were penned in groups of 18 and housed in typical stage 1 accommodation for 4 weeks and in typical stage 2 accommodation for a further 4 weeks. Pigs were offered commercial diets *ad libitum* which contained either 0 or 2x10^9 cfu/kg feed of *Saccharomyces cerevisiae* var boulardi (CNCM I-1079) (Levucell SB®) included in all diets between weaning and the end of stage 2. All diets were pelleted and commercially available and contained no other additives with similar effect as the *Saccharomyces cerevisiae* (CNCM I-1079). Pigs were offered 2.2 kg/pig of a starter 1 diet, 2 kg/pig of a starter 2 diet, 8 kg/pig of a link diet after which a grower diet was offered until the end of stage 2. In each of the eight replicates (time periods) two pens of pigs were formed (18 pigs per pen) with each pen being offered one of the two dietary treatments. Pigs were allowed a 5 day acclimatisation period after which they were weighed and performance data reported below reflects performance between the end of acclimatisation (5 days after weaning) and the end of stage 2. Pen mean values were used to determine treatment effects on live weight, daily gain, feed intake, feed conversion ratio, faecal colour, consistency and microbiology. Faecal colour and consistency was recorded at each weighing interval (weaning, 4 and 8 weeks post weaning) and faecal samples were taken at these time points to study effects on faecal microbiology (specifically major bacterial genus) using PCR and agarose gel electrophoresis. Analysis of variance (Genstat, Version 10) was used to compare treatment effects on the pig performance.

Results There was no significant effect of *Saccharomyces cerevisiae* (CNCM I-1079) inclusion on pig weight, average daily gain or average daily feed intake but the FCR of pigs offered 2x10^9 cfu/kg feed was significantly (P<0.05) improved. There were no significant effects of *Saccharomyces cerevisiae* (CNCM I-1079) inclusion observed on the profiles of the major bacterial genus found, or on faecal colour or consistency using the method described above. Whilst there was a statically significant positive effect of *Saccharomyces cerevisiae* inclusion on the crypt depth of gut tissue in the ileum, this did not translate into a significant effect in the ratio of crypt depth and villi height measurements. Overall there were no biologically significant effects on gut morphology found between treatments using pigs housed under these conditions.

Table 1 Effect of *Saccharomyces cerevisiae* (CNCM I-1079) inclusion on pig performance up to 12 weeks of age.

Treatment	Live weight (kg) (8 weeks)	Live weight (kg) (12 weeks)	Average daily gain (ADG) (g/day)	Average daily feed intake (ADFI) (g/day)	Feed conversion ratio
Control	18.5	33.8	481	798	1.67
2x10^9 cfu/kg	18.4	34.6	497	773	1.56
s.e.m.	0.26	0.46	11.1	14.5	0.028
P value	NS	NS	NS	NS	<0.05

Conclusion The inclusion of 2x10^9 cfu/kg feed of *Saccharomyces cerevisiae* (CNCM I-1079) improved pig performance (FCR) by 6% compared with when it was not included in the diet. This improvement in FCR was mainly driven by a numerical increase in ADG and numerical decrease in ADFI. These results are in agreement with other workers investigating yeast incorporation into pig diets (Mathew *et al*., 1998; Shen et *al*., 2009).

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Feeding behaviour, body composition traits and performance of pregnant beef suckler cows differing in phenotypic residual feed intake offered grass silage

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Implications Residual feed intake (RFI) is a measure of feed efficiency that is independent of body weight and level of production in pregnant beef cows. Feeding behaviour is a significant contributory factor to variation in RFI in beef cows.

Introduction Residual feed intake is a measure of feed efficiency defined as the difference between actual and predicted feed intake, based on body weight (BW) and live weight gain (ADG) and, is genetically independent of these latter two traits (Crews, 2005). Activity associated with eating, and particularly consumption of forage diets, is a potentially significant energy sink in cattle (Susenbeth *et al.*, 2004), and may explaining some of the observed variation in RFI unaccounted for by growth and body size. The objectives of this study were to examine the relationship of RFI and performance with feeding behaviour and body composition traits in pregnant beef cows offered a grass silage diet.

Material and methods Individual dry matter intake (DMI) was recorded on 47 gestating Simmental and Simmental × Holstein-Friesian beef cows [initial conceptus adjusted BW 646 kg (s.d.=69.6)] offered grass silage *ad libitum* (DM digestibility = 666 g/kg) for a period of 80 d. Body condition score (BCS), ultrasonic muscle and fat depth, and feeding behaviour, using an automated feeding system, were measured. Phenotypic RFI was calculated as actual DMI minus predicted DMI. Predicted DMI was computed for each animal by regressing DMI on conceptus-adjusted mean metabolic BW ($BW^{0.75}$) and ADG. Within breed, cows were ranked by RFI and assigned to high (inefficient), medium or low groupings by dividing them into terciles. Statistical analysis was carried out using the MIXED procedure of SAS. The model included the fixed effects of RFI group, breed and their interaction, as appropriate. The model for analysis of feeding behaviour variables included fixed effects of breed, RFI group, day of RFI measurement period and RFI × day interaction, as appropriate. Calving date was included as a linear covariate.

Results Overall mean (±s.d.) values for DMI (kg/d), RFI and conceptus-adjusted ADG were 8.41 (±1.09) kg/d, 0.01 (±0.13) kg/d, and -0.07 (±0.32) kg, respectively (Table 1). High RFI cows ate 25% and 8% more than low and medium RFI cows, respectively. Low and high RFI groups had similar (P>0.05) BW, ADG, BCS, calf birth weight and calving difficulty score. All ultrasonic fat and muscle depths were similar (P>0.05) for RFI groups except for backfat thickness change where low RFI cows gained less fat (P<0.05) than high RFI cows. Low RFI had fewer (P<0.001) daily feeding events, of longer (P<0.001) mean duration (min/feed event/d) but of shorter (P<0.001) total duration (min/d) compared to high RFI cows.

Table 1 Performance and feeding behaviour of pregnant beef cows of differing phenotypic residual feed intake (RFI)

Variable	RFI group			s.e.m.	Significance		
	High (n=15)	Medium (n=17)	Low (n=15)		Day	RFI	Day×RFI
Feed intake (kg DMI/d)	9.50[a]	8.78[b]	7.59[c]	0.178	-	***	-
RFI (kg DM/d)	0.90[a]	0.10[b]	-1.00[c]	0.106	-	***	-
Mean BW (kg)[1]	642	642	647	18.6	-	NS	-
ADG (kg)[2]	-0.09	-0.01	-0.12	0.100	-	NS	-
Initial backfat thickness (mm)	2.0	1.8	2.7	0.36	-	NS	-
Backfat thickness change (mm)	0.40[a]	0.52[a]	-0.25[b]	0.197	-	*	-
Feeding duration (min/d)	209[a]	190[b]	171[c]	3.0	***	***	*
No. feeding events/d	45[a]	37[b]	34[b]	1.0	***	***	NS
Eating rate (kg DMI/min)	0.049[a]	0.049[a]	0.047[b]	0.0007	***	**	NS

[1]BW and [2]ADG = Conceptus-adjusted mean.

Conclusion Compared to cows of high RFI, those with low RFI consumed less feed for similar levels of productivity, however, low RFI cows may have been mobilising more body fat during pregnancy. Low RFI cows spent less time engaged in feeding behaviour-related activities than high RFI cows. Daily feeding events accounted for 17% of the variation in RFI over and above BW and level of production in pregnant beef cows offered a grass silage diet.

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The effect of suckler cow genotype on energetic efficiency and maintenance energy requirements

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Implications Maintenance energy requirements for suckler cows were developed in the current study. These data add novel information to the scientific literature and will help improve the production efficiency of suckler beef systems.

Introduction The energy feeding systems currently used to formulate rations for dairy and beef cattle in the UK (AFRC, 1993) were developed using data obtained more than 30 years ago. There is evidence that current dairy cows have greater metabolic rates than their lower genetic merit predecessors (Yan *et al.*, 1997). However, there is little quantitative data available on the prediction of the maintenance energy requirements of modern suckler beef cows. The objectives of the current study were to evaluate the energy utilization efficiency of two suckler cow genotypes and to use the data gained to develop models to predict the maintenance energy requirements and address the knowledge gaps that currently exist.

Material and methods Seventeen non-lactating dairy-bred suckler cows (Limousin x Holstein-Friesian, LF) and 17 non-lactating beef composite breed suckler cows (Stabiliser, ST) were used in a two-period study, one in November 2010 to January 2011 (6 LF and 6 ST) and the other in January to March 2012 (11 LF and 11 ST). Cows were housed in cubicle accommodation for 17 days, and then moved to individual tie-stalls for an 8 day digestibility balance which included a 2 day period of adaptation, followed by the immediate transfer to indirect, open-circuit, respiration calorimeters for 3 days. Gaseous exchanges (of O_2, CO_2 and CH_4) were recorded over the last two days only. All animals were offered grass silage only diets *ad libitum*, once daily at 0900 h, throughout the study. The data obtained were analysed by one-way ANOVA with effects of experimental period removed. The linear regression between ME intake and energy retention was used to determine net energy requirements for maintenance (NE_m) for each breed.

Results There were no significant differences between LF and ST breeds for energy intake (e.g., ME intake = 93.3 *vs.*105.7 MJ/d, s.e. 6.24), faecal energy, urine energy, methane energy, heat production or energy retention (3.2 vs. 6.9 MJ/d, s.e. 5.29), or any variable in energy use efficiency in terms of energy digestibility, metabolisability, heat production over ME intake or energy retention over ME intake. Accordingly, the data for both cow genotypes were pooled and used to develop relationships between ME intake and energy retention ($R^2 = 0.52$; $P < 0.001$) (Table 1). The NE_m values derived from the 3 equations were 0.386, 0.392 and 0.375 MJ/kg$^{0.75}$ for LF+ST, LF and ST respectively.

Table 1 The relationships between ME intake and energy retention (MJ/kg$^{0.75}$) [1]

Breed	Equation	R^2	NE_m (MJ/kg$^{0.75}$)	Equation No
LF + ST	Energy retention = 0.541 $_{(0.1191)}$ ME intake - 0.386 $_{(0.1000)}$	0.52	0.386	1
LF	Energy retention = 0.538 $_{(0.1207)}$ ME intake - 0.392 $_{(0.1036)}$	0.52	0.392	2
ST	Energy retention = 0.538 $_{(0.1207)}$ ME intake - 0.375 $_{(0.1043)}$		0.375	3

[1] Equations [2] and [3] were developed for LF and ST respectively, using a common slope as breed had no significant effect on the energetic efficiency. Data in brackets are s.e. values

Conclusion There were no significant differences in energetic efficiency between dairy-bred and beef composite breed suckler cows. However, NE_m values obtained in the present study were higher than those recommended by AFRC (1993) (0.386 vs. 0.337 MJ/kg$^{0.75}$), indicating that the use of the equations of AFRC (1993) to ration current suckler cows may under-predict their maintenance energy requirements.

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Relationships between methane (CH_4) production and residual feed intake (RFI) of two divergent breeds of finishing steers offered either a concentrate-straw based diet or a silage based diet

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Implications Breed type affected feed efficiency (RFI) in finishing steers on concentrate-straw or silage based diets but did not affect methane (CH_4) output. CH_4 production was not therefore a major factor in explaining variation in feed efficiency.

Introduction Due to growing concern over global food security and increasing pressure to minimise the impact of livestock production on the environment, production efficiency is becoming increasingly important. To improve the economic and environmental sustainability of beef production systems, it is important to identify cattle which are more efficient at converting feed into saleable product. Previously we (Duthie *et al*. 2013) reported RFI of finishing steers of two divergent breed types; here we extend these measurements to CH_4 production by these steers.

Material and methods The experiment was of a 2x2 factorial design, comprising 2 steer breed types (Charolais sired (CHx) and purebred Luing (LUI)), and 2 diet types (concentrate (721, 173, 76, 19, 11 g/kg DM of barley, maize dark grains, straw, molasses and minerals, respectively) and forage (286, 205, 397, 103 and 9 g/kg DM of whole crop barley silage, grass silage, barley, maize dark grains and minerals, respectively)). The concentrate diet contained (/kg DM) 135g CP, 12.8 MJ ME, 247g NDF, and 413 g starch and the forage diet contained (/kg DM) 139g CP, 12.0 MJ ME, 374g NDF, 276g starch. Measurement of RFI has been described previously (Duthie *et al*. 2013). Subsequent to measurement of RFI and FCR over an 8 week test period, steers were allocated to respiration chambers using a replicated (3 times) randomised block design (four measurement periods (weeks) for each of the six chambers) so that allocation was balanced for live-weight, breed and diet. Six indirect open-circuit respiration chambers were used with CH_4 production being recorded for the last 48 h of a 72 h measurement period (Rooke *et al*. 2013). Measurements from two steers were rejected because of anomalously low intakes during chamber measurements. Data were analysed using Genstat using linear mixed models where the factors were the 2 x 2 arrangement of breed and diet, block and chamber. Comparisons between CH_4 and RFI were made within diets. Results of diets and breeds are reported as means and standard errors of difference.

Results Mean values for CH4 and feed efficiency are show in Table 1. Methane production was reduced on the concentrate diet, irrespective of method of expression and there were no differences between breeds. In comparison to the other treatment groups, CHx steers which received the forage diet had significantly lower RFI values (interaction, $P < 0.05$). While there was no correlation between CH_4 (g/day) and RFI for the forage diet, there was a weak positive correlation ($r = 0.39$; $P=0.02$) between CH_4 (g/day) and RFI for the concentrate diet. There was no relationship between RFI and CH_4 (g/kg DMI).

Table 1 Dry matter intakes (DMI, kg/d), CH_4 production and feed efficiency by cattle fed either forage or concentrate diets

Diet	Concentrate		Forage			Significance		
Breed	CHx	LUI	CHx	LUI	SED	Breed	Diet	Breed x Diet
DMI	10.8	9.9	9.1	8.7	0.44	*	***	NS
CH_4 (g/d)	142	147	192	182	10.5	NS	***	NS
CH_4 (g/kg DMI)	13.3	14.6	20.3	20.7	0.84	NS	***	NS
CH_4 (kJ/MJ GEI)	39.7	43.6	59.6	60.6	2.49	NS	***	NS
RFI (kg)	0.21	0.45	-0.76	0.09	0.21	***	NS	*
FCR (kg, kg)	6.98	7.06	6.98	7.06	0.33	NS	NS	NS

GEI, gross energy intake. ***, P<0.001; *, P<0.05; means with different superscripts are significantly different (P<0.05).

Conclusion LUI steers were less efficient than CHx steers in terms of feed efficiency (RFI), however no significant differences were identified between breeds for CH_4 production. Relationships between CH_4 and RFI were diet dependant and CH_4 only explained a small proportion of variance in RFI on the Concentrate diet.

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Feed intake, animal performance and net feed efficiency (NFE) in finishing Stabiliser steers

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Implications Measurement of NFE in finishing steers offers significant opportunities to select future breeding stock with improved feed efficiency characteristics.

Introduction Whilst often measured in breeding bulls, net feed efficiency (NFE) can also be determined in finishing stock for use in selection programmes where appropriate. The objective of this study was to determine voluntary feed intakes, animal performance and NFE in finishing Stabiliser steers prior to slaughter.

Material and methods A total of 79 Stabiliser finishing steers between 13-18 months of age were offered a mixed forage/concentrate complete diet (CD) *ad libitum* for an adaptation period of 4 weeks and a subsequent measurement period of 56 days *via* electronic feed intake bins (Growsafe). The CD contained (g/kg DM) wholecrop wheat (466), barley straw (38), barley (240), sugar beet pulp (77), maize distillers grains (126), molasses (43) and minerals (9) - (DM: 539 g/kg; ME: 11.6 MJ/kg DM; CP: 131 g/kg DM). Dry matter intakes (DMI) were recorded continuously by the feed intake bins, individual electronic ear tags and their associated computer software. Individual steer liveweights (LW) were determined weekly and individual carcass fat depths were determined once at the end of the recording period by ultrasound scanning. Daily liveweight gain (LWG) was determined by linear regression of weekly LW measurements over the 56 day period whilst mean feed conversion ratio (FCR) was calculated as kg DMI/kg LWG. NFE was derived for individual steers as the difference between actual DMI against estimated DMI using a multiple regression model including metabolic LW ($LW^{0.75}$), LWG, fat depth and killing out proportion (KO) following slaughter shortly after the end of the trial as predictor variables. All data was grouped into three groups where low NFE = < -0.5 sd of mean NFE, MID NFE = > -0.5 but < +0.5 sd of mean NFE and high NFE = > +0.5 sd of the mean NFE value respectively. Differences between these three groups were then tested using the residual maximum likelihood (REML) facility in Genstat 15.

Results Voluntary DMI, animal performance, FCR and NFE measurements are shown in table 1 whilst individual NFE values for all 79 steers are shown in Figure 1. No significant differences between the three groups were seen in mean $LW^{0.75}$, LWG, KO or fat depth parameters as expected in NFE derivation studies. However, low NFE steers ate less and were significantly more efficient (P<0.05) in terms of both FCR and NFE compared to the high NFE group of steers.

Table 1 DMI, animal performance and NFE in finishing Stabiliser steers grouped on the basis of NFE standard deviation

	Low NFE (n=24)	MID NFE (n=33)	High NFE (n=22)	s.e.d.	Significance
DMI (kg/d)	12.17[a]	13.06[b]	14.24[c]	0.248	***
DMI (g/kg LW)	20.0[a]	21.2[b]	22.8[c]	0.248	***
DMI (g/kg $LW^{0.75}$)	99[a]	106[b]	114[c]	1.35	***
KO (g/kg)	525	535	529	6.49	
Mean $LW^{0.75}$ (kg)	123	124	125	1.18	
LWG (kg/day)	1.45	1.48	1.44	0.087	
Mean fat depth (mm)	7.6	7.2	7.5	0.599	
FCR (kg DMI/kg LWG)	8.83[a]	8.99[a]	10.29[b]	0.596	*
NFE (kg DMI/day)	-0.85[a]	0.00[b]	+0.94[c]	0.123	***

[a,b,c] Values within rows not sharing common superscripts differ significantly

Figure 1 Individual steer NFE values (kg DMI/day)

Conclusion Low NFE steers consumed 16% less feed, had 16% better FCR and cost £28 less to feed over the 12 weeks on the NFE unit (at 165/t DM) compared with high NFE steers without significant differences in animal performance.

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Effect of reducing the starch content of cereal based rations by the partial replacement of barley with soya hulls for intensively finished beef cattle

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Implications The results of this study show that increasing the starch content of cereal beef rations from 299 to 429g starch/kg DM when fed *ad libitum* with straw optimises performance with intensively finished beef cattle.

Introduction In a study by Marsh and Brown (2007) the performance of bulls fed diets with either a high (383 g/kg starch in DM) or low (93g/kg DM) level of starch in a proprietary beef nut was evaluated. The bulls fed the high starch ration recorded significantly improved physical and financial performance. The objective of this experiment was to investigate the partial substitution of barley with soya hulls to evaluate different starch contents of intensive beef finishing diets.

Material and methods Forty two Holstein and six Continental cross Holstein bulls weighing 299kg (s.e.d = 1.5) at approximately 7 months old were allocated in a randomised block design according to breed and live weight and fed the following diets *ad libitum* through to slaughter; High Starch, containing (kg/t) 785 rolled barley, 70 soyabean meal, 70 rapeseed meal, 50 molasses, 25 minerals; Standard Starch same formulation as High Starch but with 100kg rolled barley replaced with 100kg soya hulls; Medium Starch with 200kg rolled barley replaced with 200kg soya hulls The High, Standard and Medium Starch rations were analysed to contain 429, 374 and 299g starch/kg DM respectively. The cattle were housed in straw-bedded yards with 2 pens per treatment and were selected for slaughter at EUROP fat class 3. The data were analysed using ANOVA with initial live weight and breed as covariates.

Results Bulls fed the High Starch diet recorded significantly higher ($P<0.05$) slaughter weights compared to the Standard and Medium Starch fed bulls. There was a tendency ($P=0.103$) for an increase in carcase weight with the High Starch ration. Liver abscesses are associated with mild acidosis from feeding high starch based diets (Owen *et al.,* 1998). There were no differences in liver damage scores in the study and no obvious symptoms of acidosis in the live cattle.

Table 1 Animal Performance

	High	Standard	Medium	s.e.d	Sig
Slaughter weight (kg)	574[a]	561[b]	564[b]	4.8	*
Days to slaughter	197	194	197	5.0	NS
DLWG (kg)	1.41	1.37	1.35	0.044	NS
Carcase wt (kg)	297	291	292	3.4	NS
Carcase daily gain (kg)	0.80	0.78	0.77	0.025	NS
Liver score[1]	1.23	1.11	1.08	0.223	NS

[1] Liver assessment: 1= Healthy liver, 3 = Slight abscesses, discolouration and/or swelling, 5 = Severe abscesses
The chi-squared probability is presented for the liver scores and was non-significant. The arithmetic means are presented.

Feed intakes were relatively similar and with a reduction in DLWG with the cattle fed the Standard and Medium Starch diets by the partial replacement of barley with soya hulls resulted in a deterioration of the FCR and increase in feed cost per kg gain based on the feed prices prevailing at the time of the study.

Table 2 Concentrate feed intakes, feed conversion ratio (FCR) and feed cost per kg gain

	High	Standard	Medium
Concentrate feed intake (kg)	1,907	1,918	1,932
FCR (kg: kg LWG)	6.93	7.29	7.37
Feed cost (£/kg LWG)	1.41	1.46	1.47

The FCR appears relatively high compared to the target of 5.4:1 for cereal beef production but it must be taken into consideration that the experiment did not include the period of growth from 110kg to 300kg. During this rearing phase dairy-bred bulls at Harper Adams University typically record an FCR of 3.4:1 with a DLWG of 1.52kg.

Conclusion Overall performance of all the bulls on all three treatments was satisfactory achieving recognised targets for intensive cereal beef production. The High Starch diet resulted in the highest slaughter weight, DLWG with improved FCR. It can be concluded from this experiment that diets containing 429g starch/kg DM optimises performance with intensively finished beef cattle.

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Gene expression in the insulin signalling pathway in beef bulls differing in phenotypic residual feed intake offered a high concentrate diet

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Implications Genes regulating the insulin signalling pathway are not differentially expressed in muscle tissue of beef cattle varying in residual feed intake, however, further research of genes involved in fatty acid synthesis is warranted.

Introduction Residual feed intake (RFI), defined as the difference between actual feed intake and expected feed intake based on maintenance and growth requirements, is a measure of feed efficiency that is genetically independent of growth and body size (Crews, 2005). Insulin is a highly metabolically active hormone involved in the control of nutrient uptake and storage and regulation of glucose homeostasis at the cellular level (Cheng *et al.*, 2010), therefore examining gene expression in the insulin signalling pathway (ISP) may identify potential mechanisms that contribute to variation in RFI. The objective of this study was to examine expression profiles of key genes in the ISP in skeletal muscle of beef bulls differing in RFI.

Material and methods Individual dry matter intake (DMI) and growth were measured in a total of 67 Simmental bulls, reared from birth on the research farm, [mean (±s.d.) initial body weight 431 kg (±63.7) and age 426 d (±43.1)] over 3 years. Bulls were offered concentrates *ad libitum* for 105 d. Biopsies of *M. longissimus dorsi* were harvested at the middle and end of the experimental period. Residuals of the regression of DMI on average daily gain (ADG) and mid-test metabolic body weight, using all animals, were used to compute individual RFI coefficients. Bulls were ranked on RFI and assigned to high (inefficient), medium or low groupings. Tissue samples from bulls with the highest (n=15) and lowest RFI (n=15) were selected and total RNA was extracted. Expression of key genes in the ISP was measured by q-RT-PCR with *GAPDH* and *UBQ* as reference genes. Gene expression values were normalised to the reference genes and converted to values relative to the mean cycle threshold (Ct). Data were transformed where necessary and statistically analysed using PROC MIXED (SAS) with terms for RFI group, time point, year of study, and their interaction included in the model, as appropriate.

Results The 30 bulls used for the gene expression analysis, had an initial mean (±s.d.) BW of 430 kg (±54.7) an ADG of 1.6 kg (±0.34) and DMI of 9.44 kg (±1.33) during the experimental period. Despite high RFI bulls consuming 11% more ($P<0.01$) DM than low RFI bulls, BW and ADG did not differ ($P>0.05$) between the groupings. Least square means of gene expression data are presented in Table 1. There were no ($P>0.05$) RFI × time point interactions nor was there an effect of RFI on expression of genes in the ISP. Time point had no effect ($P>0.05$) on mRNA expression of genes, with the exception of *GLUT4*, *INPPL1* and *SHC* where the relative expression of these genes increased ($P<0.05$) over time. mRNA expression levels of the transcription factor *SREBF1* tended to be positively correlated ($r=0.25$; $P=0.07$) with RFI.

Table 1 Effect of residual feed intake (RFI) and time point on the expression of genes in *M. Longissimus dorsi* of the insulin signalling pathway

	RFI			Time point			Significance	
	High	Low	s.e.m.	Middle	End	s.e.m.	RFI	Time point
INSR	0.99	0.78	0.595	0.77	0.96	0.588	NS	NS
IRS1	1.41	1.90	0.898	1.46	1.85	0.906	NS	NS
GLUT4	1.67	1.72	0.214	1.50	1.90	0.219	NS	*
INPPL1	1.47	1.41	0.300	1.13	1.75	0.296	NS	**
PI3K	1.78	1.62	0.544	1.41	1.99	0.575	NS	NS
SREBF1	3.19	2.81	0.698	2.85	3.15	0.781	NS	NS
AMPK	1.07	1.07	0.225	1.01	1.13	0.237	NS	NS
FOXO1	1.86	1.68	0.356	1.76	1.77	0.355	NS	NS
PPARG	1.47	1.08	0.780	1.47	1.08	0.766	NS	NS
SHC	1.50	1.48	0.316	1.15	1.83	0.314	NS	**

Conclusion Collectively, results suggest that differential expression of key genes of the ISP in muscle tissue is not a contributory factor to variation in RFI. However, examination expression of target genes of *SREBF1*, which are involved in adipogenesis, may explain some of the well documented differences in body fatness between high and low RFI animals.

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Response to *in vivo* glucose tolerance test in Holstein Friesian bulls during dietary restriction and subsequent re-alimentation

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Implications This study provides evidence of insulin sensitivity to a glucose challenge in cattle during restricted feeding and subsequent re-alimentation. Further work is now warranted to investigate the underlying molecular mechanisms controlling glucose metabolism in animals undergoing compensatory growth.

Introduction Compensatory growth (CG) is defined as a physiological process whereby an animal has the potential following a period of restricted feed intake to undergo accelerated growth upon re-alimentation (Hornick *et al*., 2000). A reduction in feed intake is generally associated with lower concentrations of circulating insulin as a consequence of reduced dietary nutrient intake and hepatic gluconeogenesis. The glucose tolerance test (GTT) facilitates a pancreatic response in insulin synthesis to hyperglycaemia. Therefore the objective of this study was to determine the insulin response to an intravenous glucose tolerance test in Holstein Friesian bulls during restricted feed intake and CG during subsequent re-alimentation

Material and methods This experiment was conducted as part of a larger study by Keogh *et al*. (2012). Briefly, 24 Holstein-Friesian bulls were assigned to one of two groups: (i) restricted feed allowance for 125 days (RES; n=12) followed by *ad libitum* access to feed for 55 days or (ii) *ad libitum* access to feed throughout (ADLIB; n=12). The first 125 days was denoted as Period 1 and the subsequent 55 days, Period 2. During Period 1 RES were managed to achieve a target mean daily growth rate of 0.6 kg/day. On days 90 and 36 of periods 1 and 2 respectively a glucose tolerance test was performed on all bulls. Indwelling jugular catheters were fitted and glucose was administered as a 50% solution w/v at a rate of 1g of glucose per kg metabolic body weight. Blood samples were collected at -30, -15, 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150 and 180 minutes relative to glucose infusion. Blood samples were subsequently centrifuged and plasma was assayed for concentrations of both insulin and glucose. Area under the curve (AUC) analysis was carried out for both analytes. AUC data were transformed if necessary and statistically analysed using the mixed procedure of SAS with terms for treatment and period, as well as their interaction included in the model, as appropriate.

Results Average daily gain (ADG) for Period 1 was 0.6 kg/d for RES and 1.9 kg/d for ADLIB. During re-alimentation an ADG of 2.5 and 1.4 kg/d was observed for the RES and the ADLIB groups, respectively. A treatment x period interaction ($P<0.001$) was evident for insulin AUC and this was manifested as a lower insulin response for RES in Period 1, with no subsequent difference in insulin response between treatment groups in Period 2. There was no difference ($P>0.05$) in glucose AUC between treatments in either period. Figure 1 displays the glucose and insulin response profiles to glucose administration for both animal groups across both periods.

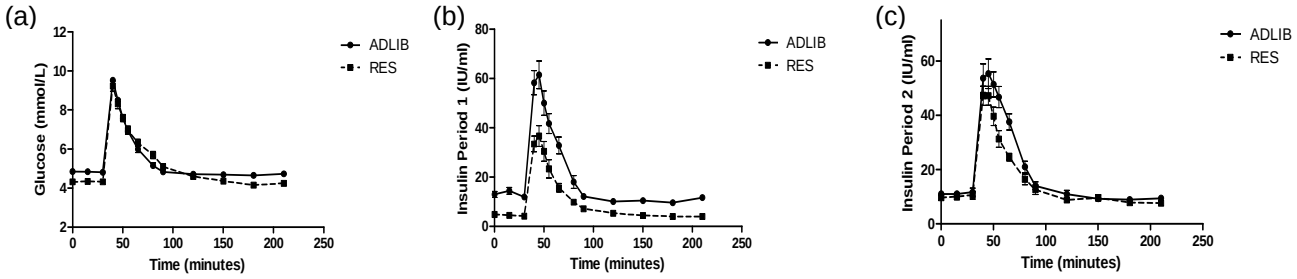

Figure 1 Response to GTT on (a) glucose (both periods combined); (b) insulin, Period 1; (c) insulin, Period 2.

Conclusion The lower insulin response for RES during Period 1, given no difference in glucose response between treatment groups, indicates insulin sensitivity to the uptake of glucose during restricted feed intake. During Period 2, RES animals were consuming more food per unit of bodyweight; however the insulin response remained lower in these animals when compared to ADLIB, suggesting a continued sensitivity to insulin in re-alimentation and compensatory growth. The increase in insulin response in RES animals in Period 2, compared to Period 1, indicates that this insulin sensitivity continues only during the initial stages of compensatory growth. This study provides evidence that insulin sensitivity occurs as a consequence of a period of restricted feed intake and potentially continues during the initial stages of compensatory growth in cattle.

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Oats for intensively finished bulls

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Implications The results of this study show that the replacement of barley with oats in intensive beef rations for dairy-bred bulls resulted in a reduction in DLWG and slaughter weights with a deterioration in FCR.

Introduction Intensively fed cattle are traditionally fed diets based on rolled barley however a number of enquiries were received at EBLEX/Harper Adams University intensive beef finishing meetings on replacing some or all of the barley with oats. Compared to barley, oats have a lower energy value (12.2 *vs*. 13.2 ME MJ/kg DM), lower starch content (420 *vs*. 590 g/kg DM) and a higher fibre content (350 *vs*. 210 g/kg NDF). This may not appear to be beneficial when it is starch that drives live weight gain with intensively fed bulls. Feeding oats however could result in reduced problems with rumen acidosis with consequential improvements in performance. The objective of this experiment was to determine the effect of full or partial replacement of rolled barley with rolled oats to intensively finished dairy-bred bulls through to slaughter.

Material and methods Forty two Holstein and six Continental cross Holstein bulls weighing 283kg at approximately 7 months old were allocated in a randomised block design according to breed and live weight and fed the following diets *ad libitum* through to slaughter; Barley diet containing (kg/t) 845 barley, 40 soya-bean meal, 40 rapeseed meal, 50 molasses, 25 minerals; Barley/Oats diet containing (kg/t) 417.5 barley, 417.5 oats, 45 soya-bean meal, 45 rapeseed meal, 50 molasses, 25 minerals; Oats diet containing (kg/t) 825 oats, 50 soya-bean meal, 50 rapeseed meal, 50 molasses, 25 minerals The Barley, Barley/Oats and Oats mixes were analysed to contain 422, 359 and 257g starch/kg DM respectively. The cattle were housed in straw-bedded yards and were selected for slaughter at EUROP fat class 3. The data were analysed using ANOVA with liveweight and breed as a covariate.

Results The bulls fed the Barley diet recorded significantly higher ($P<0.05$) slaughter weights and carcase weights ($P=0.096$) compared to the Oats fed bulls. There was a tendency for the Barley and Barley/Oats fed bulls to record higher ($P=0.093$) daily liveweight gains (DLWG) compared to the Oats fed bulls. Liver abscesses are associated with mild acidosis from feeding high starch based diets (Owen *et al.,* 1998). There were no differences in liver damage scores.

Table 1 Animal Performance

	Barley	Barley/Oats	Oats	s.e.d	Sig
Slaughter weight (kg)	593[a]	579[ab]	556[bc]	14.0	*
Days to slaughter	242	231	236	6.4	NS
DLWG (kg)	1.29	1.28	1.17	0.058	NS
Carcase wt (kg)	302	294	283	8.6	NS
Carcase daily gain (kg)	0.70	0.70	0.65	0.036	NS
Liver score[1]	1.33	1.00	1.33	0.223	NS

[1] Liver assessment: 1= Healthy liver, 3 = Slight abscesses, discolouration and/or swelling, 5 = Severe abscesses
The chi-squared probability is presented for the liver scores and was non-significant. The arithmetic means are presented.

The replacement of Barley with Oats increased total concentrate feed intake and with no improvement in DLWG resulting in a deterioration of the feed conversion ratio (FCR) and increase in feed cost per kg gain based on the feed prices prevailing at the time of the study.

Table 2 Concentrate feed intakes, FCR and feed cost per kg gain

	Barley	Barley/Oats	Oats
Concentrate feed intake (kg)	2,101	2,157	2,196
FCR (kg: kg LWG)	6.78	7.29	7.39
Feed cost (£/kg LWG)	1.16	1.32	1.51

Straw intakes were recorded for a 48 day period during the study. Individual daily intakes were 0.90, 0.67 and 0.57kg per head per day for the Barley, Barley/Oat and Oat fed bulls, respectively.

Conclusion Overall performance of the Barley fed bulls was satisfactory achieving recognised targets for intensive cereal beef production. The replacement of barley with oats resulted in a reduction in DLWG and slaughter weights with a deterioration in FCR. Based on the costs prevailing at the time of the study oats would have to cost £49-51 per tonne less than barley to justify their inclusion in a cereal based ration for intensively fed beef cattle.

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Effect of feeding a yeast culture (Diamond V XP_{LS}) on the performance of intensively finished bulls

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Implications Optimising performance with intensively feed beef cattle is essential in an effort to create a margin. The results of this study show that a yeast culture (Diamond V XP_{LS}) can increase feed intakes, DLWG, carcase daily gain and margin over feed costs.

Introduction In 2012 22.6% of all male beef cattle in the UK were reared as bulls (Pers. Comm. P Heyhoe, EBLEX) which would predominantly be finished on intensive cereal based systems at 13-15 months old. Since feed accounts for 74-85% of the variable costs of intensive beef production systems (EBLEX, 2013) and with the recent volatility in cereal prices there is a requirement to evaluate the use of supplements that could optimise performance within intensive beef finishing units. The objective of this experiment was to investigate the effect of feeding Diamond V XP_{LS} on the performance of intensively finished dairy-bred bulls. Diamond V XP_{LS} Yeast Culture, Diamond V, Cedar Rapids, IA, USA is a *Saccharomyces cerevisiae* fermentation product containing the yeast and the media on which it is grown and fermented.

Material and methods Thirty six Holstein (n=26) and Continental cross Holstein (n=10) bulls were assigned in a randomised block designed experiment to one of the following treatments: Control, bulls fed *ad libitum* barley mix containing (kg/t) 845 rolled barley, 40 soyabean meal, 40 rapeseed meal, 50 molasses and 25 minerals; $XP_{LS,}$ bulls fed the same formulation as Control but with 4kg rolled barley replaced with XP_{LS} yeast culture. Straw was offered *ad libitum* to both treatments. The Control and XP_{LS} rations were analysed to contain 846 and 845g DM, 152 and 153g CP/kg DM, 496 and 495g starch/kg DM respectively. The cattle were housed in straw-bedded yards with 2 pens per treatment and were selected for slaughter at EUROP fat class 3. The data were analysed using ANOVA with initial liveweight and breed as covariates.

Results As shown in table 1 the bulls fed XP_{LS} yeast culture recorded a significantly higher ($P<0.05$) daily liveweight gain (DLWG) and daily carcase gain.

Table 1 Animal Performance

	Control	XP_{LS}	s.e.d	Sig
Start weight (kg)	318	319	9.1	NS
Slaughter weight (kg)	570	581	11.4	NS
Days to slaughter	204	198	5.5	NS
DLWG (kg)	1.23	1.32	0.041	*
Carcase wt (kg)	300	311	8.5	NS
Carcase daily gain (kg)	0.74	0.81	0.033	*

Table 2 Cereal feed intakes, feed conversion ratio (FCR) and feed cost per kg gain

	Control	XP_{LS}
Cereal feed intake (kg)	1,719	1,792
FCR (kg feed: kg LWG)	6.82	6.84
Margin over feed costs (£/bull)	662	674

The XP_{LS} fed bulls recorded an increased feed intake consuming 0.58kg more feed per day and overall some 73kg more which will have influenced the DLWG. The FCR appears relatively high for the bulls compared to the EBLEX target of 5.4:1 but it must be taken into consideration that the trial did not include the period of growth from 110kg to 320kg. During this rearing phase dairy-bred bulls at Harper Adams University typically record a DLWG of 1.52kg with an intake of 720kg of feed with an FCR of 3.4:1.Margin over feed costs were increased by £12 per bull with XP_{LS} based on the costs prevailing at the time of the study (bulls slaughtered February-April 2012).

Conclusion Overall performance of the bulls was good achieving the EBLEX targets for intensive cereal beef production. Feeding bulls XP_{LS} yeast culture resulted in increases in feed intakes, DLWG and carcase daily gains. Based on the costs prevailing at the time of the study the highest margin over feed was recorded with the XP_{LS} fed bulls which was increased by £12 per bull.

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Modelling the environmental impacts of commercial pig production using Life Cycle Assessment

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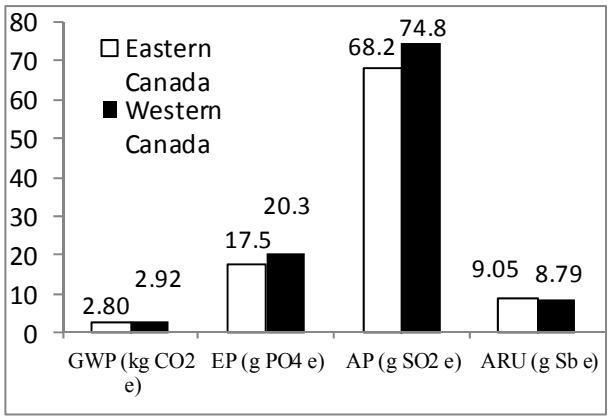
Implications A new Life Cycle Assessment (LCA) of pig production has been created, based on Canadian farm data, to assess and investigate more effective ways for the pig industry to mitigate its environmental impacts.

Introduction There is an increase pressure on the livestock industries to assess the impact they have on the environment, and to minimise any negative impacts. There are many important measures of environmental impact in addition to carbon footprint which is most frequently assessed. Here we developed a holistic method to assess the environmental impacts of Canadian pig production by conducting an LCA which assessed environmental impact using multiple impact categories. This is the necessary first step for the development of strategies that will enhance the sustainability of pig production systems.

Material and methods A cradle to farm-gate LCA was conducted to compare the environmental impact of pork production supply chains in Eastern and Western Canada. The model was structured into 3 subsections which calculated the inputs and outputs from 1) the production of feed ingredients, 2) the pig barn and 3) manure management. Typical diet formulations for these two regions were supplied by Nutreco Canada. Western diets were based typically on wheat and barley and Eastern diets based on corn. Data on herd performance including feed conversion ratio (FCR), mortality and average litter size were based on benchmark data provided by Canadian farms. The performance data represented over 100,000 finished pigs in 2012 from 10 farms in eastern provinces and 7 in the western provinces. The proportional mixture of manure management techniques used for swine manure in Canada was established using existing Government funded surveys (Statistics Canada, 2003 and Beaulieu, 2004). The environmental impact of the systems was calculated as the Global Warming Potential (GWP), Eutrophication Potential (EP), Acidification Potential (AP) and Abiotic Resource Use (ARU) for a functional unit of 1 kg of finished pig carcass weight (CW).



Results Major performance indicators such as FCR and mortality rates were very similar in both systems compared**.** EP and AP were higher for Western Canada per kg CW at farm gate (Figure 1). Their numerically higher levels in the western systems are reflective of increased levels of fertilizer input and associated emissions in the wheat and barley based diets, as well as slightly higher levels of nutrient excretion. The results for GWP and ARU showed little difference in both systems reflecting the trade off in the model between increased nutrient excretion in Western systems and the potential for this to replace synthetic fertilizer inputs to the system. For both systems around 60% of GWP and > 90 % of ARU was associated with the production of feed ingredients.

Figure 1 Environmental impact of 1kg CW at farm gate measured using the impact categories GWP, EP, AP and ARU

Conclusion The GWP results for Canadian pork in this study are comparable to the only previous LCA study on Canadian pork of 2.96 and 2.83 kg CO_2 per kg CW for Eastern and Western systems respectively (Verge *et al*, 2009). Despite the two studies using different methodologies, both appear to show lower levels of GWP resulting from Canadian systems in comparison to LCA's of pig systems in the US, with whom they share several similarities. As a first step the model has been able to represent the environmental impacts of Canadian pig production using a broader spectrum of impact indicators than used in previous studies. This will allow further work to investigate the most important aspects of the system causing variability in the impacts of pig systems and uncertainty in the calculation of these impacts.

Acknowledgements The authors would like to thank Nutreco Canada for supporting this research.

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Meta-analysis on the effects of animal and management factors influencing feed efficiency and growth in growing and finishing pigs

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Implications A number of factors that influence feed efficiency (FE) in pigs were identified, as well as how these factors interact with each other to affect FE. The results may be used to improve efficiency in growing and finishing pig systems.

Introduction Feed is the largest cost in pig production and its efficiency of conversion into pig meat underpins the UK pig industry's competitive ability. A meta-analysis on the effects of environmental and animal based factors on the FE of growing and finishing pigs can provide information on the interaction effects that are absent in individual studies. This study analysed the separate and interactive effects of factors on the feed conversion ratio (FCR), average daily feed intake (ADFI), and average daily gain (ADG) of growing and finishing pigs maintained in a variety of systems.

Material and methods Information regarding the FE of finishing pigs was collected from 54 publications in peer-reviewed journals published between 1967 and 2011. Raw data from four commercial sources were also used, leading to a total of 248 usable treatments. The peer reviewed studies were gathered using a search engine (SCOPUS) with the terms 'pig', 'finishers', 'feed efficiency', 'performance', with the literature cited checked to complete the information available. The information gathered included the outputs ADG, ADFI and FCR, as well as information regarding the animal characteristics, physical and social environment, feed composition and feeder type. Only papers from the EU were used due to differences in management and genetics between continents. A separate model was run for FCR, ADFI and ADG. Initially each factor was analysed separately using a one way ANOVA to determine which factors were significant. Significant factors were then combined in a GLM model, with all categorical factors included as fixed terms; continuous factors were also included as covariates. To begin with, only those factors which were represented across all studies were added: the remaining factors were then added incrementally, whilst at the same time removing those which were no longer significant (Averos *et al*., 2012). By doing so, the main factors which significantly contributed to FCR, ADFI and ADG remained in the model. The effect of the remaining factors on the performance parameters were then investigated with a post-hoc Tukey or, in the case of continuous factors, a scatter plot. Finally, interactions between factors were analysed. To achieve this, the number of categories within each factor was reduced on the basis of the ANOVA output; categories that were not statistically different were grouped together. The interactions were added onto the existing model individually, those which were significant were then added to the model together, remaining there if significant.

Results Whilst a number of factors were individually significant, the final models identified significant effects only: (1) for FCR = stage of growth, genetics, gender, feed crude protein and lysine (Figure 1), and temperature (Figure 2), with an interaction of group composition x bedding; (2) for ADFI = stage of growth, bedding, genetics, group size, gender, feed crude protein, group composition and temperature and an interaction of stage x diet energy, (3) for ADG = stage of growth, genetics, bedding, floor type, building type and the interactions bedding x lysine content and building type x lysine.

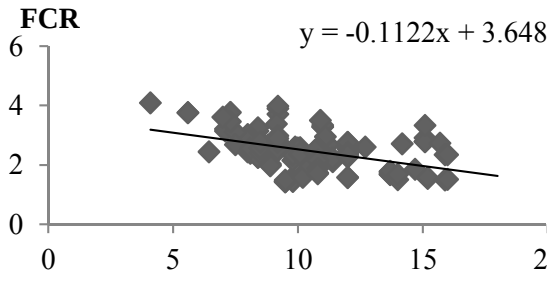

Figure 1 The effect of lysine (g/kg) on the FCR of pigs

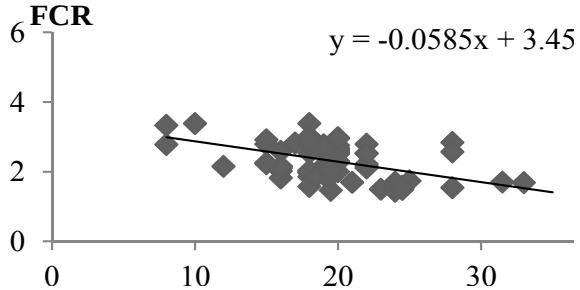

Figure 2 The effect of temperature (°C) on the FCR of pigs

Conclusion The meta analysis confirmed the significant effect of several well-known factors on the performance of growing and finishing pigs, and, importantly, the interactions between these factors, especially with bedding**.** In addition, the effects of some less established factors were noted, such as floor type, with increased ADG noted on either solid or partially slatted floors compared to fully slatted floor. The results may contribute towards the improvement of efficiency of growing-finishing pig systems by better knowledge of the various factors that influence this.

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Feeding value of bean starch concentrates for growing pigs

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Implications The assessed standardized ileal digestible amino acid and digestible energy contents of bean starch concentrates suggests it has the potential to reduce reliance on soya bean meal and wheat in growing and finishing pig diets.

Introduction Air classification separates finely ground pulse flour in fractions of different densities using an air stream (Vose *et al*., 1976). Air classification of dehulled faba bean (*Vicia faba*) produces both protein and starch concentrates as co-products. Since bean starch concentrate (BSC) will still contain moderate levels of crude protein (CP), it may be used as an alternative energy and protein source for pigs. The current experiment was designed to determine the digestible energy (DE) and standardised ileal digestible (SID) amino acid (AA) contents of BSC for use in growing and finishing pig diets.

Material and methods Twenty-four 10-wk old male pigs, weighing 46.5±1.1 kg, were housed individually and fed commercial diets for three days, before experimental diets were gradually introduced at 3.3 × DE for maintenance. The two experimental diets were either a purified nitrogen-free diet or a semi-purified diet, containing 93.5% BSC as the only protein source. Pigs were fed the experimental diets for 8 days; faecal samples were collected on days 6, 7 and 8, and ileal digesta were collected on day 8. Faeces and ileal digesta were analysed for dry matter (DM), CP, starch, gross energy, titanium (digestibility marker) and amino acids (AA, ileal digesta only). BSC DE content was calculated from feed and faeces gross energy levels, corrected for DE arising from 2% soya oil included in the test diet. Coefficients of apparent ileal digestibility (AID) values for CP and AA were corrected for basal endogenous losses derived from the nitrogen-free diet to calculate SID.

Results The BSC tested contained 16.8% CP and 1.13% Lys on a DM basis (Table 1), with similar relative Met, M+C, Thr and Trp levels to the 0.11, 0.31, 0.52 and 0.13 respectively in BSC tested elsewhere (Gunawardena *et al*., 2010) and 0.11, 0.31, 0.56 and 0.14 in whole faba beans (Houdijk *et al*., 2013). As expected, SID coefficients were greater than AID, averaging at 0.79 vs. 0.75 for the AA reported, respectively. Averaged AA SID coefficients were in line with the 0.77 from whole faba beans in feeding tables (Hazzledine, 2008), which were both considerably lower than the 0.91 reported in BSC tested elsewhere (Gunawardena *et al*., 2010). The BSC contained 52.6% starch, and its ileal and total tract digestibility was 98.1 and 99.7%, respectively. Total tract diet energy digestibility averaged 84%; corrected for soya oil contribution, this indicated a DE content of 14.22 MJ/kg DM for the BSC tested, which is very similar to DE values of whole beans (Hazzledine, 2008) though around 14% lower than the 16.5 MJ/kg DM reported in BSC tested elsewhere (Gunawardena *et al*., 2010).

Table 1 Composition and digestibility of bean starch concentrate for growing pigs

Nutrient	Composition		Digestibility coefficients		Composition
	% (in DM)	AA to Lys ratio	AID	SID	SID %
CP	16.81	-	0.77	0.81	13.54
AA					
Lys	1.13	-	0.86	0.88	1.00
Met	0.12	0.11	0.79	0.84	0.10
M+C	0.35	0.31	0.68	0.72	0.25
Thr	0.58	0.52	0.71	0.75	0.44
Trp	0.11	0.10	0.74	0.78	0.09
Starch	52.60	-	0.98	-	-

Conclusion The composition and digestibility of amino acids in BSC in combination with its moderate DE content indicate that it is a promising ingredient in grower and finisher pig diets to replace at least a proportion of soya bean meal and wheat, provided that its imbalanced AA profile and moderate DE content is accounted for through appropriate raw material inclusion, including the use of synthetic AA. These expectations are currently being tested in a growth performance trial with grower and finisher pigs.

Acknowledgements The authors thank Dave Anderson and Jolinda Pollock for technical assistance. This research is part of "Development of protein-rich and starch-rich fractions from faba beans for salmon and terrestrial animal production, respectively" (ref 101096), funded by the UK Technology Strategy Board.

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Increased aggression at mixing results in fewer injuries in stable social groups of pigs

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Implications This study provided evidence that increased aggression during mixing results in lower levels of chronic aggression in stable social groups. Mixing unaggressive pigs is likely to successfully reduce aggression at mixing, however may have welfare implications for long term group stability.

Introduction Mixing of growing pigs disrupts established social relationships and results in aggressive contests between individuals. Aggression is considered a welfare and production concern. As aggression establishes dominance relationships, it is possible that increased aggression at mixing may increase the subsequent stability of hierarchies (Rodenburg *et al*, 2010). Once pigs are mixed they will usually remain in these social groups until marketed, therefore from a welfare standpoint, there may be a worthwhile trade-off between initial heightened aggression and long term stability. This study sought to determine if there is a phenotypic link between aggression at mixing and increased long-term group stability as measured by skin lesions and if so, to identify behaviours which may deliver this benefit.

Material and methods 78 new social groups were formed by mixing 15 individuals sampled from 5 litters at an average age of 70 days (SD 4.3). The number of skin lesions was recorded for each body region (anterior, central, posterior) 24 h post-mixing (SL24h), and once again at three weeks post-mixing (SL3wk) (89.8 days [SD 5.2]). Behavioural traits were defined based on time, duration, intensity and outcome of all aggressive interactions observed via video recordings. To account for systematic influences, the effects of breed, sex and experimental batch were fitted as class variables, and body weight as a covariable. Pens, into which the animals were mixed, were fitted as a random effect. Using this model, correlations between residuals of traits were estimated to examine individual animal associations (within pen), and correlations between estimates of pen effects were used to examine between pen associations. To identify the change in aggression of animals and groups over time, these correlations were estimated between behavioural traits, SL24h and SL3wk. A multiple linear regression mixed model was developed to further explore the relationship between aggression at mixing and skin lesions, and resulted in the best model to predict lesion scores from behavioural traits.

Results For individuals, all behavioural traits of aggressiveness correlated with anterior SL24h (0.1 to 0.53; $p<0.001$), with the highest correlation for the duration of reciprocal aggression received. Central and rear SL24h were also positively associated with several measures of aggression (0.09 to 0.27; $p<0.001$). The opposite trend was found for SL3wk, as several aggressive behavioural traits were negatively correlated with skin legions, but at a lower magnitude at this time. Anterior and central SL3wk correlated with the most behavioural traits. The strongest relationships were observed for the central body region (-0.07 to -0.14; $p<0.05$) and the weakest for the posterior region (-0.06 to -0.08; $p<0.05$). Between groups of animals (between pens), the duration and number of reciprocal encounters, and the number of pigs encountered correlated positively with anterior SL24h (0.34 to 0.67; $p<0.01$) and correlated negatively with central SL3wk (-0.28 to -0.38; $p<0.01$). The proportion of phenotypic variance attributed to pen effects was small (0.03 to 0.23; $p<0.05$). The direction of the regression coefficients estimated by multiple regression linear models agreed mostly with those obtained from correlation analyses; however fight success and initiation of non-reciprocated attacks were associated with fewer SL24h and the number of pen mates a pig was bullied by was associated with slightly higher central SL3wk.

Conclusion Within aggressive cohorts at mixing, animals with a high fight success rate received slightly fewer SL24h than their equally aggressive, but unsuccessful pen mates, while animals that avoided aggression altogether received the fewest SL24h. This trend was reversed at 3 weeks. While high fight success at mixing predicted the lowest SL3wk, involvement in aggression, even when unsuccessful, predicted lower SL3wk than animals which avoided aggression altogether. This suggests that animals with no fighting experience are lower in social rank to those which have been defeated. Correlations at the between pen level provide evidence that the more aggression the group experiences at mixing, the fewer average SL3wk it suffers. It may be that increased aggression at mixing leads to less ambiguity over dominance position, resulting in fewer outbreaks of aggression under stable social conditions. This raises the ethical dilemma that increased acute aggression during mixing may actually decrease chronic aggression in groups and thus benefit long term welfare of the group.

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The provision of multiple chewing ropes to pigs housed in large groups and the effects on oral fluid sample representation

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Implications Providing more ropes (~1:20) to large populations of young pigs kept in straw-based accommodation improves oral fluid sample representation from a 30 minute sampling. Increasing rope number (>1:100) does not affect sample representation for finishing pigs in straw-based accommodation, but is higher than current blood sampling protocols.

Introduction Oral fluid (OF) has been well established in the US as a biological sample which can be easily collected from pigs without veterinary presence in order to test for a number of key diseases. The presentation of a cotton rope to a group of pigs results in chewing interactions whereby OF is deposited onto the rope and can be wrung out by hand for subsequent laboratory testing. Current sampling protocols in the US are validated for conventional indoor slatted husbandry systems with small pen sizes (n<30). In the UK, straw-based accommodation with large group size is widely used due to its scale of operation and welfare benefits, but might reduce sampling efficiency (Seddon *et al*. 2011). The aim of this study was to investigate the pen representation in OF samples from larger pig populations, and the manipulation of this by changing the number of cotton ropes offered.

Material and methods Four pens each of commercial weaned (n=150-200) and finishing (n=80-100) pigs (Large White x Landrace x Pietrain) in straw-bedded housing were presented with either 1, 2, 4 or 8 cotton ropes (Outhwaites Ropemakers UK) on four separate occasions according to a balanced Latin Square design. 25% of the pigs in each population were individually spray marked for identification during the rope presentation periods. Ropes were presented for 30 minutes during which time the pigs were observed, and the number of marked pigs that engaged in rope chewing was recorded. Analysis of Variance was performed using Minitab Version 16 software to assess the effects of different rope numbers on the number of pigs to chew and volume of fluid collected.

Results In weaned pigs, increasing the number of ropes lead to significantly increased numbers of pigs chewing ($F_{(3,6)}$ = 13.93, P=0.004). This was not observed in the finishing pigs ($F_{(3,6)}$ = 0.16, P = 0.916), although 50% representation was still achieved. The provision of multiple ropes resulted in increased OF volumes in both weaned ($F_{(3,6)}$=13.54, P=0.04) and finishing ($F_{(3,6)}$=10.47, P=0.008) pigs.

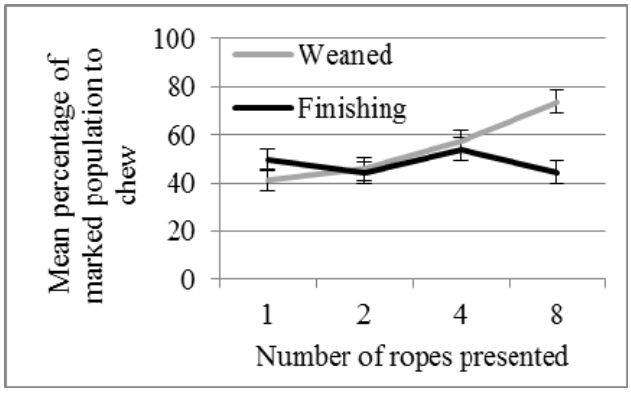

Figure 1 The mean percentage of the marked population (±SEM) of weaned and finishing pigs to engage in rope chewing when different numbers of ropes were offered

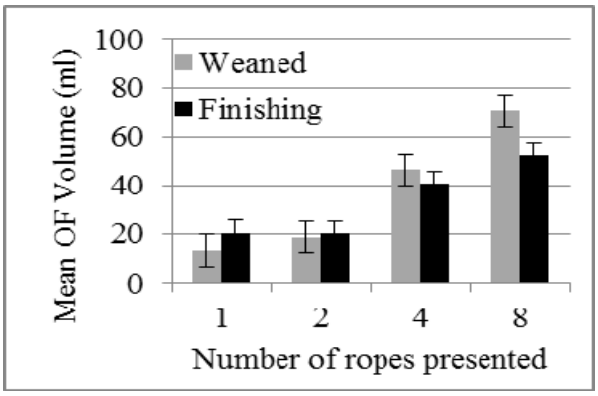

Figure 2 The mean volume of OF collected (± SEM) from weaned and finishing pigs when different numbers of ropes were offered

Conclusion Increasing the number of ropes presented to large populations of young pigs in straw-bedded housing lead to greater numbers of pigs chewing ropes, and thus better sample representation than offering a single rope. In finishing pigs, sample representation did not change when more ropes were offered.

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Pig performance and Salmonella prevalence pre and post slaughter when finisher pigs are offered organic acid in feed

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Implications The inclusion of organic acid in the feed improves pig performance and reduces the probability of *Salmonella* being excreted on farm. However, it does not suppress the impact of the effects of transport since no reduction in *Salmonella* prevalence in the animals gut post slaughter was found when it was offered to pigs.

Introduction *Salmonella is* recognised as a food-borne pathogen of worldwide importance. EU Zoonoses Regulation targets *Salmonella* reduction in products of animal origin in all phases of production and distribution. Among animal products, pork products are also considered a significant source of human infection. The inclusion of organic acid in pig diets has been found in a number of studies to reduce the excretion of *Salmonella* from pigs while on farm due to its bacteriostatic activity (EFSA, 2010). However, it is realised that *Salmonella* excretion increases significantly between the farm and the abattoir (Korsak *et al*, 2003) and therefore the risk of cross contamination increases. The aim of this study was to assess the effectiveness of organic acid inclusion in finisher pig feed to reduce the prevalence of *Salmonella* in pig faeces pre and post slaughter. An additional aim was to assess the effect of organic acid inclusion on pig performance.

Material and methods A total of 44 pigs ((LR x LW) x PIC337) originating from pens where pen faeces was found to be positive for *Salmonella* were individually housed and used over two time periods. For analysis representative samples of faeces were collected from the pen floor and sent for *Salmonella* analysis. Pigs were transferred to individual pens with concrete flooring, at 11 weeks of age. Pigs were offered one of two dietary treatments which either did, or did not include organic acid in a normal finisher pig diet. The main ingredients in the finisher diet were (g/kg) 394 Barley; 360 wheat; 188 soya; 20 molaferm; 11.6 limestone; 10 vegetable oil; 6.1 mono DCP; 5 minerals and vitamins, 2.3 lysine; 2.8 salt and 0.4 methionine. The acid used was a combination of formic and propionic acid added at an inclusion rate of 2g/kg. Diets were steam pelleted and offered to pigs in dry form from 11 to 20 weeks of age. Pigs were randomly assigned to dietary treatment so that each treatment was balanced for weight and gender. Faecal samples were collected from the individual pens that pigs were housed in on the day before slaughter. Faecal samples were also collected from individual pig gut (colon) at the point of evisceration, 45 minutes after slaughter. Faecal samples were analysed for presence of *Salmonella* according to International Organization for Standardization (ISO) method 6579 (ISO 6579:2002, Annex D, Horizontal Method for the Detection of *Salmonella* spp.). Pig performance data was analysed using analysis of variance in Genstat version 10 with each pig representing an individual replicate. The presence or absence of *Salmonella* in pig faeces pre and post slaughter was analysed using generalised linear mixed model methodology.

Results Feed conversion ratio of pigs was significantly improved in the late finishing period resulting in an overall improvement of 5.6% ($P<0.05$) in FCR during the finishing period (Table 1). Pre slaughter *Salmonella* prevalence results suggested that when organic acid was included in the finisher pig diet, the probability of *Salmonella* excretion on farm tended ($P<0.1$) to be lower (9%) compared with when it was not included in the diet (32%) (Table 1). With regard to post slaughter there was no significant difference in the predicted probability of *Salmonella* presence whether organic acid was included in the finisher diet or not (Table 1).

Table 1 Effect of organic acid inclusion on pig performance and *Salmonella* prevalence during the finishing period.

Treatment	Finish weight (kg)	Average daily gain (g/day) 11-finish	Average daily feed intake (g/day) 11-finish	Feed conversion ratio	Salmonella prevalence pre slaughter %	Salmonella prevalence post slaughter %
Control	88.7	899	2171	2.43	31.8	68.2
Organic acid	88	900	2074	2.30	9.1	54.6
SEM	1.93	23.7	58.8	0.041		
P value	NS	NS	NS	<0.05	<0.1	NS

Conclusion Whilst this study found a tendency for organic acid to decrease *Salmonella* prevalence pre slaughter, However, organic acid inclusion had no significant impact on the prevalence of *Salmonella* in the animal gut/faeces post slaughter and as such could not suppress the suggested effects of stress on *Salmonella* shedding imposed during transport from farm to abattoir.

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RNA degradation in tissues collected from pigs subjected to two different slaughtering methods

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Implications Obtaining high quality RNA from carcasses sent through conventional slaughter means not only can the RNA be used in downstream analyses but the carcasses can be sold on to recover costs.

Introduction There are high costs involved in the use of livestock in research. Therefore if the carcasses can be sold on for human consumption the costs of the experiment can be recovered. However for studies involving the need to utilise intact RNA, using commercial post-mortem carcass processing (adhering to the Welfare of Animals (Slaughter or Killing) (WASK) Regulations 1995 and associated EU regulations) could be problematic. RNA starts to degrade once the animal is dead, so it has been considered imperative to take tissue samples as soon as possible. Some downstream analyses require the RNA to be of a certain quality, as assessed by RNA integrity number (RIN) (Udvardi *et al*., 2008; Guerau-de-Arellano *et al*., 2012). The objective of the current study was to determine whether commercial processes of scalding and dehairing carcasses leads to increased RNA degradation due to the increased temperature and time to tissue collection through the assessment of the RIN values of the total RNA extracted from different tissues of the pig.

Material and methods Twelve male Camb12 type pigs weighing 59.5±3.56 kg were assigned to two groups, those that went through the scalding tank and dehairer (S) and those that did not (NS). One pig from group NS did not complete the trial. The pigs had access to feed and water *ad libitum* for one week. The NS group were transported in fours to the place of slaughter, where they were brought in individually, stunned, then exsanguinated and eviscerated. The same protocol was followed on the following day in a different building for S, however between exsanguination and evisceration the animals were put through a 60°C scalding tank and dehaired which made the protocol 20 minutes longer than for NS (5 minutes). For both groups, after evisceration, samples of liver, pancreas, jejunum, ileum and colon tissue were taken as quickly as possible. All tissue samples were snap frozen in liquid nitrogen and stored at -80°C for later analysis. Each tissue sample was crushed in liquid nitrogen and stored at -80°C prior to total RNA being extracted using the High Pure RNA Tissue Kit (Roche Diagnostics, Mannheim, Germany) and the RNeasy Mini Kit (QIAGEN, Hilden, Germany). The concentrations were then quantified using the NanoDrop ND1000 Spectrophotometer (Thermo Scientific, Waltham, MA). The samples were then assessed for quality using the Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, CA).This quantified the amount of degradation by classifying each sample with a RIN value. Data were analysed by one-way ANOVA using Genstat 15th Edition (VSN International Ltd, Hemel Hempstead, UK) to assess the effect of scalding and dehairing on the RINs of each tissue collected. Significance was accepted at $P<0.05$.

Results Mean RIN data are shown in Table 1 and clearly show that there was no effect of the different slaughter practices on the RIN values for each tissue (all P-values >0.05). The pancreas was the only tissue to have degraded RNA and this effect was equivalent in both groups.

Table 1 Mean (±SD) RIN (RNA Integrity Number) values for each tissue RNA was extracted from. *n=6, □n=5, ‡n=4.

Tissue	S	NS	P-value (ANOVA)
Liver	7.14 ± 0.305*	6.85 ± 0.995‡	0.551
Pancreas	2.40 ± 0.158□	2.30 ± 0.200‡	0.459
Jejunum	6.58 ± 2.148*	8.06 ± 0.568□	0.172
Ileum	7.73 ± 0.787*	8.48 ± 0.206‡	0.108
Colon	8.77 ± 1.300*	8.48 ± 1.359□	0.729

Conclusion The lack of difference between S and NS sample RIN values suggest that RNA extracted from carcasses subjected to different slaughter practices can be used in downstream analyses as in our experience RIN values >6.5 are acceptable for most assessment of gene expression, the exception being RNA from the pancreas. In turn the carcasses can be sold on for profit to be reinvested back into the research, potentially making the study more ethically acceptable.

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Assessing animal welfare – in sow herds using data on meat inspection, medication, or sow mortality

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Implications Data selected for their relevance for animal welfare from data on meat inspection, medication and mortality, respectively were not associated with a herd level welfare index based on animal based measurements. This indicates that animal based measurements are necessary for assessing animal welfare in a sow herd.

Introduction Today farm animal welfare has political attention, and citizens have great concerns on animal welfare in pig production. However, assessing animal welfare at herd level is complicated and expensive when on-farm animal based welfare measurements are used. If sow welfare at herd level could be assessed by using existing data this would be cheap. Further if we could select key-parameters from only one source of data, e.g. data on meat inspection, medication, or mortality it would be a simple way to identify sow herds with poor welfare. The aim of this study was to investigate the association between a sow welfare index inspired by the Welfare Quality® (2009) protocol for sows, with selected data from meat inspection, medication, or mortality.

Material and methods In this study 63 sow herds were visited and a welfare protocol was used to collect data for a multi-dimensional sow herd welfare index that has been developed. In Denmark, much herd data are collected and stored in central databases. In this study the data on meat inspection, medication, and mortality were included. The data were collected in a one year period before the sow herds were visited and the welfare protocol was completed. Data on meat inspection were obtained from abattoirs and the incidence of all slaughter codes together was calculated so that each herd had one meat inspection incidence. Data on medication were included as the sum of doses antimicrobial and analgesic medicine used per sow per year. Data on mortality was the incidence of dead sows divided by herd size.

The association between the welfare index score with data on meat inspection, medication, or mortality on the above mentioned 63 herds was analysed and the coefficient of determination was calculated.

Results

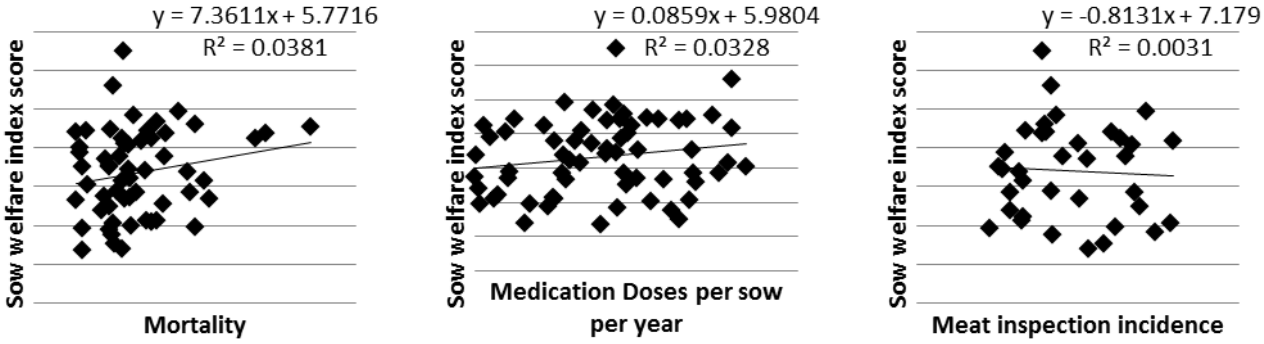

Figure 1 The linear association between sow welfare index score with data on meat inspection (Range: 0.23-0.79), medication (Range: 0-16.77), or mortality (Range: 0.05-0.34). The linear relationships and the coefficients of determination are located in the upper right corner.

Conclusion There were no simple relationships between a multi-dimensional sow herd welfare index with welfare relevant data from meat inspection, medication, or mortality records.

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The commercial viability of crossbreeding modern and traditional pig breeds

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Implications The crossing of commercial and traditional pig breeds can be undertaken as a means to ensure the future sustainability of intensive pig production through the effective utilisation and productive improvement of both groups.

Introduction Intensive pig production is based upon the utilisation of a limited number of modern international breeds, rigorously selected for desirable performance characteristics of economic value. This has caused the emergence of several rare, traditional breeds, owing to the fact that anecdotal evidence suggests that they do not fit the productivity parameters of those few breeds considered as commercially efficient. The crossbreeding of modern and traditional breeds could achieve the preservation of genetic variation for valuable traits and improve the productive performance of both groups (Bonneau and Lebret, 2010). The aim of the study was to evaluate the performance and financial differences between Large White and Gloucester Old Spot crossbred progeny, to determine the commercial viability of hybrid crossing between a rare and modern breed.

Material and methods A total of 78 slaughter progeny were generated from 8 JSR Genepacker 90: Large White x Landrace (LW x L) sows, artificially inseminated using Large White (LW) or Gloucester Old Spot (GOS) purebred semen, from several different terminal sires. The trial populations comprised of 39 LW x (LW x L) crossbreds (LW X), and 39 GOS x (LW x L) crossbreds (GOS X). The progeny were weaned at 4 weeks (28 ± 3 days) in mixed-sex breed groups of 39 individuals per pen, with fully slatted flooring, two bite-type drinkers and were fed *ad libitum* in two four-space feeders. The pigs were transferred to the grower accommodation at 15 weeks (105 ± 3 days), and housed in 4 mixed-sex single breed groups. Each grower pen had a raised covered 'kennel type' lying area, bedded with straw, with a concrete floor. All 4 groups were fed *ad libitum*, in double spaced feeders, with two bite-type drinkers per pen. The pigs were transferred to the finishing accommodation (same layout and management as grower) at 19 weeks (133 ± 3 days), in the previous 4 groups, where they remained until 23/24 weeks (162 ± 8 days). All pigs were offered the same commercial pelleted diets *ad libitum*, with the quantity of feed added per day and removed between feed changes measured throughout all stages. The pigs were weighed using calibrated weighing scales, on a weekly basis from birth to slaughter. The backfat thickness of each pig was recorded individually, on an alternate week basis, from week 14 to the day of slaughter, at the P2 site, 65mm from the edge of the dorsal midline, at the point of the last rib (Mullan *et al.,* 2009), using a Renco® Lean-Meater®. The approximated cost of producing a kg of pig meat, for each breed, was calculated from collected farm data on variable costs and fixed costs, which were adapted from the BPEX average GB financial performance figures. The two-sample t-Test assuming equal variances was conducted for; birth, weaning, slaughter and carcass weight, average daily gains, feed conversion efficiency and P2 backfat depth, to assess significant differences between sample means.

Results There were significant breed effects on average daily gain from birth to weaning and weaning weight ($P < 0.001$). The result for lifetime growth rate demonstrated that the GOS X grew slower in comparison to the LW X, however there was no difference in average daily gain from weaning to slaughter, with both groups concurrently reaching slaughter weight (~100kg) and achieving similar carcass deadweight (~75kg). The feed consumption of the GOS X was slightly higher than the LW X; however there was no significant difference between feed conversion efficiency. The GOS X had greater carcass backfat depth at slaughter ($P <0.001$). The price paid per kilogram of deadweight for the LW X was £1.43, in comparison with a premium of £1.53 for the GOS X. The approximate costs of production per kilogram of deadweight for the LW X were £1.64, in comparison to £1.68, for the GOS X.

Conclusion The crossbreeding of the modern hybrid dam (LW x LR), with a rare GOS sire, can be exploited to achieve similar productive and economic results, in comparison with the commercial hybrid. A higher return per kilogram of deadweight can be achieved for the traditional and commercial hybrid, due to the supplementary premium paid over the prevailing market price.

Acknowledgements This study was funded by the BBSRC.

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Blood collection methods: Vacutainer® selection for serum or plasma mineral analysis (pigs)

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Implications Selection of Vacutainer® collection tube is important for the quantification of blood minerals in pigs, as there are some significant differences in values obtained using different products.

Introduction The use of mineral concentration as an indicator of health is a tool widely used by veterinarians and animal scientists. The optimal choice of sample type and collection protocol is dependent on the mineral under investigation (Herdt and Hoff, 2011). If blood is to be used, it can either be allowed to clot (for serum) or prevented from clotting (for plasma) prior to analysis. BD Vacutainer® tubes contain a range of anticoagulants and have a product line specifically for trace element analysis. A study was conducted to assess differences in determined blood mineral concentration dependent on the choice of collection tube and internal coating.

Material and methods All experimental procedures were approved by Harper Adams University Research Ethics Committee and regulated procedures were conducted in compliance with the Animals (Scientific Procedures) Act 1986. Blood was collected by jugular venipuncture from four weaned pigs, fed on a wheat based commercial diet, in three different BD Vacutainer® tubes (Becton, Dickinson and Company: Oxford, UK). These were one serum trace element tube (CAT), one trace element tube spray coated with K_2EDTA and one standard tube spray coated with lithium heparin (LH). Tubes were allowed to stand at room temperature for two hours before centrifugation at 1000 x g for ten minutes at 4^0C. Plasma and serum were pipetted into 2.0 ml eppendorfs and stored at 4^0C overnight prior to storage at -20^0C. Defrosted plasma and serum was vortexed and diluted 1:20 with 1% HNO_3 prior to elemental analysis using an X series 2 ICP-MS in standard mode, running Plasma Lab software (Thermo Scientific Inc, USA). Calculations were performed in Excel 2010 (Microsoft Corporation) and analysed in GenStat sixteenth edition (VSN International Ltd) using ANOVA blocked by pig and followed by Tukey test where significance was indicated.

Results There were no differences in blood mineral P, Ca, Mn, Fe, Cu, Se or Mo concentrations when obtained from serum or plasma. Zinc concentrations were lower in serum than in either of the plasma samples (P=0.01). Magnesium concentrations were lower in the LH tubes over the two trace element tubes (P=0.002). K concentration was elevated in the K_2EDTA coated tubes (P=0.001) but was also different between serum and LH plasma (P<0.05). The concentration of Na was higher in the K_2EDTA coated tube compared with the serum tube (P=0.04) but neither were different to LH plasma. Concentrations of Co obtained from the serum samples were lower (P=0.03) than those found in LH plasma but not from K_2EDTA plasma. Variation between pigs was generally low (<20%) with the exception of Fe (41.8%) and Co (31.8%), although the mean values were within their expected ranges.

Table 1 Comparison of pig blood serum and plasma minerals collected into three types of Vacutainer®

	Na mmol/l	Mg mmol/l	P mmol/l	K mmol/l	Ca mmol/l	Mn µmol/l	Fe µmol/l	Co µmol/l	Cu µmol/l	Zn µmol/l	Se µmol/l	Mo µmol/l
Serum	48.96^{a}	1.15^{B}	5.88	6.01^{B}	3.00	0.20	34.90	0.014^{a}	32.16	13.45^{A}	2.10	0.42
K_2EDTA	51.12^{b}	1.24^{B}	5.82	22.26^{C}	2.98	0.23	25.20	0.015^{ab}	30.93	15.90^{B}	2.05	0.41
LH	49.29^{ab}	1.02^{A}	5.52	4.76^{A}	3.02	0.14	20.00	0.017^{b}	30.90	16.33^{B}	2.13	0.51
S.E.D.	0.686	0.035	0.145	0.352	0.075	0.044	4.880	0.0008	0.542	0.719	0.059	0.049
P-Value	0.04	0.002	0.10	<.001	0.88	0.19	0.06	0.03	0.25	0.01	0.46	0.17
Pig CV%	0.9	10.4	2.1	2.9	3.6	4.7	41.8	31.8	18.2	12.5	4.0	13.9

*means within columns with common superscripts are not significantly different (P<0.05)

Conclusion Determination of most major minerals in the pig can be done from either plasma or serum, blood plasma is however recommended for Zn and Co quantification due to the higher values obtained. Additionally, a trace element Vacutainer® is recommended for blood collection for the determination of Mg concentration.

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Reduction of androstenone in organic entire male pigs by reduction of weight and age

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Implications Challenges regarding boar taint is an actuality within organic entire male pig production. However, concentration of androstenone in entires could be minimized by reduced weight as within the conventional system.

Introduction Castration is a routine procedure in Denmark performed primarily due to risk of boar taint. Boar taint is an off-flavour and off-odour of pork, mainly caused by elevated concentrations of androstenone and/or skatole in the adipose tissue of entire male pigs. A screening of organic entire male pigs showed sorting out percentages of 68 % based on skatole, androstenone and human nose (Maribo 2012), and there is reason to believe that the organic farming system will be challenged in relation to production of entire male pigs. The organic pig production system is different from the conventional system in numerous ways, e.g. stocking density, bedding, feed, production efficiency *etc*. and it is uncertain if the initiatives used for reduction of boar taint in the conventional system will also be applicable within the organic production system. Previous research within conventional farming has found an effect of slaughter weight on androstenone levels (Fàbrega *et al*, 2011; Aluwé *et al*, 2011). Reducing the slaughter weight might be a future management strategy for reducing the risk of boar taint in organic entire male pig production. The objective of the study was to investigate if the level of boar taint decreases with a decrease in slaughter weight and/or age at slaughter of organic entire male pigs.

Material and methods The study included 1467 entire male pigs (LYxD) reared in parallel on 5 organic farms. The experiment was structured into 4 batches and was conducted over a two year period. Each batch consisted of approx. 90 pigs allocated into four experimental pens. The pigs in a pen were sent for slaughter in two steps. Live weight was registered at the farms before slaughter. Carcass weight was registered at the slaughter house. Age was determined by days from farrowing to slaughter. A neck fat sample of each pig were analysed by the HPLC method to detect the androstenone and skatole concentration and by the Human Nose method (trained personnel sniffing a heated sample of neck fat) to detect samples with smell (ranging from 0-2).

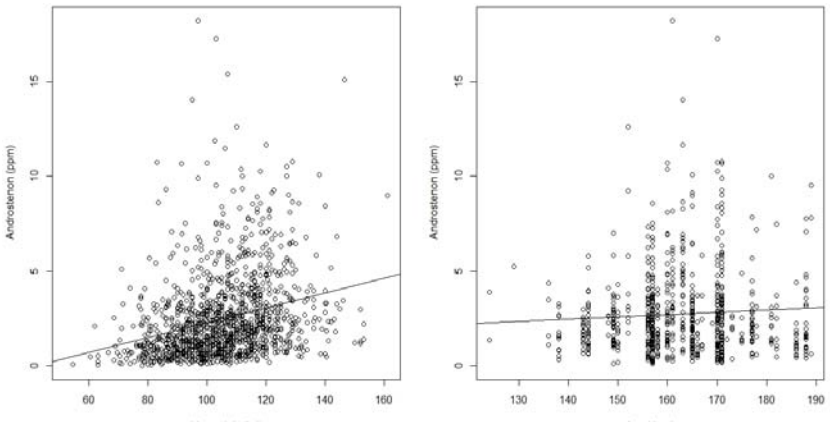

Figure 1 Concentration of androstenone as a function of live weight and age.

Results A significant effect of both live weight and carcass weight on androstenone level was found (P<0.001), with reduced weight leading to reduced androstenone concentration. A preliminary analysis revealed no direct age effect on androstenone levels (figure 1). A significant effect of herd was found (P<0.001). For an average weight of 115 kg, 74% of the pigs were sorted out based on androstenone concentration above 1ppm, 11 % based on skatole above 0.25 ppm and 19% based on the human nose method. Reducing slaughter weight reduces the percentage of sorted out carcasses. Regarding the results of the human nose method a diagnostic test showed a sensitivity of 24% and a specificity of 94%, demonstrating that a high amount of carcasses with concentrations below the threshold limits for skatole and androstenone (0.25 and 1 ppm respectively) are classified as negative (>1) by the human nose method, whereas only a low amount of carcasses with concentrations above the thresholds are classified as positive. This indicates that the androstenone threshold maybe could be raised to be above 1 ppm, or that the androstenone concentration is not as important for boar taint odour as previously presumed.
All presented results are preliminary.

Conclusion A reduction in slaughter weight reduces the level of androstenone and by that the percentage of animals sorted out at the slaughter line. Reducing weight could therefore be an element in a management strategy for reducing the risk of boar taint also in organic pig production. However, herd differences indicate that individual management procedures affect the level of boar taint.

Acknowledgements The study was funded by the Green Development and Demonstration Programme. The authors thank the participating organic farmers for their cooperation.

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Primary Culture of Porcine Intestinal Epithelial Cells

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Implications Intestinal epithelial cells are targets and/or effectors for many physiological and pathological processes, including novel drugs. Reliable methods for culturing primary epithelial cells *in vitro* would be of enormous value for studies of these processes and/or for screening biologicals without the use of animals. Recent, landmark advances in culturing human and rodent cryptoids should be developed in target animal species.

Introduction Intestinal epithelial cells facilitate immune and inflammatory responses through pattern recognition, antigen presentation and cytokine release. In many cases, the outcomes of disease are determined by the initial response of these cells to injury and the subsequent effects on cells of the immune system and of the extracellular matrix. Studies of these cells are critical for understanding immune mediated disease, host-pathogen interactions, oral tolerance and the development of mucosal vaccines (Shimazua *et al*, 2012). However, the majority of cell lines used in studies are derived from foetal, tumour or virally transformed cells and exhibit significant differences in gene and protein expression, and in overall function, from those in intestinal epithelium *in vivo* (Langerholc *et al*, 2011). It is, therefore, important to establish a robust method for primary culture of intestinal epithelium to allow direct studies of these cells and of their interactions with other, more easily culturable cells. High levels of differentiation, rapid turnover and dependence on their natural cellular interactions make these cells difficult subjects for primary culture. Recent work in humans and mice has described a system using inhibitors of cellular differentiation to culture multicellular 'cryptoids' which retain cells with the phenotype of epithelial stem cells (Yui *et al*, 2012; Aly *et al*, 2013). Porcine intestinal epithelial cells may be a suitable model for those of other monogastric species, including humans, and recent work in pigs has identified potential novel approaches to establishing them in culture.

Material and methods All pigs used in this study were 5-6 months old and were killed by electrical stunning followed by exsangunation in the University of Bristol research abattoir. Segments of jejunum and spiral colon were removed before scalding. The mucus layer and the mucosa were removed by scraping and centrifuged at low speed to remove small particulate debris. Larger material was incubated with collagenase at 37 ^{o}C to digest extracellular matrix. After washing, residual particulate material was cytospun and stained with monoclonal antibodies identifying a range of cellular molecules (E-cadherin, Polymeric Ig receptor, cytokeratin 18 and CD45). Isotype-specific secondary reagents were used to allow co-visualisation of the different molecules. Tissue culture plates were coated with human type IV (basement membrane) collagen, and seeded with isolated crypt epithelium. After one week, collagenase (100U/ml) was applied to the wells and incubated for 1hour to detach the cultured cells from the Type IV collagen. Cytospins of recovered cells were immunostained as before for cell identification.

Results Jejunal and colonic crypt structures were successfully isolated from the intestinal tract of adult pigs. Recovered structures were stained and imaged as separate z-stacks for red, green, blue and for differential interference contrast (DIC). Out of focus information was removed by post-processing and three-dimensional structures visualised. Recovered structures retained the morphology of intact crypts, including the crypt lumen, and staining with E-cadherin, pIgR and cytokeratin confirmed their epithelial nature. In short-term cultures, viable cells were present in cultures from both jejunum and colon after seven days, indicating that this approach may allow longer term culture of multicellular ‘cryptoids’. In future, the use of semi-solid medium and supplementation with additional factors affecting growth and differentiation will be assessed.

Conclusion Intact crypt epithelial structures can be recovered from pig intestine and maintained over short periods. This technique may be extended in future by inclusion of inhibitors of differentiation.

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Comparison of the microbial population in faeces of rabbits and guinea pigs by next generation sequencing

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Implications Faecal bacterial populations from rabbits and guinea pigs (two caecotrophic species) were compared in order to investigate possible differences in the nutritional role of the microbes. Significant interspecies differences were observed.

Introduction The rabbit is a global presence; it is the third most common companion animal in the world and the most economically important caecotrophic species as it is farmed for meat production in a number of countries. The rabbit gut microbiota is considered to be unique (Abecia *et al*., 2005; Monteils *et al*., 2008; Michelland *et al*., 2010) and specifically adapted to enable caecotrophy but factors affecting the bacterial population are not fully understood. Other than the rabbit, the gut microbiota of caecotrophs has been scarcely studied. Diet is typically thought to influence GI tract bacterial populations but the extent of the effects on the performance of caecotrophy and the structure of bacterial populations throughout the GI tract varies with age, health, gestation, *etc*. (de Blas and Wiseman, 1998). Currently, nutrition advice is fairly standardised across many caecotrophic species, based on the assumption of similar inter-species digestive microbiota. It is also unknown how the rabbit's gut microbiota compares to that of other caecotrophic species. The current work aims to investigate the degree of similarity between microbes in the faeces of two caecotrophic animals (the rabbit and guinea pig) and determine if there is a "caecotrophic-specific" core microbial population found in caecotrophs other than the rabbit. Such information may help with nutritional studies in other caecotrophic species.

Material and methods This metagenomic analysis study applied 16S *rRNA* gene sequencing, using an Ion Torrent PGM (Life Technologies), to perform both inter- and intra- species comparisons of the caecotrophic gut microbiota. Rabbits and guinea pigs were housed in the same pen and had access to the same food. Fresh faeces were collected from the first six adult animals from each species which was seen defecating. DNA was extracted from all samples using a QIAamp Stool Mini Kit, the 16S *rRNA* V1-V2 gene region (400 bp amplicon) amplified by PCR and the samples sequenced. Data were normalised to give uniform numbers of bacterial sequences, per faecal sample, and sequences were classified into operational taxonomic units (OTU) and identified to genus level using the Ribosomal Database Project (RDP) and EBI databases. The relative abundance of respective each genus was then compared between samples using Kruskal–Wallis one-way analysis of variance and Mann-Whitney U tests.

Results Significant differences were found between the rabbit and guinea pig faecal bacterial populations ($P<0.001$) but, when individual OTU were compared, only nine OTU were significantly different ($P<0.05$) in prevalence between rabbits and guinea pigs. The proportional prevalence of the two most numerous phyla (*Firmicutes* and *Bacteroidetes*) in the rabbit were typical for most mammals. However, the guinea pig contained approximately 80% *Bacteroidetes* species, considerably more than other mammals (Ley *et al*., 2008).

Conclusion This study observed that the guinea pig and rabbit gut microbiota have significant differences in structure (and, possibly, function) and that individuals can vary in their gut microbiota. In addition, variation between individuals existed between animals. These differences in the microbial populations utilising feed given to animals imply that dietary and nutrition advice provided for rabbits cannot automatically be applied to other caecotrophic species.

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In vivo digestibility of diets containing fungi biodegraded cowpea husks by growing rabbits

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Implications Fungi biodegraded cowpea husks will contain less lignin and other insoluble fibre fractions; therefore will be better utilized by rabbits to fill their guts and to produce VFAs for energy production.

Introduction Cowpea husk, a veritable feed stuff for rabbits is lignocellulolytic in nature, however, degrading with fungi holds prospects of reducing the lignin content thereby enhancing more soluble fibre. In the rabbit, nutrients can be hydrolysed by the endogenous intestinal enzymes and/or hydrolysed by enzymes produced by the microbiota in the hind gut. This hydrolysis can enhance the digestibility of feed stuff of high fibre such as cowpea husk. Hence this study determined the digestibility of fungi-biodegraded cowpea husk by growing rabbits.

Material and methods Cowpea husks (CH) were collected from designated centres, milled (1.0 mm sieve) and degraded through anaerobic fermentation by single and mixed cultures of selected fungi as follows: *Aspergillus niger* (ASP), *Rhizopus oligosporus* (RHZ), *Trichoderma reesei* (TRI), *A. niger +R. oligosporus* (ARH), *A. Niger + T. reesei* (ATR) and *T. reesei + R. oligosporus* (TRH) (Adedire *et al*., 2012). Seventy experimental rabbits were allotted on weight equalisation basis (average weight of 550g) into 7 treatments, with each treatment having 5 replicates and 2 rabbits per replicate. The rabbits were managed intensively for a period of 10 weeks in wooden cages of approximately 76 x 62x 42cm dimension. Fermented and unfermented crop residues were incorporated into the experimental diets to supply 10% crude fibre while the whole diet supplied 15% crude fibre. Other major ingredients in the diet were wheat offal, maize and soya bean meal. At the 9th week of the experiment, 6 rabbits (with weights closer to the average weights of the treatments) per treatment were selected for digestibility trials which lasted for a period of 7 days. Experimental feed and faeces collected during the trials were subjected to proximate analysis (AOAC, 2000) to determine apparent nutrient digestibility. Data obtained were statistically analysed with the one-way ANOVA in a completely randomised design using SAS (2002) statistical software package.

Results The results showed high digestibility of ether extract and calcium while ADF was relatively lower (on DM basis). Observation across the treatments revealed that fermented cowpea husk were better digested than UCH with the highest rate of digestibility in TRH.

Table 1 Apparent digestibility in rabbits of nutrients from fungal treatment of cowpea husk

	UCH	ASP	RHZ	TRI	ARH	ATR	TRH	s.e.m.
Dry Matter	62.87^{d}	67.58^{c}	68.80^{b}	69.33^{ab}	69.39^{ab}	69.73^{a}	69.85^{a}	0.452
Crude Protein	58.62^{b}	61.90^{a}	61.95^{a}	62.22^{a}	62.05^{a}	62.08^{a}	62.24^{a}	0.221
Crude Fibre	54.74^{d}	58.22^{c}	58.71^{c}	59.00^{c}	60.55^{b}	60.61^{b}	63.41^{a}	0.522
NDF	46.02^{c}	59.26^{b}	59.58^{b}	62.39^{a}	59.31^{b}	59.64^{b}	62.82^{a}	1.343
ADF	13.29^{c}	31.01^{b}	31.35^{ab}	31.33^{b}	31.33^{b}	31.33^{b}	32.67^{a}	1.762
Ether Extract	76.07^{a}	76.06^{abc}	76.07^{a}	76.06^{abc}	76.04^{bc}	76.03^{c}	76.06^{abc}	0.633
Ash	55.74^{a}	54.68^{b}	54.71^{b}	54.64^{b}	54.70^{b}	54.77^{b}	54.76^{b}	0.741
Calcium	63.78$_{b}$	64.24^{ab}	64.23^{ab}	64.26^{ab}	64.35^{a}	64.30^{ab}	64.41^{a}	0.373
Phosphorus	57.55	57.63	57.64	57.64	57.87	57.94	58.16	0.361

Note: $^{a-d}$, means on the same row with different superscript are significantly ($p<0.05$) different. UCH = unfermented cowpea husk

Conclusion In this study, rabbits on cowpea husk fermented with TRH were better able to digest crude fibre NDF, ADF, calcium and phosphorus than rabbits receiving other dietary treatments.

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Nutrient intake and digestibility of weaner rabbits fed graded levels of roasted pigeon pea meal

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Implications There is great potential of dietary inclusion of roasted pigeon pea meal an unconventional feed stuff in compounding rabbit feed in the future, particularly in those countries were conventional feed ingredients are expensive.

Introduction In Nigeria, conventional feedstuffs are expensive which has led to the search for cheap and locally available unconventional feeding materials. It is very obvious that the conventional sources of feed can no longer adequately meet the needs of the fast growing livestock industry. Pigeon pea is a legume crop of interest to many researchers in several countries of the world to use as a source of livestock feed ingredient. Akande and Adeleye (2013) reported that performance of rabbits were not negatively affected by up to 30% dietary inclusion of roasted pigeon pea meal.

Material and methods Forty Dutch x Chinchilla male and female rabbits with an average initial weight of 725g and between 5 and 7 weeks old were allocated to four dietary treatments. Each treatment had ten rabbits and five replicate per treatment in a completely randomized design (CRD). Pigeon pea seeds were roasted at about 80^0C for 3-5 minutes. After cooling, the seeds were then milled in a hammer mill. The processed pigeon pea meal (PPM) was used in compounding iso-nitrogenous and iso-caloric experimental diets. Treatment 1 (control) was corn-soybean based diet with 0% PPM while treatments 2, 3 and 4 contained 10, 20 and 30% PPM in the diets respectively. This research was conducted at the Rabbit Research House of the Abubakar Tafawa Balewa University, Bauchi state, Nigeria. In the fourth week of the feeding trial, faecal collection was done for seven days. The faeces were dried, bulked and weighed for nutrient digestibility determination. The acid detergent fibre (ADF) and neutral detergent fibre (NDF) of the samples were determined by the method of Goering and Van Soest (1970). Data obtained were subjected to the analysis of variance (Steel and Torrie, 1980).

Results There was no significant effect of pigeon pea meal based diets on daily live weight gain and nutrient intakes of rabbits (Table 1). Dry matter intake (DMI), organic matter intake (OMI) and acid detergent fibre intake (ADFI) had values ranging from 43.5 - 53.1g, 40.0 - 48.3g and 5.8 - 7.1g respectively for rabbits fed both 30 and 10% PPM based diets. Results for OMI and ADFI followed the same trend as observed in the DMI. CPI values varied from 7.2 to 8.9g and that of NDFI was from 10.4 to 11.2g. Results of this study also showed that digestibility of nutrients were not significantly affected by dietary treatments (Table 1). This is similar to the findings of Oso *et al.* (2012) who reported the inclusion of up to 50g/kg of processed pigeon pea meal in the diet of their experimental animals without adverse effect on nutrient digestibility.

Table 1 Daily live weight gain, nutrient intake and digestibility of rabbits fed graded levels of roasted pigeon pea meal

	Dietary levels of PPM (%)				
Parameters	0	10	20	30	SEM
Daily live weight gain (g)	14	16	13	12	1.1^{NS}
Dry matter intake (g)	50.3	53.1	52.6	43.5	2.19^{NS}
Organic matter intake (g)	45.8	48.3	48.0	40.0	2.00^{NS}
Crude protein intake (g)	8.9	8.8	8.9	7.2	0.38^{NS}
Acid detergent fibre intake (g)	6.7	7.1	7.0	5.8	0.29^{NS}
Neutral detergent fibre intake (g)	10.5	10.5	11.2	10.4	0.47^{NS}
Dry matter digestibility (%)	73.8	75.2	70.9	72.0	1.67^{NS}
Organic matter digestibility (%)	79.9	80.4	78.1	79.6	1.84^{NS}
Crude protein digestibility (%)	86.4	83.7	84.5	84.0	0.71^{NS}
Acid detergent fibre digestibility (%)	30.0	30.3	28.0	29.1	1.73^{NS}
Neutral detergent fibre digestibility (%)	49.5	43.2	45.8	48.2	1.30^{NS}

SEM = Standard error of mean ; NS = Not significant

Conclusion The results showed that pigeon pea meal can be successfully included up to 30% in the diets of weaner rabbits without adverse effect on nutrient intake and digestibility

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Growth performance of weaned rabbits fed diets supplemented with varying levels of baker's yeast (*Saccharomyces cerevisiae*)

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Implications Rabbits fed a diet supplemented with 60 g of *Saccharomyces cerevisiae* per kg of basal diet consumed more feed and had a higher total body weight gain. The most efficient feed:gain ratio was also recorded for rabbits fed the diet supplemented with 60 g of *Saccharomyces cerevisiae* per kg of basal diet.

Introduction The indices of productivity of rabbits found in Nigeria, both for growth and reproductive performance, are inferior to those of their temperate counterparts. Low levels of antibiotics have been used over the years in rabbit production as both growth promoters and for the prophylactic treatment of disease. However, the over-use of antibiotics for disease prevention and growth promotion in animal production has been implicated in the emergence of antibiotic-resistant pathogens and antibiotic residues in animal products, which is a public health concern (Falcão-e-Cunha *et al.,* 2007). *Saccharomyces cerevisiae* is a probiotic and a possible strategic alternative because of its availability, safety and cheapness. It contains a lot of proteins, carbohydrates, lipids, vitamins and minerals. The present study was aimed at evaluating the effect of *Saccharomyces cerevisiae* on the growth performance of weaned rabbits, reared under intensive management system in Nigeria.

Material and methods Sixty (60) weaned crossbred (New Zealand White x Chinchilla) rabbits in equal number of males and females, aged between 5 - 6 weeks with average initial live weight of 612.73 ± 60.84 g (mean ± SD) were used for this experiment, which lasted for 12 weeks. The rabbits were housed in a well-ventilated rabbitry in three tier-wire hutches. Each hutch measured 70 x 60 x 50 cm in length, width and height, respectively. The sixty rabbits were divided by simple randomisation into five treatment groups of 12 animals per treatment, after balancing for live weight and sex. The five treatment groups were allotted to the five experimental diets in a completely randomized design. Each treatment with 12 rabbits was divided into three replicates, each comprising four rabbits. Diet 1 (basal diet), designated as TRT_1 and without supplementation with *Saccharomyces cerevisiae*, served as the control diet. Diets 2, 3, 4 and 5, designated as TRT_2, TRT_3, TRT_4 and TRT_5, respectively, were supplemented with *Saccharomyces cerevisiae* at the rate of 20, 40, 60 and 80 g per kilogram of basal diet, corresponding to 2 x 10^9, 4 x 10^9, 6 x 10^9 and 8 x 10^9 cfu/kg of basal diet, respectively. A commercial baker's yeast, Vahine® (Avignon, Monteux, France), containing *Saccharomyces cerevisiae* was used as the dietary supplement. The diets were not pelleted. The diet and clean fresh water were offered *ad libitum*. Rabbits were weighed individually at the commencement of the experiment and, thereafter, on a weekly basis. Each morning, feed that was not consumed was weighed before cleaning the individual hutches. The amount of feed consumed was calculated as the difference between the quantity of feed offered and quantity not consumed. At the end of the experiment average daily feed intake, daily weight gain and feed:gain ratio gain were computed.

Results Rabbits fed diets supplemented with *Saccharomyces cerevisiae* at 60 g per kg of basal diet consumed significantly ($P < 0.05$) more feed (84.51 g/day) and had the highest total body weight gain (908.08 g) while rabbits that were fed diets supplemented with *Saccharomyces cerevisiae* at 20 g per kg of basal diet gained the least weight (558.92 g). Rabbits fed diets supplemented with *Saccharomyces cerevisiae* at 60 g per kg of basal diet also had the most efficient ($P < 0.05$) feed:gain ratio (7.82).

Table 1 Growth performance of weaned rabbits fed varying levels of *Saccharomyces cerevisiae*-supplemented diets

	Saccharomyces cerevisiae, g/kg of basal diet						
	0.00	20.00	40.00	60.00	80.00	s.e.m	P
Initial body weight (g)	662.50	600.50	600.83	594.17	605.67	7.85	n.s.
Feed intake (g/day)	64.85 ^{d}	68.63 ^{c}	82.53 ^{a}	84.51 ^{a}	73.90 ^{b}	1.11	<.0001
Daily weight gain (g/day)	7.97 ^{c}	6.65 ^{d}	8.85 ^{b}	10.81 ^{a}	7.75 ^{c}	0.22	<.0001
Total Body weight gain (g)	669.67 ^{c}	558.92 ^{d}	743.75 ^{b}	908.08 ^{a}	651.08 ^{c}	18.75	<.0001
Final body weight (g)	1332.17 ^{c}	1159.42 ^{d}	1344.58 ^{b}	1502.26 ^{a}	1256.75 ^{c}	20.00	<.0001
Feed:gain ratio	8.14 ^{c}	10.32 ^{a}	9.33 ^{b}	7.82 ^{c}	9.54 ^{b}	0.19	<.0001

Conclusion Baker's yeast containing *Saccharomyces cerevisiae* at 60 g per kg of basal diet improved the performance of the rabbits and could be a valuable and qualitative growth promoter for rabbits at that level.

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Multiple regression analysis for the prediction of milk yield in dairy goat herds in Greece

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Implications The dearth of prediction models in dairy goat sector is a major obstacle for evidence-based decision making policies and strategic planning at farm level. The present study proposes a useful tool for the prediction of milk yield potential in dairy goat herds, based on basic characteristics of farm structure and overall management schemes.

Introduction Dairy goat farming in Greece is a trademark of agricultural activities dating back to ancient era. The national flock in Greece which is ranked first with 39.8% of the total EU goat census. It has played a vital role in maintaining rural tradition and self-sufficiency of rural population. But, the existing high diversity in farm structure and management schemes remains unexploited. The latter results in *a priori* approaches for the classification of farms based mainly on assumptions and subjective observations. Moreover, the lack of objective typology of farms is a further barrier for the development of prediction models regarding farms' production outputs and the development of a benchmarking system for dairy goat production systems in Greece. Hence, the design of prediction models need to be based on primary data collected on-site, from representative farms. The objective of the study was to develop a prediction model of annual milk yield per goat using structural and management characteristics of goat farms as predictors.

Material and methods A total of 100 dairy goat herds (37,000 goats) were randomly selected from 20 prefectures and were surveyed from September 2011 to November 2012. Data were collected during pre-scheduled on-farm visits, using a case-specific questionnaire. It comprised questions about livestock, labour, facilities and equipment and general management practices (land use, grazing and feeding management *etc*.). A multiple stepwise regression analysis was used in order to assess the relationship between milk yield and various potential predictors assigned to farms' structural and management characteristics as described below (Model 1).

$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7$ e

where: Y: milk yield per goat per lactation, b_0: intercept, b_1-b_7: coefficients of: prolificacy X_1, concentrate per livestock unit X_2 (tn), goats replacement rate X_3 (%), facilities and equipment score X_4 (1 to 10), average grazing distance X_5 (km), cultivated land per livestock unit X_6 (ha) and weaning age (days), respectively, e: random residual.

Results Six out of the 7 variables that firstly assigned into the model, were finally used as predictors for the analysis (Table 1). The model with all six predictors accounted for about 56% of milk yield per goat per lactation variance (R^2=0.558) and produced F (6, 93) = 19.56, P<0.001. Table 1 shows summary statistics, correlations and results from the regression model. A significant positive regression coefficient was found for prolificacy (P<0.001), concentrates per livestock unit (P<0.001), goats replacement rate (P<0.001) as well as for facilities and equipment score (P<0.05). Average grazing distance and cultivated land per livestock unit had significant negative regression coefficients at P<0.05 level, whereas, weaning age did not contribute significantly to the multiple regression model.

Table 1 Summary statistics, correlations and results for the predictors from the multivariate linear regression analysis

	Unstandardized coefficients		Standardized coefficients		95% CI for B			Collinearity statistics
Variable	B	S.E.	Beta	P	Lower bound	Upper bound	Partial correlation	Tolerance
Intercept	-199.9	56.0			-311.0	-88.8		
Prolificacy	161.2	35.0	0.35	***	91.6	230.7	0.43	0.81
Concentrates per livestock unit	31.4	7.7	0.34	***	16.2	46.6	0.39	0.68
Goats replacement rate	732.0	219.1	0.24	***	297.0	1167.1	0.33	0.90
Facilities and equipment score	10.9	4.2	0.20	*	2.5	19.2	0.26	0.79
Average grazing distance	-3.7	1.8	-0.15	*	-7.3	-0.1	-0.21	0.92
Cultivated land per livestock unit	-28.5	14.1	-0.17	*	-56.4	-0.6	-0.21	0.71

*P≤0.05, ***P≤0.001

Based on the results of the multiple linear regression analysis, the suggested model for the calculation of potential milk yield per lactation is described as: Potential milk yield = $161.2 \cdot X_1 + 31.4 \cdot X_2 + 732.0 \cdot X_3 + 10.9 \cdot X_4 - 3.7 \cdot X_5 - 28.5 \cdot X_6 - 199.9$.

Conclusion The prediction of farm production outputs can be a reliable tool for the decision making process. However, the variables which will be used as predictors for the development of such prediction models need to be carefully selected and assessed on terms of objectivity and effectiveness. In any case, national scale surveys need to be considered in order such models to be developed as useful tools for the development of benchmarking systems for dairy goat production systems in Greece.

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Nutritional performance of West African dwarf goats fed wild sunflower (*Tithonia diversifolia*) leaf meal supplemented diet

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Implications Animals fed up to 10% of their diet as wild sunflower leaf meal (WSFLM) showed superior performance over those fed this as 20 or 30% of their diet, in terms of crude protein digestibility and nitrogen utilization.

Introduction Major constraint to livestock productivity in sub-Saharan Africa has been identified to be that of costly quality feeds and the effect of season (drought). As a result, the focus is on the search for alternative cheap feed resources that are readily available, especially during drought periods, and which do not compete with man's dietary demands. Wild sunflower is a freely growing shrub in Nigeria with limited processing demand as livestock feed (Odedire and Oloidi, 2011; Odunsi and Farinu, 1997). The aim of this study therefore, was to assess the nutritional response of West African dwarf (WAD) goats fed a wild sunflower leaf meal supplemented diet.

Material and methods Twenty growing WAD goats, averaging 6.94kg weight with ages ranging between 5 and 7 months, were used for the study which lasted 112 days. Goats were housed in individual pens in an open sided and well ventilated building with a concrete floor. Animals were randomly allotted to four treatment diets in a completely randomized design, with five goats per treatment. Pen floor was covered with 5cm of wood shavings to absorb urine and for easy removal of waste. Wild sunflower leaves were harvested prior to the flowering stage of the plants and air – dried for seven days and then milled. The leaves were incorporated into a concentrate diet at 0, 10, 20 and 30% levels of inclusion. The compounded diets were offered to the goats as supplement to a basal diet of *Panicum maximum* forage at 0.05 of live weight.
Growth related measurements were taken and recorded on a weekly basis, after an initial two – week period of adaption, while data on feed intake and refusals were recorded on a daily basis and feed offered adjusted to reflect any corresponding change in the weight of the animals. A digestibility trial was carried out between days 98 and 112 of the experiment. Goats were transferred to metabolism cages with facilities for separate collection of urine and faeces. Faeces and urine were collected each morning before feeding. Total faeces of individual pens were collected, weighed and recorded and ten percent of the faecal samples per animal were taken and dried in a forced-draught oven at 70^{o}C for 72 hours. The daily stored samples of faeces were bulked, thoroughly mixed, ground and sub-sampled for chemical analysis. The volume of urine voided for the 14 – day period was also recorded and subsamples preserved in a deep freezer for laboratory analysis. Samples of feeds and faeces were oven – dried at 70^{o}C for 72 hours and sub samples taken and ground in a mortar for proximate analysis as described by AOAC (2005). Data obtained were analyzed using the General Linear Model of SAS (2006) and Duncan New Multiple Range Test of same package was used to test for significant differences among means.

Results Table 1 presents the summary of the goats' nutritional performance on WSFLM supplemented diets. The crude protein contents of the diets were similar except for the control and 10% WSFLM diets ($P < 0.05$). There was no significant ($P > 0.05$) difference in the feed intake, as well as average daily gain of all the goats irrespective of the diet fed. Goats on the control and 10% WSFLM diets were able to utilize their nitrogen better ($P < 0.05$) than those on 20 and 30% WSFLM diets.

Table 1 Performance of WAD goats fed concentrate diets containing graded levels of WSFLM

Parameter	Control	10% WSFLM	20% WSFLM	30% WSFLM	SEM	P
Feed Intake (g/day)	311.5	310.5	306.2	305.2	3.87	0.6949
Average daily gain(g)	27.65	31.73	27.14	26.12	5.86	0.9145
Diet Crude Protein (g/100g)	17.20^{a}	15.60^{b}	15.50^{c}	15.46^{c}	0.01	0.0001
Diet Crude Protein Digestibility	0.685^{a}	0.681^{a}	0.626^{b}	0.580^{b}	0.02	0.0164
Urinary Nitrogen (g/day)	1.10^{a}	1.01^{a}	0.66^{b}	0.36^{c}	0.04	0.0012
Nitrogen balance (g/day)	4.10^{a}	3.65^{a}	3.01^{b}	2.98^{b}	0.37	0.0032
Nitrogen utilization	0.540^{a}	0.534^{a}	0.516^{b}	0.517^{b}	2.72	0.0223

$^{a, b, c,}$: Means within row with different superscript are significantly different ($P< 0.05$)

Conclusion WSFLM can serve as a possible dry season feed source for WAD goats. Its inclusion at 10% of the diet of WAD goats gave the best performance.

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Restricted feeding of goats during the last third of gestation modifies reproductive parameters in their female offspring

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Implications Underfeeding often occurs in gestating goats and this could have an influence on the future productivity of their female offspring

Introduction Nutritional requirements during the last third of gestation are very high in goats due to the high prolificacy of these animals. In addition, the conceptus acquires 80% of its birth weight in the last 2 months of gestation (Conway *et al*., 1996). Nutrient availability can be low at the end of gestation due to: poor feed intake due to compression of the rumen by the foetuses, under-development of the rumen papillae, which reduces the rate at which the products of rumen fermentation-synthesis are absorbed (Mayer *et al*., 1986), the use of poor quality feedstuffs, feed cost-cutting by farmers and, finally, goats can be particularly competitive at the feed trough if feed resources are limited, so that some individuals may not have enough access to the feeding trough (Conway *et al*., 1996).

Material and methods Sixty Alpine and Saanen dairy goats were artificially inseminated after synchronization and gestation was confirmed at 60d by ultrasonography. Starting at 89±3d gestation, the goats were allocated to one of two dietary treatments: control (C, n=30) or restricted (R, n=30) according to breed, age, liveweight (LW) and body condition score (BCS). During feed distribution all the R goats were individually restrained by a neck-lock for 30 min to ensure that they were able to eat undisturbed. The C group was fed *ad libitum* a TMR designed to cover requirements for the last third of gestation (INRA, 1988). The R group was given the same TMR and they received 50% of the amount of feed given to the C for 4wk, 60% for 1wk, 70% for 1wk and 80% the last 2wk of pregnancy. The R group also received *ad libitum* barley straw to limit potential behavioural problems. The diets were given at 07:00h and 15:00h. The full experimental setup has been published (Laporte-Broux *et al.,* 2011). At birth, kids were immediately separated from their dam and given *ad libitum* two meals of good quality pooled colostrum on the first day and then received a milk replacer *ad libitum* until 2mo of age. The female kids were weaned and reared together for the remainder of the experiment. They were synchronised at 8mo of age and mated. Serial blood samples were taken at mating and afterwards to measure plasma progesterone (P4). Subsequent litter size was measured in these animals. A SAS mixed model for repeated measures was used to analyse the data with diet, breed, sampling time and interaction terms as fixed effects. Dam was used as a random effect.

Results All the female offspring from R and C were pregnant after mating. There was an interaction diet*sampling time for plasma P4 (P=0.02). P4 was lower after mating in the offspring produced by the R *vs*. C dams (Table 1). There was no significant difference between the average number of kids born to these animals (R, 2.1±0.8 *vs*. C, 2.4±0.6, P>0.05).

Table 1 Effect of dam feeding level in late gestation on progesterone concentrations (ng/mL) in their female offspring after oestrous synhronisation and mating

Day	mating	+2	+4	+6	+8	+10	+15	+20	s.e.m.
Control, n = 17	<0.03	<0.03	8.0^{a}	9.8^{a}	10.6^{A}	9.9	8.7	8.0	0.38
Restricted, n = 15	0.1	0.5	6.9^{b}	8.6^{b}	9.7^{B}	9.5	8.7	8.7	0.36
P value	n.s.	n.s.	0.040	0.028	0.097	n.s.	n.s.	n.s.	

Conclusion Dietary feeding level of dams during gestation affected circulating P4 concentrations in their offspring after mating. This did not appear to be caused by differences in ovulation rate (number of progesterone secreting *corpora lutea*, CL) since prolificacy was the same between treatments. If there was no difference in CL numbers between dietary groups then there may have been an effect of gestational dietary programming on offspring: hepatic catabolism of P4 (R>C) or CL secretion of P4 (R<C).

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Physiological factors affecting the electrical conductivity of goat milk

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Implications Milk quality in goats can be greatly improved by monitoring the electrical conductivity of their milk which is an indirect indicator of nutritional and management practices applied in practice.

Introduction Milk electrical conductivity (MEC) is considered as a low-cost, rapid diagnostic tool of subclinical mastitis in dairy goats. However, current information is based on small-scale studies, whereas the role of several physiological factors on MEC in goat's milk is unclear. In the present study we measured MEC from dairy goats on a large scale experiment.

Material and methods A total of 562 dairy goats from five dairy herds were randomly selected. The goats belonged to two indigenous Greek breeds and one foreign breed, which was well-adapted to the climatic conditions of Greece. The study took place during the last two months of lactation. Each goat was subjected to a detailed clinical and udder examination. Composite milk samples (50 ml) were obtained after complete hand-milking the udders of each individual goat. The samples were then sub-divided into two test tubes. One was used for pH and MEC (at 25° C) measurements and the other for determination of milk chemical composition and SCC. A second sample of milk (50 ml) was obtained directly from the udder and was used for Total Bacterial Count (TBC). Fat, protein and lactose concentration were measured using Milkoscan, whereas Somatic Cell Counts (SCC) and TBC were measured by Fossomatic and Bactoscan, respectively. SPSS 18© was used for statistical analyses. The latter included descriptive and analytical statistics (univariate analysis of covariance). In the developed model, fat, protein, lactose concentration, SCC, TBC and pH were forced as covariates. Values of SCC and TBC were log-transformed.

Results Mean concentration of fat, protein and lactose as well as average values for SCC, TBC, pH and MEC are presented in Table 1. Mean values of SCC and TBC were higher in flocks of Damascus and Indigenous goats. Table 2 shows the effects of the parameters estimated in Model 1. The effects of fat, protein and lactose concentration were significant and negative ($P<0.001$). Moreover, the random effect of herd on MEC was, also, significant ($P<0.001$). Milk pH and animal age tended to influence MEC ($P=0.097$ and $P=0.088$, respectively). The effects of log SCC and log TBC were not significant.

Table 1 Descriptive statistics (mean, standard deviation) of milk traits in the three dairy goat breeds.

	Skopelos (n=112)		Indigenous (n=276)		Damascus (n=174)	
Milk trait	Mean	S.D.	Mean	S.D.	Mean	S.D.
Fat (%)	5.3	0.96	4.1	1.24	4.2	0.95
Protein (%)	3.8	0.42	3.7	0.46	3.6	0.47
Lactose (%)	4.5	0.20	4.4	0.23	4.2	0.24
Somatic Cell Count $x10^6$/ml	918.8	2158.61	2493.7	4192.81	3771.1	4611.70
Total Bacterial Count $x10^6$/ml	56.7	236.10	101.2	291.71	152.7	324.17
pH	6.4	0.06	6.5	0.07	6.5	0.11
Milk electrical conductivity (mS/cm^2 at 25 °C)	5.4	0.37	6.0	0.44	5.6	0.40

Table 2 The effects of Model I standardized parameters on milk electrical conductivity.

Estimated parameter	Coefficient	Degrees of freedom	F-value	P-value
Intercept	-0.313	1	0.0	0.988
Age	*	3	2.2	0.088
Herd	**	4	151.6	0.000
ZFat	-0.462	1	216.1	0.000
ZProtein	-0.192	1	61.9	0.000
ZLactose	-0.610	1	747.0	0.000
ZSomatic cells count (log)	-0.036	1	1.225	0.269
ZTotal bacterial count (log)	-0.001	1	0.001	0.976
ZPh	-0.039	1	2.762	0.097

*B-values for age: 1 Year=0.329, 2 Years=0.115, 3 Years=-0.069, 4 Years= Reference category
**B-values for herd: Herd 1=0.237, Herd 2=0.640, Herd 3=0.949, Herd 4=-0.704, Herd 5=Reference category

Conclusion MEC is not a reliable diagnostic tool for subclinical mastitis associated with high SCC and TBC in dairy goats. Milk quality is associated with MEC at the end of lactation; the observed differences among herds on MEC are probably associated with differences in nutrition and other management practices (e.g. milking procedure).

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Factors affecting lamb mortality on a sheep breeding farm

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Implications The knowledge of when and how lamb mortality occurs and the specific causes of lamb mortality could help keep the mortality rate at a minimum to provide extra income and support food security/the survival of many rural farmers.

Introduction The profitability of any sheep enterprise is largely determined by the number of lambs sold per ewe. These laudable goals are not fully achieved due to high lamb mortality in the flock, thus reducing the return on investments (Binnsa *et al*., 2000). The study seeks to determine the effect of factors such as sex, year of lambing and stage of weaning as well as disease conditions on lamb mortality in the study area.

Material and methods The project was carried out at the Ejura Sheep Breeding Station, in Ghana. Secondary data was collected on post mortem records of lambs and weaners from 2008 to 2012 on monthly and yearly basis. Data on total number of lambs born, mean birth weight, number of weaned animals, mean weaning weight and total deaths were collected from 2008-2012 on monthly and yearly basis. Descriptive statistics such as the mean, standard deviation, minimum and maximum was estimated using SPSS version 16. T-test was employed at 1 and 5 % probability levels in the analysis to determine the effect of sex on lamb mortality and the stage of weaning on mortality. Chi-square test was used to determine the association between year of lambing and lamb mortality. The major causes of mortality, the total and percentage occurrences of mortality in the study area were estimated using Microsoft office excel 2007.

Results There was no correlation between year of lambing and lamb mortality. There was a significant difference ($P< 0.01$) between the mean % mortalities when compared in ewe and ram lambs, Table 1. There was a significant difference ($P< 0.05$) between the % and mean % mortalities for pre (1-4months) and post-weaners (4-6months) Table 2. Diarrhoea was the most important cause of lamb mortality with a total of 75 occurrences representing 33.63 % Figure 1. Pneumonia was responsible for 30 occurrences followed by plant poisoning at 27 occurrences representing 13.45 and 12.11 %, respectively.

Table 1 Effect of sex on lamb mortality

	Ewe lamb	Ram lamb
Total lambs born	1247	1242
Total deaths	73	84
% mortality	5.85	6.76
Mean % mortality	5.51[b]	6.19[a]
SD	2.63	4.85

Table 2 Effect of stage of weaning on lamb mortality

	Pre-weaners	Post- weaners
Total number	2489	4060
Total deaths	157	66
% mortality	6.3[b]	1.63[a]
Mean % mortality	5.87[b]	1.64[a]
SD	3.71	0.81

[a, b]means within rows with different superscripts differ significantly; ($P<0.05$) ($P<0.01$).

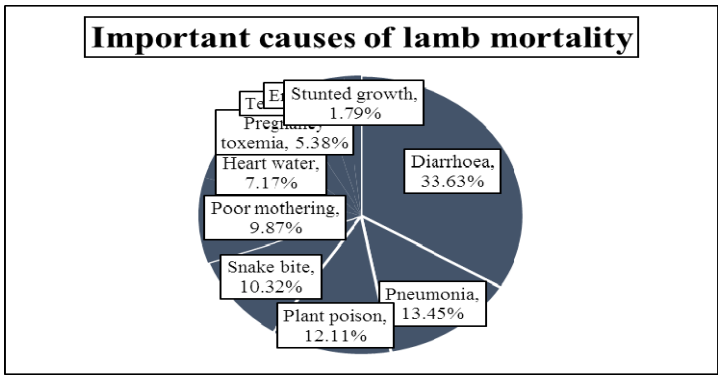

Figure 1

Conclusion The results from the study showed that higher mortality occurred in ram lambs than in ewe lambs. Pre-weaning mortality was higher than post weaning mortality. Diarrhoea, pneumonia and plant poison were the major causes of mortality in lambs and weaners in the study area.

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Breed, but not maternal under nutrition, affects markers of immune status in lambs

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Implications There are striking differences between Suffolk and Scottish Blackface lambs in various indices of health and immune status including faecal worm burdens, 'dagginess' and the expression of cytokines by the immune system.

Introduction The immune systems of mammals that give birth to precocious offspring, such as sheep, cattle and pigs, develop predominantly in utero and hence represent a potential target for pre-natal programming. We have shown that lambs born to under-nourished Suffolk, but not Scottish Blackface, ewes had higher strongyle faecal egg counts at weaning than lambs born to maintenance-fed ewes (Rooke at al., 2010), implying that offspring of different breeds respond differently to pre-natal challenges. The objective of this study was to determine the effects of pregnancy under-nutrition on measures of lamb health and immune status in two breeds of sheep.

Material and methods Twenty-eight Scottish Blackface or Suffolk lambs carried by mothers fed either a maintenance diet or a 0.75 maintenance diet between days 1and 90 of gestation (2x2 design: n=6-8 lambs per breed x pregnancy nutrition combination) were assessed for faecal egg counts (FEC) and DAG score (on a scale of 0 (no faecal soiling) to 4 (extensive soiling and dags on hocks)) between 3.5 and 4 months of age. The lambs were slaughtered between 5.5 and 6 months of age when the thymus, spleen, lymph nodes and whole blood were collected. Lamb, thymus and spleen weights were recorded. Lymphocytes were separated from whole blood and their numbers determined. Lymph nodes (pooled within animals) were snap-frozen and stored at -80^{0}C. RNA was extracted from lymph nodes and the expression of nine cytokines regarded as hallmarks of immune competence (transforming growth factor-β (TGF-β), interleukin (IL)-21, IL-7R, IL-2, IL-4, IL-6, interferon-γ (IFN-γ), IL-23α and Epstein-Barr virus induced gene 3 (EBI3) determined by qPCR, with18S and β-actin used as reference genes. Gene expression data are presented as normalised relative expression. When required, data were log or square root transformed to overcome scale effects. Data were examined for correlations and analysed by analysis of variance with slaughter weight fitted as a covariate.

Results There was a positive correlation between FEC and EBI3 ($P<0.0005$). There were negative correlations between FEC and both IFN-γ and IL-21 (both $P<0.05$), between DAG score and IFN-γ, IL-2 and IL-23α (all $P<0.05$) and between thymus weight and EBI3 ($P<0.01$). Suffolk lambs had higher DAG scores (1.615±0.38 versus 0.667±0.23; $P=0.028$), higher FEC (1352.75±273.71 eggs/g faeces; $P<0.001$) and higher expression of EBI3 (1.113 ± 0.09 versus 0.795 ±0.045; $P=0.002$) compared to Scottish Blackface lambs. Expression of IL-2 was lower in lymph nodes from Suffolk than Scottish Blackface lambs (1.506±0.20 versus 2.117 ± 0.17; $P=0.029$), respectively. No other parameters measured varied between breeds. Although there were no effects of maternal nutrition, the breed x treatment interaction affected thymus weight ($P=0.012$), such that Suffolk control-fed lambs had lighter thymus weights than lambs from the other three groups.

Conclusion This is believed to be the first study to show striking differences in indices of health and immune status between two commercial breeds of sheep prevalent in UK flocks. Furthermore the study reveals important associations between routine observations of health (FEC and DAG scores), immune organ development and the expression of key cytokines by the immune system. The lack of an effect of prenatal nutrition was unexpected. However the breed x nutrition effect on lamb thymus weight is of interest, as the thymus, which begins development during early gestation in sheep, has been proposed as a possible mediator of the effect of pre-natal nutrition on immune competence in later life (Cronje, 2003).

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Impact of environmental temperature on *in vitro* establishment rate of *Teladorsagia circumcincta*

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Implications Environmental conditions may modify the rate of gastrointestinal nematode infectivity in small ruminants.

Introduction Climate change is predicting to increase environmental temperature (T) and relative humidity (RH), and weather conditions are expected to have more extreme patterns. This has already affected diseases by changing their epidemiological patterns, leading to more clinical disease outbreaks (Taylor, 2012). Parasitic gastroenteritis, caused by gastrointestinal nematodes, is present in every farming enterprise worldwide and causes serious economic losses and decreases animal welfare. Whilst environmental sensitivity of free-living stages of gastrointestinal nematodes has been investigated (O'Connor *et al*., 2006), it is not known whether the rate of infection establishment is sensitive to environmental change. Here, we assess on the impact of different environmental T during the development of infective *Teladorsagia circumcincta* larvae (L_3) on their establishment rate.

Material and methods Faecal samples were collected from 3 donors sheep, infected with 10,000 L_3 *T. circumcincta* susceptible strains. The samples were homogenized and divided in 2 equal groups (A and B). Group A and B culture conditions were 15 °C and 27 °C, respectively, for 12 days adjusted at 70% RH. After 12 days, L_3 were retrieved from the coprocultures under the same conditions that the cultures were held. The L_3 were exsheathed and baermannized at 38 °C in 0.85% physiological saline to remove *Strongyloides papillosus* contamination. A third group of larvae (C) was derived form a coproculture of 10 days at 23 °C without RH adjustment, and after retrieval L_3 were stored at 4 °C for 2 months. L_3 from group C were also exsheathed but were not baermannized. ~700 exsheathed L_3 on average of A, B and C were each applied on triplicate sheep abomasal explants, using three sheep for each of two experimental rounds, and incubated at 38 °C for 3 h in an oxygen-enriched incubator as described previously (Jackson *et al*., 2004). This resulted in six biological replicates for each of the three conditions investigated. Tissue explants and culture plates were then washed and rinsed to retrieve L_3 that did not establish on the tissue (X). The number of L_3 in X was estimated through counting L_3 in a 10% sub-sample. The tissue explant was incubated in a digestion solution for 12 h at 38 °C, followed by processing with 16% HCL, diethyl ether and Lugol solution, to release the tissue associated L_3 (Y). The rate of larval establishment (LE, in %) was estimated as $(Y/(X+Y) \times 100\%$. The square-root arcsine transformation was applied to LE (as proportion) prior to statistical analysis through ANOVA. Experimental round and total number of L_3 retrieved were initially used as block and covariate, respectively, but omitted from the final model used as they did not significantly affect LE. A set of orthogonal contrast statements was used to assess the effect of controlled L_3 culturing *per se* (C vs (A+B)) and the effect of T through controlled L_3 culturing (A vs B).

Results Table 1 shows that the controlled culturing *per se* did not affect *in vitro* L_3 establishment rate, but that treatment A resulted in a significantly greater *in vitro* L_3 establishment than treatment B ($P < 0.05$). It was qualitatively observed that culture conditions A resulted in a smaller amount of L_3 than B arising from coproculture.

Table 1 *In vitro* L_3 establishment rate on abomasal explants of larvae cultured at 70% relative humidity and 15 °C (A) or 27 °C (B) or under uncontrolled conditions (C).

					P-values	
Group	A	B	C	s.e.d.	C vs A+B	A vs B
√arcsine LE	0.49	0.24	0.35	0.11	0.829	0.041
LE% (arithmetic mean)	29.21	10.57	14.22			

Conclusion This data support the view that environmental conditions under which gastrointestinal nematodes develop from egg into infective larvae can impact on establishment rate, and potentially resulting in variation in the impact of parasitism on sheep performance and welfare. This will be further examined under different T and RH combinations, and outcomes are expected to inform models predicting the impact of environmental change on parasite epidemiology.

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In vivo validation of the antiparasitic properties of medicinal plants

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Implications This work suggests that *Albenzia anthelmintica* and *Dodonea angustifolia* may contribute to plant-based parasite control strategies by small holder farmers in Ethiopia and other countries where these plants are indigenous.

Introduction Despite the wide ethno-veterinary use of plant-based parasite control strategies in developing countries, scientific evidence on their efficacy and side effects is scarce. We have identified a large number of plants originating from South Omo region of Ethiopia with purported anti-parasitic activity (Tolossa *et al*, 2013). Here, we validate *in vivo* the anthelmintic efficacy and performance side effects of two plants, *Albenzia anthelmintica* (AA) and *Dodonea angustifolia* (DA), which both showed very high anthelmintic efficacy *in vitro* (Athanasiadou *et al*, 2013).

Material and methods Eighty 4-month old indoor reared, individually housed Suffolk-cross lambs were either dosed with 15,000 infective *Teladorsagia circumcincta* larvae (I) or sham-infected with water (SI) on day 1. Lambs were drenched once either with 400 ml of water alone, or 400 ml of water containing either 50 or 100g of either dried AA roots (AA50 and AA100) or DA leaves (DA50 and DA100) on day 36. Animals were offered nutritionally improved straw pellets *ad libitum* and 1 kg of lamb concentrate pellets daily. Intake of straw pellets was measured twice weekly and lambs were weighed weekly. Faecal samples were collected weekly up to day 35 and twice weekly after that, to assess the number of eggs per gram fresh faeces (FEC). Mean daily feed intake over days 1-21 (pre-patent period), 22-35 (patent period), 36-42 (wk 1 post drenching) and 43-49 (wk 2 post drenching) were analysed using ANOVA with pre-infection daily feed intake as covariate. FEC were square root transformed prior to repeated measurements ANOVA using pre-drench FEC as covariate

Results Infection did not affect feed intake during days 1-21, though tended to reduce feed intake during days 22-35 (P=0.09; Table 1). Post-drenching, AA100 lambs had lower intake compared to the others during days 36-42 though this effect disappeared during days 43-49 (Table 1). Infection and plant treatment did not affect live weight gain (P>0.10; data not shown). Time and plant treatment tended to interact on FEC (P=0.07); AA100 and DA50 lambs showed lower FEC at 2 time points post-drenching compared to the other lambs (Figure 1).

	Feed intake (g/d)				
	Pre- drenching			Post- drenching	
Infection	Days 1-21	Days 22-35	Plant Treatment	Days 36-42	Days 43-49
SI	2054	2071	Water	2275	2216
			AA50	2234	2288
			AA100	2071	2138
			DA50	2291	2251
			DA100	2300	2264
I	2021	2015	Water	2240	2241
			AA50	2230	2243
			AA100	2118	2230
			DA50	2232	2188
			DA100	2255	2326
s.e.d	21	34		72	74
P-values					
Infection	0.11	0.09	Infection	0.55	0.66
			Plant	<0.01	0.17
			Interaction	0.84	0.60

Table 1 Feed intake during the whole experiment

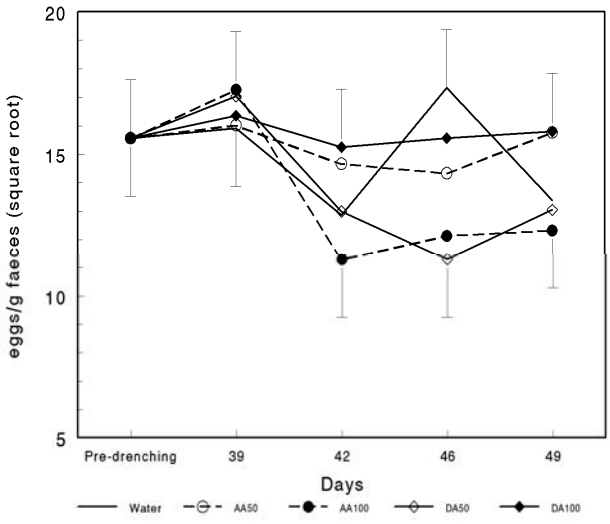

Figure 1 FEC of parasitized lambs. Bars are s.e.d of means

Conclusion Drenching with AA and DA reduced FEC though high levels of AA also resulted in short-lived penalties on feed intake. A full cost-benefit analysis would help determine whether such anthelmintic benefits outweigh the costs of the short-lived penalties on intake in parasitized animals.

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Performance of three Nigerian indigenous sheep experimentally infected with *Trypanosoma vivax*

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Implications The welfare of Nigeria indigenous sheep has been continually threatened with trypanosomes of which Trypanosoma vivax (T.vivax) is one of the most dangerous and least studied in sub-Saharan Africa.

Introduction West African Dwarf sheep have been known to be the only trypanotolerant sheep breed in Nigeria (Osaer *et al*., 1994), hence there is a need to study if other breeds are also tolerant to trypanosomes especially *T. vivax*. The aim of the study was to determine the response of Nigerian sheep breeds to *T. vivax* infection.

Material and methods This experiment was carried out in accordance with the animal production ethics as approved by the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. A total number of 24 sheep were used for the experiment, 5 sheep of each breed were infected and 3 sheep of each breed were used for control. The sheep were housed and reared in a well fenced semi-intensive environment. They were fed basal diets consisting of forages in the morning and concentrates (16%) in the afternoon. The experiment was subjected to a 2x3 factorial arrangement. *T. vivax* used molecularly detected from cattle blood was inoculated into mice and later into goat. Goat blood was screened for parasitemia every day till parasitemia level was high. On 14-day post infection, 0.5ml of *T. vivax* infected blood of goat was used to infect 15 trypanosome-free sheep (5 of each of Yankassa, West African Dwarf and Ouda). Blood samples were collected via the jugular vein and packed cell volume (PCV) was estimated using a haematocrit centrifuge and reader during the infection and treatment phases. The weights of animals were measured using weighing balance. PCV and live-weight were monitored twice weekly for 6 weeks. **Treatment phase**-Treatment was carried out using diminazine aceturate at a dosage of 7mg/kg. Liveweights, PCV levels and parasitemia clearance were monitored twice weekly for 60 days in order to know the response to treatment. Data were analysed using a two-way analysis of variance and means were separated using Duncan Multiple Range Test. Probability levels <0.05 were described as significant. Minitab 16 statistical software was used.

Results There was no breed effect on PCV and Liveweight of sheep infected with *T. vivax* during the infection phase. However, PCV of infected sheep were significantly lower from days 7-42 during infection phase and days 0-25 during treatment phase. At the treatment phase, the infected sheep only had a reduced liveweight (control-26.72kg, Infected-22.28kg; P=0.02) on day 0 of the treatment phase (first day of treatment). There was also no breed effect on liveweight of sheep infected with *T. vivax* during treatment phase.

Table PCV and Live-weight of sheep experimentally infected with *T. vivax* at Infection phase

	PCV(%)					LIVE-WEIGHT (kg)			
Days	Control	Infected	SEM	P.value	Days	Control	Infected	SEM	P.value
0	26.6	27.1	1.21	0.70	0	23.56	24.00	1.14	0.76
4	28.0	25.8	1.73	0.30	4	23.33	23.73	1.33	0.81
7	27.7	23.3	1.48	0.03	7	24.83	22.20	1.45	0.16
11	26.4	21.7	1.43	0.02	11	24.67	24.73	1.35	0.97
14	26.7	19.7	1.66	0.003	14	25.00	25.00	1.40	1.00
18	26.0	18.6	1.25	<0.01	18	26.72	23.70	1.23	0.07
21	24.3	18.2	0.78	<0.01	21	24.56	24.22	1.26	0.84
25	25.6	18.6	1.21	0.002	25	25.61	23.75	1.94	0.50
28	25.8	18.3	1.20	0.002	28	23.44	21.06	1.04	0.13
32	25.9	18.4	0.80	<0.01	32	23.00	21.03	1.24	0.28
35	24.8	17.0	0.83	<0.01	35	24.28	23.44	1.26	0.68
39	25.0	18.0	1.21	0.002	39	26.17	24.56	1.66	0.55
42	25.22	20.50	1.23	0.02	42	25.78	26.67	1.72	0.78

Conclusion The effect of trypanosomes on PCV of WAD, Ouda and Yankassa breeds of sheep was not significantly different showing no breed effect on trypanosome infection. So also, the study showed that the PCV of animals infected with *T. vivax* parasite could get better only after three weeks post-treatment

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Bovine Tuberculosis: host-pathogen interactions

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Implications Understanding host-pathogen interactions and the nature of the immune response induced by infection and/or vaccination is key to the development of improved control strategies for bovine tuberculosis.

Introduction Bovine tuberculosis (TB) caused by infection with *Mycobacterium bovis* (*M. bovis*) continues to increase in incidence in UK cattle herds with significant economic impact. Current control strategies including the skin test and slaughter regime are insufficient to control the disease and additional measures are required. Vaccination has been proposed as a cornerstone of future TB control strategies. The only licenced TB vaccine for humans is BCG (Bacille Calmette Guerin) which has reported variable efficacy in both humans and cattle. We have investigated the potential for BCG to protect calves against bovine TB and studied the underlying immune responses that may account for the success or failure of BCG vaccination.

Material and methods Cattle were vaccinated with BCG as neonates or as adults. At varying time-points post-vaccination cattle were infected with *M. bovis* and subsequently assessed for the presence of TB lesions and bacterial colonisation. Immune responses were compared in vaccinated-protected and vaccinated-infected cattle to determine immune correlates of protection. These parameters included function, phenotype and number of specific cell populations and cytokine expression profiles.

Results BCG vaccination of neonatal calves induced strong cell mediated immune responses and alterations in the function and phenotype of innate cells which drive protective adaptive immunity. These responses were less evident in adult cattle suggesting that targeting of specific types of immune cells in young calves contributes to the effectiveness of BCG as a protective vaccine [1]. In experimental challenge studies BCG vaccination of neonatal calves reduced the number, size and distribution of TB lesions and significantly reduced the level of bacterial colonisation in the respiratory tract [2,3]. The protection induced by BCG was shown to last for at least 12 months indicating relatively long duration of immunity [4].

Conclusion BCG vaccination of young calves induces a significant degree of protective immunity against infection with *M. bovis* and could contribute significantly to the control of bovine TB. The success of BCG vaccination is correlated with the induction of specific cell types and further investigation of these responses could lead to the identification of improved vaccines to stimulate specific parts of the immune response for improved immunity against infection.

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Transcriptional profiling and DNA methylation analysis of CD4+ T cells from *Mycobacterium bovis* infected cattle

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Implications Identification of genes involved in regulating the cell-mediated response to tuberculosis in cattle.
Understanding of whether DNA methylation changes occur as a result of *Mycobacterium bovis* infection-specific epigenetic marks could be potential targets for improving animal health in the future

Introduction Cell mediated immunity, in particular activation of Th1-type $CD4^+$ T cells and production of Th1-type cytokines are essential for protection against bovine tuberculosis (BTB) (Pollock *et al.* 2005). Whilst omic approaches have aided in the study of the immune response to BTB, transcriptomic analysis of $CD4^+$ T cells during infection has not been carried out in cattle, to date. DNA methylation which is an essential epigenetic modification for regulating gene expression has been reported to change in response to disease as well as during cellular differentiation of CD4+ T cells (Absher *et al.* 2013). Analysing the transcriptome and corresponding DNA methylation patterns in this crucial cell type contributes to the understanding of the immune response to infection as well as the role of DNA methylation in regulating this response. Therefore, the aim of this study was to identify both differentially expressed genes in circulating CD4+ T cells and genome wide DNA methylation changes that regulate T cell function in response to BTB.

Material and methods Age-matched Holstein-Friesian bullocks from the TB reactor herd at the Department of Agriculture, Food and the Marine, Co. Kildare, Ireland as well as from a farm free of TB >5 years were blood sampled and $CD4^+$ T cells were isolated using an antibody against CD4 (clone CC8). Total RNA was extracted from these cells from both the healthy (n=5) and TB infected (n=5) animals and RINs >8 obtained. Illumina TruSeq mRNA libraries were prepared from 250ng total RNA from each sample. In parallel, genomic DNA from the same cells was purified and EpiQuest RRBS libraries were prepared from 500ng genomic DNA. Both sets of libraries were sequenced on the Illumina HiSeq platform. The RNA-seq dataset was mapped to the genome using Tophat v2.0.8 and count data was collected using HTSeq-count. Differential gene expression analysis was carried out using EdgeR and pathway analysis was carried out using IPA (Ingenuity). The DNA methylation dataset was quality and adapter trimmed using Trim Galore, mapped to the genome using Bismark and analysed using the mapped sequence analysis tool Seqmonk and the UCSC genome browser.

Results Ninety-three genes were differentially expressed in $CD4^+$ T cells between BTB infected and healthy control cattle (adjusted p value, <0.05). Seventy-one genes were upregulated in the infected animals and 22 genes were downregulated. Upregulated immune genes include CCL4, CXCR4, the lymphocyte activation marker CD69 and marker of Dc maturation CD83. DNA methylation levels across genes and promoters were calculated and all biological replicates were found to have similar methylation levels across these features. Gene bodies were found to have a median DNA methylation level of 60% whereas promoter regions were less methylated, with a median methylation level of 20%. Ongoing analysis is examining differentially methylated cytosines and whether any relationship exists between the differentially methylated regions and the observed gene expression levels.

Conclusion This study has uncovered differential expression of genes in a key cell subset regulating the cell-mediated immune response to BTB infection. Some of these genes are known to encode for proteins involved in cell mediated immunity and T cell function. Global methylation levels across genes and promoters were the same for both groups however interesting differences in methylation of specific genomic regions is emerging between groups. The ongoing examination of the relationship between site specific DNA methylation and gene expression will provide a novel insight into the role of methylation in the regulation of T cell function in cattle with TB.

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Dynamics of the major acute phase proteins in milk during a *Streptococcus uberis* mastitis challenge

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Implications The dynamics of acute phase proteins, haptoglobin and serum amyloid A highlights their potential usefulness in monitoring inflammation and resolution of infection in udders of dairy cows, using milk samples

Introduction The use of acute phase proteins (APP) in the diagnosis of mastitis, which is the most costly disease affecting the dairy industry, is becoming important as an alternative and complementary procedure to somatic cell counts (SCC) which have been shown to vary with other non-inflammatory conditions of the udder in the dairy cow. The most important APP in milk associated with inflammatory conditions of the mammary gland are mammary associated serum amyloid A3 (M-SAA3, SAA) and Haptoglobin (Hp) (Eckersall *et al.,* 2001). *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus uberis* have been recognized as some of the most prevalent causes of bovine mastitis. Streptococcal mastitis in particular has been observed to be difficult to control (Zadoks, 2007). Previous studies on *S. uberis* mastitis have shown the sensitivity of milk APP in indicating the presence of inflammation in response to infection, although they were measured for only 12 hours after infection (Pedersen *et al.,* 2003). The aim of this study is to further evaluate the usefulness of the APP, Hp and SAA for identifying new infections and in reflecting the stage of disease during the resolution of infection, following an intramammary challenge, as well as to gain a better understanding of host response to *S. uberis* mastitis as it relates to secretion of these APP.

Material and methods Intramammary challenge of six (6) udder quarters of six mid-lactation cows were carried out using a host-adapted *S. uberis* strain FSL Z1-048 (Tassi *et al.,* 2013). All quarters were shown to be free of *S. uberis* infection by bacteriology prior to challenge. Quarters adjacent to those challenged, were used as controls and inoculated with sterile phosphate buffered saline (PBS). Milk samples collected at six time points; just before challenge (0 hours), 36, 42, 57, 81 and 312 hours (h) post-challenge, was defatted and stored at -20ºC prior to assay for the APP. Hp was measured by an in-house enzyme linked immunosorbent assay (ELISA) protocol optimized and validated for milk Hp measurement, while for SAA a commercial ELISA kit was used. Means and standard error of means (SEM) of APP were determined using Excel version 2007.

Results Hp concentrations in milk peaked at 57 h in all infected quarters, increasing more than several hundred-fold from basal values (< 6 ng/ml), peak SAA were noticed at the 81 h. In control quarters, however, APP remained at basal levels for the entire period of challenge. The Hp course for the IMC closely trailed the dynamics of cytokines such as TNFα, IL-8 and IL-1β in the milk as demonstrated by Tassi *et al.* (2013). The Hp and SAA concentrations had not reverted to basal levels by the end of the collection period at 312 h (Figure 1).

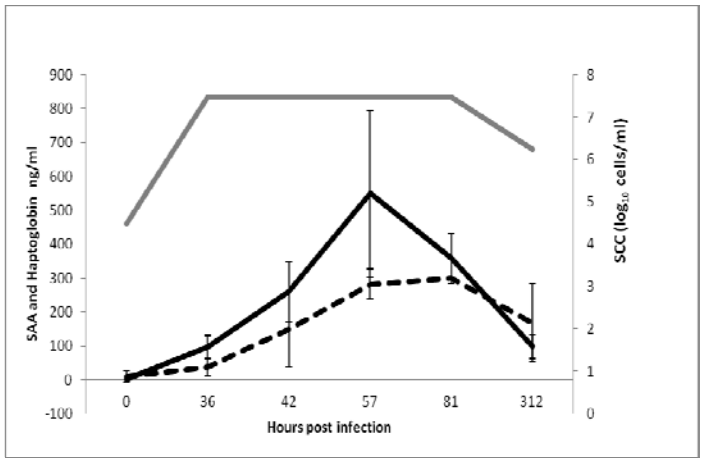

Figure 1 Mean ± SEM of Hp (solid black line), SAA (dashed black line) and $\log_{10}$SCC (grey line).

Conclusion Hp and to a lesser extent MAA, closely mirrored the trend of bacteriological counts (not shown) as it dropped in comparison to SCC; hence, in addition to its potential as a marker for inflammation, Hp (and SAA) assay, could also be indicative of resolution in mastitis conditions. Further investigation is required by including non-resolving cases.

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Host responses to virulent and avirulent strains of *Clostridium perfringens* as measured *in situ* in broilers

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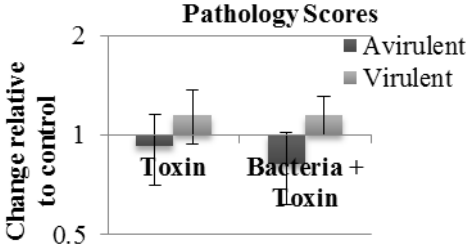
Implications Early host responses to *Clostridium perfringens* do not appear to depend on strain virulence; further development of this work can inform disease prevention strategies, e.g., vaccine development and feed additives.

Introduction Necrotic Enteritis (NE) is an enteric disease of broilers, which has major financial implications for the poultry industry. Antibiotics are commonly used as a treatment for NE but there is pressure in all areas of meat production to reduce their use. The main causative agent is *C. perfringens* type A, which express several toxins, including NetB. Host responses to the disease are not well described, due to the complex pathogenesis and difficulties in reproducing the disease experimentally. In a recent time course experiment (Russell *et al*, 2013) the temporal expression of genes related to innate responses and disease pathogenesis were identified following exposure to crude *C. perfringens* toxin over 4 hours. Here, we use an *in situ* broiler model (Athanasiadou *et al*, 2011) to investigate early host response to a virulent (CP4), NetB positive, and an avirulent (CP5), NetB negative, strain of *C. perfringens* (Thompson *et al*., 2006) and determine host responses in the absence and presence of the bacteria.

Material and methods Twenty three-week-old Ross broilers were assigned to receive the virulent (n=10) or the avirulent strain (n=10). Three, *in situ*, isolated duodenal loops were prepared surgically in anaesthetised birds (Athanasiadou *et al*, 2011). Each loop within a bird was injected with one of three different preparations: control (bacterial growth medium); *C. perfringens* crude toxin alone; or a *C. perfringens* bacteria and crude toxin combined. Four hours post-injection birds were euthanized and intestinal segments removed for histology and gene expression analysis. Hematoxylin and eosin-stained sections were scored (1-3) for pathology, with 1 = "no pathology". Expression of genes related to cell death (*FAS* & *GIMAP8*), immune cell activity (*BCL6, NBL1, B-LA*) and innate immunity (*IL-6*) was assessed. Fold change in gene expression between control and the other treatments were calculated and log transformed before a two-way ANOVA was carried out to determine differential expression between strains (Avirulent vs Virulent) and loop treatments (Toxin vs Bacteria + Toxin).



Results Pathology scores of treated loops were similar to control chambers, with sections from birds that received the virulent strain showing slightly higher scores. *B-LA, BCL6* and *FAS* expression were differentially expressed as a consequence of the loop treatment (p=<0.001) but there was no difference between the virulent and avirulent strains. *GIMAP8, NBL1* and *IL-6* gene expression was similar between all comparisons.

Figure 1 Average pathology scores shown as change relative to control at 4h.

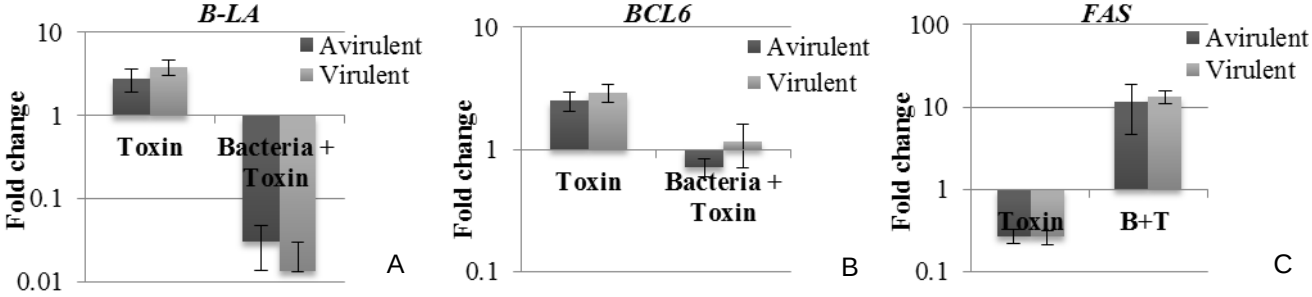


Figure 2 Gene expression (A, B & C) at 4h shown as fold change from the control. Fold change <1 indicates a decrease and >1 an increase in expression compared to control loops.

Conclusion The results from this study show few changes in pathology and gene expression between virulent and avirulent strains of *C. perfringens*. The addition of bacteria in the loops resulted in changes in the expression of some of the genes investigated. Expression of *B-LA* and *BCL6*, genes associated with antigen presentation and inflammatory signalling in macrophages respectively, was down-regulated in loops that included bacteria and the response was similar in both strains. This down-regulation may be indicative of bacterial survival mechanisms against the host's innate immune responses.

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Response to *Teladorsagia circumcincta* infection in Scottish Blackface lambs selected for divergent phenotypes for resistance

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Implications A phenotypic selection model reliably identified lambs that differed in susceptibility to gastrointestinal nematode infection. Sequencing of the abomasal lymph node transcriptome from resistant and susceptible lambs (based on the model) identified a number of genes differentially expressed between resistant and susceptible animals.

Introduction Gastrointestinal nematodes (GIN) are a serious cause of morbidity and mortality in grazing ruminants. Infected lambs have a reduced ability to absorb nutrients from the gastrointestinal tract resulting in ill-thrift and occasional death. Sub-clinical infection adds to the production losses, in the form of reduced growth rate and light, under-finished carcasses. Anthelmintic drenching has been the method of choice for nematode control for the last 50 years. However, consumer concerns about food products from animals subjected to chemical treatment, combined with the inevitable evolution of anthelmintic resistant nematodes means alternative, sustainable methods of nematode control are required. A sustainable method of nematode control is to select for genetically resistant individuals. Selection on phenotype (faecal egg count, FEC) requires detailed trait measurement. This is time-consuming, unappealing and expensive. Selecting resistant animals would be simplified if animals could be selected by genotype; this could also accelerate genetic gain. A detailed understanding of the genes and mechanisms involved in protective immunity and the factors that regulate this response would also aid the development of effective and sustainable nematode control methods, such as immunomodulatory anthelmintics. The aim of this study was to identify genes and biological process mediating the response to nematodes.

Material and methods Purebred lambs born in a Scottish Blackface flock at the Teagasc Hill Farm in Leenane, Co. Mayo, Ireland were used to identify individuals that differ in resistance to GIN. Lambs, grazed together from birth, were monitored for FEC (2 samples from 2 independent natural infections). These observations were used to identify the most resistant and susceptible individuals at about 7 months of age. Mixed model procedures were used to estimate the individual animal values for ln(FEC+25). This selection procedure was replicated, in time, across 2 cohorts (~90 lambs/cohort) with 10 resistant (Low FEC) and 10 susceptible (High FEC) individuals selected per cohort. The selected lambs were challenged with 30,000 *T. circumcincta* larvae (L3) under controlled conditions. One of the cohorts was used to verify that the difference in resistance (High v. Low) was reproduced by monitoring FEC until 71 days post-infection (DPI), when they were slaughtered. The other cohort was used to define the acute responses to infection; these lambs were slaughtered at either 7 or 14 DPI. Response to infection was monitored by measuring FEC (every 2 or 3 days), serum pepsinogen (weekly) and haematology parameters (weekly) over the course of infection in the cohort slaughtered at 71 DPI and at slaughter for the other cohort. Worm burden at slaughter was determined in all cases. The data were analysed, following transformation where appropriate, by fitting linear models with animal effects as random where repeated measures were involved. Genes associated with GIN resistance were identified using transcriptional profiling of the abomasal lymph node.

Results For the cohort slaughtered at 71 DPI the susceptible group had significantly higher FEC than the resistant group ($P < 0.05$), validating the selection method. The peak difference between the two lines occurred 8 weeks post infection (330 vs 100 EPG). Worm burden was not significantly different at 7 or 14 DPI between the resistant and susceptible groups consistent with previous studies, which showed that the time taken for a resistant animal to expel the worms is greater than 14 days (Hassan *et al*. 2011). A higher number of circulating basophils were observed in resistant animals, characteristic of an anti-parasitic immune response and induction of a Th2 type immune response. Monocyte numbers and serum pepsinogen also increased from 7 to 14 DPI. A number of genes (21) were shown to be differentially expressed between resistant and susceptible animals and these were involved in the immune response to infection and the positive regulation of type 2 immune response; demonstrating the importance of these biological processes in mediating GIN resistance.

Conclusion A selection model was developed that reliably identified Scottish Blackface lambs with divergent phenotypes for resistance to gastrointestinal nematodes. Differences were observed in FEC and circulating immune cells between high and low FEC lambs challenged with *T. circumcincta.* Sequencing of the abomasal lymph node transcriptome identified genes differentially expressed between high and low FEC animals. A number of these genes have previously been reported as being involved in mediating resistance to gastrointestinal nematode infection.

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Urinary cortisol and cortisone concentrations in Holstein Friesian young bulls as markers of pre-slaughter handling

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Implications Young bulls held in lairage for a longer period (≥90 minutes) had lower levels of urinary cortisol and cortisone. Longer lairage holding periods may allow animal recovery from stressors prior to slaughter.

Introduction Pre-slaughter stress of cattle including mixing, transport, lairage and handling by stockmen are major factors resulting in detrimental meat quality (Ferguson and Warner, 2008). Cortisol is the principle hormone utilised as a marker to assess stress from exposure to pre-slaughter animal handling practices. During stressful situations type 2 11-β-hydroxysteroid dehydrogenase (11β-HSD) converts cortisol to the inactive metabolite cortisone preventing damage to mineralocorticoid receptors from over-exposure to cortisol (McWhinney *et al*., 2010), suggesting that cortisone itself may find use as an additional assessment marker of pre-slaughter stress. This study aimed to ascertain if urinary cortisol and cortisone measurements could be used to evaluate stress pre-slaughter of young Holstein Friesian bulls and help identify optimum lairage handling conditions to minimise animal stress.

Material and methods A total of 298 under 16 month old Holstein Friesian young bulls were selected at random at a commercial abattoir over a 3 month period (May to August). Information on lairage durations prior to slaughter of all animals was obtained. Urine was obtained at the evisceration stage post mortem, placed immediately on dry ice and stored at -80^{o}C prior to analysis. Urinary unconjugated cortisol and cortisone concentrations were determined by Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (UPLC-MS/MS) analysis as described by McWhinney *et al*., (2010) with minor modifications. Urine sample preparation involved a solid phase extraction (SPE) process prior to separation and analysis on an Acquity UPLC system coupled to a Micromass Quattro Premier XE (Waters, UK) mass spectrometer maintained in electrospray ionisation positive (ESI+) mode with multiple reactant monitoring (MRM) acquisition. Quantification was performed using an extracted matrix matched calibration curve that was plotted using the response factor (analyte area (internal standard concentration / internal standard area)). Cortisol and cortisone concentrations in urine were reported relative to creatinine concentrations determined using a colorimetric reaction modified from Jaffe's Method. Statistical analysis was performed using ANOVA to determine the effect of three different lairage durations on urinary cortisol and cortisone levels, and Pearson correlations conducted to assess the relationship between urinary cortisol/cortisone concentrations in individual animals.

Results Concentrations of urinary cortisol observed in monitored animals ranged from 5.4 to 346.1 ng mg creatinine^{-1} and for urinary cortisone from 3.3 to 259.7 ng mg creatinine^{-1}**.** A significant correlation (P <0.001) was found between measured cortisol and cortisone levels in urine with an R^2 = 0.72. Urinary cortisol and cortisone levels were significantly lower (Table 1) in young bulls held in lairage for more than 90 minutes compared to animals held for shorter time periods.

Table 1 Urinary cortisol and cortisone concentrations in groups of young bulls held in lairage for different durations.

Lairage duration	≤45min	>45<90min	≥90min	SEM	Significance
(n)	(112)	(150)	(36)		
Cortisol (ng mg creatinine^{-1})	83.7^{a}	67.6^{a}	35.7^{b}	3.85	***
Cortisone (ng mg creatinine^{-1})	49.62^{a}	49.28^{a}	26.54^{b}	2.43	***

*** P<0.001; means with common superscripts are not significantly different; SEM, standard error of mean

Conclusion Wide variations in urinary cortisol and cortisone values were found in young bulls pre-slaughter, which were shown to be associated with the duration of holding within lairage. The significant correlation found between cortisol and cortisone levels indicates the potential usefulness of utilising cortisone measurements alongside cortisol to provide additional stress measurements. However, cortisol measurements were shown to best reflect the actual length of time animals spent within lairage. The lower levels in longer lairage may represent recovery from previous stressors or that this longer lairage did not involve additional stress. Further work is required to interpret the urinary cortisol response in relation to both animal welfare and meat quality.

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Characterising the fatty acid profile of beef supplied through a premium Welsh beef supply-chain

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Implications Increasing unsaturated and decreasing saturated fat intake is beneficial to human health. Forage feeding improves fatty acid (FA) profile of beef (Morgan *et al*, 2013). This study illustrates this trend within a commercial setting.

Introduction Efficient management and use of forage is a key driver in the nutritional management of cattle since it is a relatively cheap feed. However during the finishing period, it may be necessary to supplement forage with nutrient dense concentrates to maximise weight gain, meat yield and fat cover. The particular beef supply-chain which participated in this study use an exclusive concentrate blend which is fortified with vitamin E, and is offered to the beef producers either as a straight or as part of an on-farm mixed compound. The objective of this study was to determine the FA profile of beef supplied through a forage-based beef supply chain, and identify the effects seasonality of diet has on the FA profile.

Material and methods A total of 116 meat samples were collected on 6 different sampling dates between December 2011 and October 2012. Animals were slaughtered at one of three registered abattoirs used by the supply-chain. Samples of 1-2cm thick were collected at the 10th rib loin area from the hind-quarter of both sides of the carcass 7-9d post-mortem, and categorised based on finishing ration of the animal into one of six dietary regimes: Grass silage & compound feed (SB), Grass silage & straight (SN), Grass silage, straight & home-grown cereal (SNC), Grazed grass (GG), Grass & compound feed (GB) and Grass, straight &/or home-grown cereal (GNC). FAME analysis was carried out on freeze-dried meat as detailed by Lee *et al.* (2012). Abnormally distributed data were log transformed and analysed using GenStat14 by an unbalanced one-way analysis of variance (ANOVA). Mean comparisons carried out via least significant difference (LSD).

Results SNC was highest whereas GNC was lowest for SFA (1122.02 vs. 645.65 mg/100g), MUFA (1066.60 vs. 625.17 mg/100g) and Total FA (2716.44 vs. 1690.44 mg/100g). SNC was also highest for 18:2n-6 while GG was lowest (94.19 vs. 62.18). The same trend was found for PUFA content however this was not significant. GG was highest for 18:3n-3 (30.90mg/100g), however GB was marginally higher than GG for EPA+DHA (20.54mg/100g).

Table 1 Fatty acid profile (mg/100g) of beef muscle supplied through a premium Welsh beef supply-chain, categorised into different dietary finishing regimes.

	Diet								
	SB (n=21)	SN (n=18)	SNC (n=15)	GG (n=24)	GB (n=18)	GNC (n=20)	SED	P	LSD (P<0.05)
Saturated FA[1]	2.98^{bc} (963.83†)	3.03^{bc} (1066.60†)	3.05^{c} (1122.02†)	2.91^{abc} (814.70†)	2.89^{ab} (776.25†)	2.81^{a} (645.65†)	0.070	**	0.140
Monounsaturated FA[2]	2.95^{b} (895.36†)	3.00^{b} (997.70†)	3.03^{b} (1066.60†)	2.91^{ab} (820.35†)	2.91^{ab} (803.53†)	2.80^{a} (625.17†)	0.075	*	0.149
Polyunsaturated FA[3]	2.22 (167.11†)	2.23 (169.82†)	2.27 (184.93†)	2.21 (162.93†)	2.22 (166.72†)	2.21 (161.81†)	0.019	NS	0.037
C18:2n-6	1.85^{a} (70.47†)	1.93^{b} (84.53†)	1.97^{b} (94.19†)	1.80^{a} (62.81†)	1.81^{a} (65.16†)	1.81^{a} (64.27†)	0.029	***	0.058
C18:3n-3	27.48^{b}	20.22^{a}	20.51^{a}	30.90^{b}	30.51^{b}	28.14^{b}	2.218	***	4.396
EPA+DHA	18.31^{bc}	15.42^{a}	16.82^{ab}	20.31^{c}	20.54^{c}	19.02^{bc}	1.188	***	2.355
Total	3.37^{bc} (2328.09†)	3.41^{bc} (2570.40†)	3.43^{c} (2716.44†)	3.32^{abc} (2070.14†)	3.30^{ab} (2013.72†)	3.23^{a} (1690.44†)	0.063	*	0.126

[1]ΣC12:0+C14:0+C16:0+C18:0; [2]ΣC16:1c9+C18:1t11+C18:1c9; [3]ΣC18:2n-6+C18:3n-3+C20:4n-6+C20:5n-3+C22:5n-3+C22:6n-3;
†Geomean; NS–not significant; *P<0.05; **P<0.01; ***P<0.001; ^{abc}Values not sharing common superscripts differ significantly (p<0.05)

Conclusion Beef which has been finished on grass-based diets has a higher C18:3n-3 and EPA+DHA content and a lower SFA, MUFA, C18:2n-6 and total FA content, relative to beef finished on silage-based diets. Further investigation is required into commercially-viable strategies to enhance the FA profile of beef, in view of achieving the guidelines regarding n-3 and n-6 labelling set out by EFSA.

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The relationship between slice shear force and tenderness of beef assessed by an un-trained consumer sensory panel

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Implications We present an equation to convert the slice shear force values of cooked beef in to tenderness scores given by an un-trained UK consumer panel. The equation could be used by operators to aid in the interpretation of slice shear force test results.

Introduction The tenderness of whole beef cuts is particularly important to beef consumers and consequently to beef value chains (producers and processors) because it is a key driver of repeat purchase events (Grunert, 2005). Differences in beef production and processing increase variation in the intrinsic quality of whole beef cuts, yet the production of high-quality beef with a high level of consistency is a goal for beef value chains. Trained sensory panels are often used to assess meat eating quality, but training may enable panellists to perceive quality attributes in a different way to untrained consumers – who are the target market. Although considered to be the gold-standard mode of assessment, sensory panels (trained and un-trained) are labour intensive and expensive to operate, making them unsuited for routine quality assessment on a large scale. The Tenderscot tenderometer, which was designed for use by meat processors, can be used by a single operator to perform the slice shear force (SSF) test (Shackelford *et al.* 1999) as a proxy measure for tenderness. Tenderometer values can be used to determine levels of variation, but the relationship between the SSF test values and un-trained consumer panel assessments remains largely uncharacterized, particularly for UK consumers. Without this information, operators cannot relate SSF values into a consumer tenderness score. The aim of this research was to explore the relationship between tenderness assessed by an un-trained consumer panel and the SSF test assessed using the Tenderscot.

Material and methods 106 consumers (29% men, 71% women) who eat beef steaks at least once a month were recruited for the panel. 58% were in the 20-39 age group and 42% were in the 40-54 age group. The demographic of the panel was: 33% ABC1 (upper-lower middle class) and 67% C2DE (skilled working class-non working), 66% had children in the household, 34% did not. Panellists had not received sensory training. To maximise variation in beef tenderness, samples of fillet, sirloin and brisket were sourced for the trial which was conducted over two consecutive days. Animal information and aging time were unknown. For each batch of three consumers, two samples each of beef brisket, fillet and sirloin were prepared into steaks approximately 60 x 50 x 25 mm in dimension, which were seared and cooked by a professional chef to a core temperature of 71°C in an oven**.** After cooking, each steak was prepared into four 50 x 10 x 25 mm samples so that the long axis was perpendicular to the muscle fibre direction. The first three sets of samples from each batch were given to three consumers on alpha-numerically encoded paper plates, and the fourth sample set was used for the SSF test using a Tenderscot machine. Each panellist was invited by a certified interviewer to sample the six samples of beef in a random order, then to rank them in order of tenderness, then to score them for tenderness / overall liking on a 0-100 scale, where 0 = very tough / extreme dislike and 100 = very tender / liked very much. Analysis for differences between cuts was conducted using ANOVA with batch, cut and sample and a batch-by- cut interaction using Genstat 12.1. A relational analysis was obtained by plotting the SSF values against the tenderness score (0-100 scale).

Results As expected there were clear differences between the beef cuts for all parameters (Table 1). The relationship between consumer tenderness score (0-100) and SSF is characterised by the equation: Tenderness = 95.8 (± 4.22) – 0.22 (± 0.027) * SSF (r = -0.68, $P < 0.001$). The constant (95.8) is not significantly different ($P > 0.05$) from 100 so for operational purposes a more convenient equation is: Tenderness =100 – 0.25 (± .010) * SSF.

Table 1 Consumer scores and slice shear force for beef

Meat Quality Parameter	Brisket	Sirloin	Fillet	sed[a]
Tenderness Rankings (1-6)	5.1	3.4	2.0	0.12
Tenderness Scores (0-100)	40.0	65.1	83.4	1.87
Overall Liking Scores (0-100)	41.4	63.2	80.4	1.87
Slice Shear Force (N)	206.5	142.5	90.7	4.19

[a] = standard error of the difference between predicted means.

Conclusion Both un-trained consumers and the Tenderscot SSF test detected differences between cuts. Although the correlation between SSF and consumer tenderness (0-100) was not strong, as undertaken in this experiment, the SSF test correlates with tenderness and overall liking asses by untrained consumers. Using the equation presented, SSF results can be placed in a UK consumer context, but more research is needed to gain a better understanding of the relationship.

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Meat quality traits of crossbred lamb loins sired by high and low muscle density rams (as measured by computer tomography)

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Implications The use of *in-vivo* muscle density as a selection criteria, can cause differences in saturation, redness and yellowness of meat colour traits in crossbred lamb loins.

Introduction Muscle density as assessed by computer tomography (CT) has a negative genetic correlation with intramuscular fat (Karamichou *et al*., 2006). The aim of this study was to evaluate the meat quality of lambs sired by rams selected for extremes (high and low) of muscle density.

Material and methods CT scanning was carried out to select five high and five low muscle density Abermax™ (Innovis bred F1 Texel x Charollais) rams out of a sample of 120 ram lambs; these differed by 3 standard deviations. Semen from these rams was used to inseminate 230 North Country Mule ewes, which lambed over a 10 day period (2012). Slaughter lambs were selected on commercial criteria (target carcass weight: 18-21kg and fat class 2-3L). Lambs were reared on grass and weaning occurred at 22 weeks (mean age 154d ± 0.08 se). Ultimate pH was taken at 48h post slaughter on the *M. longissimuis dorsi* at the fourth lumbar vertebrae location. Right lamb loin (lumbar vertebra region) and whole left loin samples were excised and aged for 7 days post mortem and were subsequently frozen. Right rear loin were defrosted for 16 hours and cooked via water bath method until an internal temperature of 75°C was reached. These samples were then chilled overnight and measured for Shear Force (10 measurements per loin) using the MIRINZ tenderometer (Tenderscot). The left loin was analysed for colour, with the 9 steak pieces (2cm depth) left to bloom for 1h. Colour traits were determined using a Minolta Chroma Meter CR-400. Data were analysed in GENSTAT 15 using a REML model with sire as a random effect. Muscle density was fitted as a fixed effect along with sex, slaughter batch, dam age (2yr vs. older), rear type (single/twin/artificially reared), MyoMAX™ status (P<0.2) and slaughter weight as a covariate.

Results Low muscle density lamb loins tended to be lower in shear force (P=0.067; Table 1). Significant colour parameters included saturation, redness and yellowness. Low muscle density sired progeny had higher values in these colour measures.

Table 1 Meat quality trait results for progeny produced from high and low muscle density Abermax™ rams

		Sire muscle density group			
Trait	n	Low	High	s.e.d	P-Value.
Shear Force (N)	200	29.64	31.97	1.100	0.067
Ultimate pH (LV4)	205	5.71	5.69	0.011	0.162
Colour - Saturation (C*)	178	17.76	17.12	0.204	0.012
Lightness (Log L*)	181	43.45 (1.64)	43.15 (1.64)	0.003	0.290
Redness (Log a*)	178	16.22 (1.21)	15.70 (1.20)	0.005	0.012
Yellowness (b*)	178	6.80	6.49	0.122	0.033

Logarithm values in parentheses

Conclusion Low muscle density progeny had a tendency for lower shear force (P=0.067). Karamichou *et al*., (2006) found muscle density was negatively correlated with shear force. Low muscle density lamb loins had significantly increased colour saturation, redness and yellowness. High muscle density sired lambs have increased lean tissue (Price *et al*., 2013). Bünger *et al*., (2009) found that Texel sheep compared to Scottish Blackface sheep have higher muscle density, total fibre number and an increase in fast, glycolytic (white) fibres in combination with a strong decrease in slow, oxidative (red) fibres. Selection for increased lean or muscle density may influence meat quality colour traits, possibly via a difference in fibre type and therefore cause an effect in meat quality.

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Fatty acid composition of loins of crossbred lambs sired by high and low muscle density rams (as measured by computer tomography)

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Implications The use of *in-vivo* muscle density as a selection criteria, is unlikely to alter fatty acid content of lamb loin.

Introduction Lamb meat quality may be improved upon via genetic selection. *In-vivo* measures of meat quality are needed. Muscle density as assessed by computer tomography (CT) has negative genetic correlation with intramuscular fat (Karamichou *et al*., 2006). The aim was to evaluate the fatty acid composition of lambs sired by rams selected for extremes (high and low) of muscle density.

Material and methods CT scanning was carried out to select five high and five low muscle density Abermax™ (Innovis bred F1 Texel x Charollais) rams out of a sample of 120 ram lambs; these differed by 3 standard deviations. Semen from these rams was used to inseminate 230 North Country Mule ewes, which lambed over a 10 day period (2012). Slaughtered lambs were selected on commercial slaughter criteria (target carcass weight: 18-21kg and fat class of 2-3L). Lambs were reared on grass and weaning occurred at 22 weeks (mean age 154d ± 0.08 se). Loin samples (taken at the last rib of the thoracic vertebra region), were taken at 68h post slaughter and frozen. Loin samples were analysed for fatty acid composition via the direct bimethylation method (Lee *et al*., 2012). Total intramuscular (IMF) fatty acids is the sum of all fatty acids quantified. The concentrations of individual fatty acids were then grouped into sub-classes of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids (PUFA; as described in general nonclamenture), sum *n*3 and sum *n*6 associated fatty acids. Ratio's were also calculated. Data were analysed using GENSTAT 15 using a REML model with sire as random effect. Muscle density was included in every model. along with fixed effects (P<0.2) sex, slaughter batch, dam age (2yr v. older), rear type (single/twin/artificially reared) MyoMAX™ status and slaughter weight fitted as a covariate.

Results Lamb loins were low in fat content (2.5% and 2.3%; low and high muscle density sired progeny respectively). The low muscle density progeny were generally fatter (apart for sum *n*3 and sum *n*6 fatty acids) although this difference was not significant. Similar effects were seen in further analysis using the covariates 48 hour cold weight, ultrasonic fat depth (LV3) and carcase fat grade.

Table 1 Adjusted logarithmic transformed fatty acid means for progeny produced from high and low muscle density Abermax™ rams, logarithm values in parentheses, g/100g Fresh weight (FW), with s.e.d and P-Value.

Fatty acid	n	Low MD predicted means mg/100g FW (Log_{10})	High MD predicted means mg/100g FW (Log_{10})	s.e.d	P-Value
Total IMF	192	2494.59 (3.40)	2317.39 (3.37)	0.028	0.236
SFA	192	1039.92 (3.02)	952.80 (2.98)	0.031	0.242
MUFA	192	922.57 (2.97)	835.60 (2.92)	0.034	0.209
PUFA	192	224.39 (2.35)	223.87 (2.35)	0.008	0.885
Sum C18:1 *trans*	192	107.15 (2.03)	92.90 (1.97)	0.033	0.104
Sum *n*3*	192	112.72 (2.05)	113.76 (2.06)	0.033	0.845
Sum *n*6**	192	105.20 (2.02)	105.93 (2.03)	0.010	0.858
PUFA:SFA	192	0.20 (-0.69)	0.22 (-0.66)	0.028	0.197
*n*6:*n*3	192	0.93 (-0.03)	0.93 (-0.03)	0.012	0.963

*sum *n*-3 (C18:3*n*-3, C20:3*n*-3, C20:5*n*-3, C22:5*n*-3, C22:6n-3) **sum *n*-6 (C18:2*n*-6, C18:3*n*-6, C20:3*n*-6, C20:4*n*-6, C22:4*n*-6)

Conclusion There was no evidence in this study that progeny sired by high and low muscle density were significantly different in total or grouped fatty acid content. The total IMF content of crossbred lambs in this study were higher in Total IMF content than Scottish Blackface lambs (Karamichou, *et al.,* 2006b). Further analysis will be conducted on the same samples investigating fatty acid proportions.

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Evaluation of Rosé and Cereal Beef Production for Holstein bulls

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Implications The results of this study which compared the performance of Holstein bulls finished on either a Cereal or a Rosé Beef system found that latter to be a viable system. However a market outlet should be secured before entering into Rosé Beef production for dairy-bred calves since some abattoirs do not accept bulls under 12 months old.

Introduction Some 453,000 dairy-bred bull calves were born in Great Britain in 2012 and it was estimated that 55,000 were shot at birth (Pers. Comm. Ms. Butcher, AHDB/EBLEX). It is also estimated that 91,000 bulls were slaughtered as bobby calves. The undesirable performance characteristics of Holstein bull calves are well recognized with poor conformation grades (P+/-O), low killing out percentages (50-52%) and poor meat to bone ratios compared to Continental crosses. The reason for the slaughter of so many calves is simply due to lack of profitability with rearing these breed types on a barley beef system due to high cereal prices (and fixed costs), and historically very low beef prices for P+/-O grade carcasses. The objective of this experiment was to compare the performance of Holstein bulls finished on either a conventional Cereal Beef system at 13-15 months old against a Rosé Beef system with bulls slaughtered less than 12 months old.

Material and methods Thirty six Holstein bulls weighing 112kg were allocated in a randomised block design according to live weight to one of the following treatments: Cereal, bulls reared on a conventional cereal beef system and selected for slaughter at a target EUROP fat class of 3; Rosé Beef, bulls slaughtered less than 12 months old. The bulls were fed an intensive beef compound feed (Primebeef Premium, Wynnstay Group Plc) *ad libitum* through to slaughter with straw available from racks. The compound feed was analysed to contain 167g CP and 382g starch per kg DM. The cattle were housed in straw-bedded yards. The data were analysed using ANOVA.

Results Compared to the Cereal bulls, the Rosé bulls recorded significantly higher ($P<0.01$) DLWGs and were slaughtered at significantly lower ($P<0.01$) slaughter and carcase weights with a lower ($P<0.05$) fat classification. The Rosé bulls were slaughtered at a significantly younger ($P<0.05$) age with the Cereal bulls taking an extra 65 days to be selected for slaughter.

Table 1 Animal Performance

	Cereal	Rosé	s.e.d	Sig
Slaughter weight (kg)	528	489	17.4	*
Age at slaughter (days)	424	359	7.4	***
DLWG (kg)	1.22	1.37	0.057	**
Carcase wt (kg)	269	246	8.6	*
Kill out (g/kg)	509	503	19.0	NS
Carcase daily gain (kg)	0.69	0.75	0.032	*
Conformation[1]	2.0	1.8	0.23	NS
Fat class[1]	2.9	2.4	0.28	*

[1]EUROP carcase classification: Conformation: P+=1 and E=7, Fat class: 1=1 and 5H=7.

The feed conversion ratio (FCR kg feed: kg LW gain) of the Rosé bulls was improved from 5.72 to 5.14 with total concentrate feed intakes reduced by 443kg/bull. The gross margins per bull were similar however with the earlier slaughter of the Rosé bulls the margin per 'bull place' was improved by £48 based on the feed and finished beef prices prevailing at the time of the study (bulls slaughtered Jan-April 2014).

Table 2 Concentrate feed intakes, FCR and financial appraisal

	Cereal	Rosé
Concentrate feed intake (kg)	2,381	1,938
FCR (kg: kg LWG)	5.72	5.14
Carcase price (£/kg)	3.45	3.43
Gross Margin/Bull (£)	269	276
Gross Margin/Bull/Year (£)	233	281

Conclusion Rosé Beef production offers a viable system of production for Holstein bull calves provided there is a market outlet since some abattoirs do not accept bulls under 12 months old.

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From food to feed: Food industry co-products in animal nutrition as functional feed ingredients for enhanced meat quality

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Implications Incorporation of food industry co-products as functional feed ingredients in animal nutrition presents a good market potential for the production of value added meat with "clean and green" label product differentiation.

Introduction Production of food industry co-products and waste has been estimated at approximately 36 million tonnes per year in the EU countries in 2010, and it follows an increasing trend. Thus, reutilisation of food industry co-products is a food and feed industry priority with a significant environmental, social and economic impact. Moreover, consumers that demand the production of "clean", "natural" and "eco/green" label food products (Grunert, 2005; Joppen, 2006; Karre *et al*., 2013) are also willing to pay significant premiums for such products. Food industry co-products are promising sources of functional compounds with favourable technological or nutritional properties (Schieber *et al*., 2001). Additionally, developments in the biorefinery industry have led to the production of phytochemicals and other value-added products that could be used in animal nutrition as functional feed ingredients. On the other hand, the global market of feed ingredients is expected to grow due to population growth and increased consumption of meat in the developing countries offering, thus, emerging and challenging roles for the application of food industry co-products in livestock production. However, this is a new class of feed ingredients and there is limited knowledge on their aspects of application and functions. The objective of this work is to present the recent developments in the field of utilisation of food industry co-products in livestock nutrition in relation to meat quality, and to consider the current limitations and where future research work could be targeted.

Material and methods Scopus, a database of peer-reviewed research literature was searched using the default search field "Title-Abstract-Keywords" and a variety of search terms such as meat, co-products, by-products, pomace, pulp, etc., excluding review and conference papers and including only studies that the full text was available. Selected papers refer to studies that the food industry co-products were included in the feed as dietary supplements and not as main feed ingredients. Research was narrowed in the decade 2003-2013 to encompass modern animal production trends. In the end, 15 studies that the reported data met the selection criteria were included in this overview.

Results Food industry co-products have been mainly used in the diets of poultry (9 studies) and swine (4 studies) and to a lesser extent in the diets of rabbits (2 studies). The applied co-products were grape pomace, tomato pomace, olive pomace, olive oil by-products, cranberry pulp, chokeberry pomace, citrus by-products (hesperidin) and mushroom by-products. In all cases, the primary intended function was to serve as an antioxidant since lipid oxidation is one of the main parameters affecting the sensory properties (appearance and eating quality) of meat along with the fact that fruit co-products are good sources of natural antioxidants due to their high phenolic content (Rice-Evans *et al*., 1997). In most cases the desirable antioxidant protection was achieved following a dose-response pattern but at lower effectiveness in comparison to the commonly used antioxidant vitamin E. Other beneficial effects of the applied food industry co-products were improvements in the fatty acid composition of intramuscular fat for long term health and disease prevention i.e. lower saturated fatty acid content and higher polyunsaturated fatty content, and increased content of conjugated linoleic acid (CLA). In some cases, meat colour variations were observed and they were mainly associated with an increase in red colour intensity. The quality of the co-product as well its processed form (fresh, dried, extract, etc) are important parameters influencing its successful function.

Conclusion Current experience shows that food industry co-products can be effectively used in farm animal nutrition for the production of meat with improved oxidative stability and in some cases functional properties. Production of "natural" and "green" meat is a promising area for the meat industry. However, the feed industry needs to embrace this trend and incorporate food industry co-products into feed mixtures. Future work could be targeted to the determination of optimum supplementation levels in relation to species, production system and feedstuff composition. Research should be broadened on the antimicrobial function of these products as alternatives of antimicrobial agents in the meat products. Seasonal nature of many of the food industry co-products and endogenous differences in their composition in respect to their botanical origin and storage conditions may also present limiting factors towards extensive application in farm animal nutrition.

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Effect of dietary supplementation of flaxseed on fatty acid profile in broilers

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Implications Dietary supplementation with n-3 PUFA improves the n-3 PUFA in chicken meat which could be beneficial for human health. Vitality of living cells depends on dietary lipids which are incorporated into phospholipid layers of cellular membranes. n-3 PUFA are reported to compete with arachidonic acid (AA, n-6 fatty acid) for this incorporation. Since AA is responsible for up-regulation of eicosanoids such as leukotrienes, this competitive inhibition down-regulates inflammation responses related to many diseases and disorders such as cardiovascular disease, increased triglycerides, blood pressure, thrombosis, atherosclerosis, stress, mental problems, asthma and rheumatoid arthritis (Calder, 2006, Leaf and Kang, 2001).

Introduction Recently, there has been interest in enrichment of poultry meat with flaxseed to increase consumption of omega-3 fatty acids in human. Studies in the literature showed that dietary supplementation of fatty acids in chickens can modulate fatty acid profile in meat tissues and eggs. For example, Zelenka *et al*.(2008) investigated the effect of increasing levels of linseed oil in the diet on the content of fatty acids in breast and thigh meat of chickens. 1, 3, 5 or 7% of linseed oil were fed to broiler chickens from 25 to40 days of age.

Material and methods One day old Cobb 500 broiler chicks were raised in the Poultry Research Farm at Sulaibiya experimental station, Kuwait. Dietary supplementation of flaxseed, 15 % of diet, started at two different ages. One supplementation started at 7 days of age and the other started at 14 days of age. In both cases, supplementation was until the end of the cycle at 35 days of age. Each treatment was replicated five times (n=5, 17 birds/replicate). All the batteries were located in one room. 85 birds were used in each treatment. The broiler chicks were fed, *ad libitum*, a starter diet from hatch until 7 days of age (1 week), a grower diet from 8 to 21 days of age (2-3 weeks), and a finisher diet from 22 to 35 days of age (4-5 weeks). At slaughter, samples of breast and leg (thigh) were stored for fatty acid analysis by gas chromatography.
Differences in the effects of the different treatment groups were analysed using one-way analysis of variance (ANOVA) and the general linear model procedure of Minitab was applied. When significant differences occurred ($P \leq 0.05$), mean treatment differences were identified by pairwise comparison using Bonferroni tests.

Results Fatty acid analysis of breast and thigh tissues of 35 days-old broilers fed flaxseed dietary supplementation at 7 and 14 days of age showed almost the same trend in both of the tissues, see Tables 1. The highest proportion of DHA was observed in breast of broilers fed flaxseed at 14 days of age, followed by those fed flaxseed at 7 days of age; no DHA was detected in breast of controls. However, significantly different. The highest proportion of α-linolenic acid (C18:3n3) was observed in broilers fed flaxseed at 7 days of age, followed by those fed flaxseed at 14 days of age. The lowest proportion of α-linolenic acid was for control samples. The effect of the flaxseed on the ratio of n-6:n-3 was expectedly significant.

Table 1 Fatty acid composition of breast/thigh tissue of 35 days-old broilers fed flaxseed dietary supplementation at 7 and 14 days of age

	mg of fatty acid in 1g sample			
Fatty acid	Control Breast/thigh	7 days Breast/thigh	14 days Breast/thigh	P value Breast/thigh
C18:3n3	0.24 ± 0.15/1.02 ± 0.55	0.48 ± 0.57/4.07 ± 1.40	1.91 ± 0.44/3.62 ± 1.81	0.113/0.360
C22:6n3	0/ 0.04 ± 0.03	0.14 ± 0.10/0.34 ± 0.15	0.33 ± 0.08/0.35 ± 0.19	0.136/0.356
∑n-6	4.48 ± 1.50/14.35 ± 2.5	0.96 ± 1.40/6.81 ± 2.55	3.79 ± 1.08/8.10 ± 3.29	0.249/0.192
∑n-3	0.24 ± 0.12/1.16 ± 0.53	0.63 ± 0.66/4.41 ± 1.53	2.24 ± 0.51/3.98 ± 1.98	0.114/0.374
n-6/n-3	**19.2 ± 1.90 /12.72 ± 2.5**	**1.64 ± 2.09/2.93 ± 1.41**	**1.63 ± 1.62/2.33 ± 1.83**	**0.002/0.002**

Conclusion Enrichment of chicken meat with flaxseed at 15% of diet reduced the ratio of n-6:n-3. This reduction is recommended by nutritionists worldwide. No significant difference was observed in case of individual fatty acids, although numerical differences exist in some cases.

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Comparing chemical and amino acid profiles of four commercial freshwater fish species

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Implications This study will provide nutritional information about freshwater fish species which would assist the farmers and food processors in determining the market price and suitability of these fish species for various processing and storage techniques. Indeed the nature and extent of chemical components in a fish may influence its quality during post-harvest processing and storage.

Introduction Various freshwater fish species are an essential source of food and livelihood for many people, especially those living along the River Indus of Pakistan. Fish are considered as an import protein source for both humans and animals, because they contain high quality nutrients such as essential amino acids (EAA). While, fish contribute to the food security and poverty alleviation in rural and urban areas of Pakistan, little is known about the nutritional value of the fish from the Indus River. Better knowledge of the nutritional value of these fish could help understand the meat quality of different fish species to determine their market price and demand. Therefore, this study examined four commercially preferred fish species (*Labeo or L. rohita*, *Aorichthys aor or A. sarwari, Channa or C. marulius, Wallago or W. attu)* from the River Indus for their chemical and amino acid contents.

Material and methods The study was carried out in the Mianwali District of Pakistan. The fish was purchased from the commercial fish sale point, immediately after fish landing. Twenty four samples of similar weight (1500±55g) of four fish species (*L. rohita*, *A. sarwari, C. marulius, and W. attu*) were selected by involving six fish per fish species. The fish were killed by percussive stunning, dissected, filleted and stored at -20°C until further analysis. Moisture and total fat (F) were expressed as g /kg according to the standard methods. Crude protein (CP) was determined by using the Dumas method on Leco nitrogen determinator. Total carbohydrates (g C/kg DM) were determined by subtracting the sum in g /kg of F, CP and ash contents (A) from 1000. Gross energy value of each sample was determined by multiplying the CP, F and total C contents with their respective energy values of 24, 39 and 18 MJ/kg of fish samples by using the equation: Caloric value = (24CP + 39F + 18C). Amino acids were determined with an amino acid analyzer (Biochrom-30 ion-exchange analyser). The data were statistically analysed by using ANOVA and Tukey's post-hoc test in Minitab16 software to test the effect of fish species on the chemical and amino acid compositions of different fish muscles at $P<0.05$.

Results Table 1 shows mean chemical compositions which differed significantly for different fish species ($P<0.05$). Table 2 shows the mean EAA contents of four fish species. All known EAA were observed in these fish species except tryptophan which was lost during acid hydrolysis of the sample. EAA were comparable or greater than the WHO/FAO standards for fish muscles. The most abundant AA in these fish was Lysine followed by Leucine, with the least amount for Histidine. None EAA like Aspartic acid, Serine, Glutamic acid, Glycine, Alanine, Proline and Tyrosine were also present in variable amounts in these fish.

Table 1 Mean (±SE) chemical (g/ kg DM, unless stated otherwise) composition of muscles of four fish species

Composition	*Labeo rohita*	*Aorichthys aor sarwari*	*Channa marulius*	*Wallago attu*
Dry Matter (g/kg)	233±8.3^{a}	230±7.1^{b}	224±8.2^{c}	212±7.5^{d}
Fat	211±8.2^{a}	193±6.4^{c}	199±7.4^{b}	139±5.2^{d}
Ash	66±2.24^{a}	48±2.22^{c}	45±2.25^{d}	59±2.31b
Crude Protein	713±17.8^{d}	750±19.5^{b}	742±18.6^{c}	796±21.1^{a}
Total Carbohydrates	9.6±0.41^{b}	9±0.43^{c}	13±0.51^{a}	6±0.3^{d}
Energy (MJ)	25.5±0.62^{c}	25.7±0.53^{b}	25.8±0.61^{a}	24.6±0.51^{d}

(Means with different letters in rows differed significantly)

Table 2 Mean essential amino acids as g/kg DM in muscles of four fish species

Fish Species	Arginine	Phenylalanine	Isoleucine	Leucine	Lysine	Methionine	Threonine	Histidine	Valine
L. rohita	49.5	33.0	38.3	65.3	77.6	24.0	34.2	18.1	39.7
A. sarwari	47.5	31.6	36.1	63.5	74.8	22.7	32.2	22.7	39.6
C. marulius	47.7	32.1	36.7	64.3	77.3	23.2	34.5	17.6	37.5
W. attu	49.9	33.8	38.8	66.3	77.3	23.6	35.1	20.8	41.0
SEM	0.21	0.24	0.33	0.41	0.51	0.20	0.31	0.45	0.33
WHO/FAO Std.	-	-	28	66	58	25	34	19	35

SEM= Standard Error of Means; Std. = Standards for fish muscles. n=24

Conclusion Selected fish species appeared to be of high nutritional value and good source of protein and AA. These fish species could be used for promoting good health and disease prevention in fish consuming people.

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Fatty acid profiles of freshwater *Catla catla* fish harvested from upstream and downstream locations of river Ravi, Pakistan

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Implications This study signifies a change in fatty acid composition of the fish species in response to urban pollution loads within a relatively small segment of the river. This change in fatty acid composition of fish implies that the river water pollution can affect nutritional quality of fish and subsequently the health of fish consuming communities.

Introduction Fish is one of the best sources of fatty acids which are not synthesized in human body while they are essential for the growth and development. Water of the Ravi has lost its vigour that used to be its identity. Ill management of untreated industrial effluents and urban sewage that are dumped through different channels in the river have mainly contributed to this situation since the past few decades (Shakir *et al.,* 2013a, b). The aim of this study was to find out the impact of river Ravi pollution on nutritional quality of fish. For this purpose, fatty acid composition of *Catla (C.) catla* inhibiting known unpolluted upstream and polluted downstream sites of the river Ravi were assessed. This fish species was selected because the local communities prefer this fish due to its taste, resistance to harsh environments and economic importance in this selected region.

Material and methods Fifty four fish specimens of comparable sizes were collected from an upstream (Siphon=A) and two downstream (Shahdera=B, Sunder=C) sites of the river Ravi during both low (winter) and high (post monsoon) flow seasons. Physiochemical parameters and metal concentrations of water and sediments collected during the same sampling earlier have been reported by Shakir *et al.* (2013a, b). Fat was extracted from freeze dried muscle samples by soxhlet apparatus and fatty acid methyl esters were prepared for analyses by Gas chromatography according to Jabeen and Chaudhry (2011), where the peaks were identified by using external standards. The data were statistically analysed by Minitab software to compare the effects of site, season and site x season interaction on different fatty acid profiles at $P<0.001$ (***), $P<0.01$ (**) and $P<0.05$(*). Tukey's test was used to compare means by using relevant standard errors of means (SEM) at $P<0.05$.

Results Muscle of the *C.catla* sampled from the three locations contained total fats in the range of 1.6 to 1.8% during low flow and from 1.64 to 1.77 % during the high flow season. Highest saturated fatty acids (SFA), but lowest polyunsaturated fatty acids (PUFA) were recorded at site C. Monounsaturated fatty acids (MUFA) ranged from 27 to 44%. The omega (ω)-3 and 6 showed decreasing trends at downstream sites (table 1). Drastic decrease of health promoting unsaturated fatty acid in the meat of fish captured downstream during both the low and high flow seasons, clearly demonstrates the changes as a function of industrial and urban pollutants.

Table 1 Mean fat (%) and fatty acid profiles (% of total fat) of muscle tissues of *Catla catla* sampled from different sites of river Ravi, during low and high flow seasons

Site	Siphon (A)		Shahdera (B)		Sunder (C)		SEM and significance		
Flow season	Low	High	Low	High	Low	High	Site	Season	Site x Season
Fat	1.78	1.77	1.62	1.73	1.60	1.64	0.024	0.030^{*}	0.041
SFA	59.51^{b}	55.59^{c}	61.10^{b}	54.48^{cd}	53.06^{d}	68.58^{a}	0.242^{***}	0.197^{**}	0.342^{***}
MUFA	28.81^{d}	29.12^{d}	31.61^{c}	37.81^{b}	43.88^{a}	26.99^{e}	0.059^{***}	0.048^{***}	0.083^{***}
PUFA	11.68^{b}	15.29^{a}	7.30^{c}	7.71^{c}	3.05^{d}	4.43^{d}	0.247^{***}	0.201^{**}	0.349^{*}
ω 3	4.91^{b}	7.38^{a}	3.08^{c}	3.72^{c}	1.06^{d}	1.60^{d}	0.106^{***}	0.086^{***}	0.149^{**}
ω 6	6.63^{b}	7.77^{a}	4.03^{c}	3.89^{c}	1.90^{d}	2.70^{d}	0.142^{***}	0.116^{*}	0.201^{*}

Means within the same row with different letters differed significantly ($P<0.05$)

Conclusion It is obvious that site A fish specimens contained the highest levels of Omega-6 and 3 PUFA suggesting that this fish species could be used as a source of healthy diet for humans. However, reductions in total PUFA in response to the magnitude of different pollutants in river Ravi may affect negatively the health of the fish consuming communities of this and other areas of this region.

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Diagnosis and surveillance of infectious diseases in wildlife (WildTech)

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Implications The project has provided the unique opportunity for dissemination of new information on diagnosis and epidemiology of wildlife diseases to workers in the fields of human and veterinary health.

Introduction It is well known that more than 70% of infectious diseases originate from wildlife and changes in land usage and animal and human population movements all contribute to the risks of exposure to zoonotic diseases**. T**he EU-funded project WildTech (Novel Technologies for Surveillance of Emerging and Re-emerging Infections of Wildlife) addresses the problem of the increasing prevalence of new and emerging diseases arising from wildlife which affect both farm livestock and man. We have applied nucleic acid (NA) microarray technology and high throughput serological screening to detect known and novel infectious agents in wildlife populations. This knowledge is applied to monitor and model patterns of wildlife disease spread and the risks associated with them. Ultimately this epidemiology framework will be used to reduce the risk of further potential epidemics by application of a generic action plan developed by this project in case of emerging epizootics among wildlife. The work is supported by the development of a wildlife disease data management system with mapping capability for use in Europe and beyond.

Recent research findings will be presented that relate the application of rapid and accurate diagnostic technologies to assess the spread of selected priority diseases (proof of concept) using historical samples and those collected during the project. The presentation summarises the objectives and progress of the WildTech project, with contributions from our 13 international Partners.

Summary of Project Progress NA and serology arrays have been fabricated and tested to detect pathogens in wildlife. Non array-based technologies (e.g. proteomics, luminex arrays, next generation sequencing) have also being investigated. Validation requirements and potential applications of these new methods for wildlife disease surveillance are being analysed. We have developed the new technologies in our Partner laboratories and delivered the Standard Operating Procedure for processing and transportation of tissue and serum samples. Large numbers of samples have been processed either for evaluating / validating the developed arrays, or for surveillance.

The epidemiology aspect of the project has delivering the mathematical, statistical and epidemiological tools necessary for a pan-European wildlife disease surveillance design, testing and support. Tasks undertaken and in progress include qualitative risk assessment for developing wildlife sampling strategies, epidemiological analysis of historic and new field data to quantify spatial and temporal patterns of disease incidence (prevalence and geographic distribution) and assessing the consequence of changing pathogen distributions using statistical and dynamic modelling. Finally, the evidence derived from these risk assessments will form the basis of recommendations for appropriate and proportionate management and policy actions.

The WildTech database has been developed. The goal is to have sample data and array results stored and accessed for epidemiological analysis that can be further developed to form part of a pan-European surveillance system. Wildpro® (the open-access electronic encyclopaedia on the health and management of free-ranging and captive wild animals, and (re)-emerging infectious diseases), continues to be updated with new pathogens as part of the WildTech project.

Two technology transfer workshops have taken place to introduce the basic principles underlying the new technologies being developed by the WildTech project: the first was a theory-based workshop held at the joint EWDA/WDA conference in Lyon in July 2012. The second was a hands-on wet-lab workshop held at the AHVLA in October 2012, attended by 8 colleagues from our Associate and Collaborative Partners.

Our pool of Associate and Collaborative Partners is growing and they, along with our Project Partners, continue to provide us with samples for surveillance and technology validation.

Conclusion In addition to bringing together diagnostic and surveillance approaches within the EU, the WildTech project is providing a valuable resource for information transfer between the western countries (USA and Canada particularly) and the non-EU countries elsewhere including Russia. Importantly, there are USA, Canadian and Russian groups who are fully integrated into the project. Technology transfer is a key component of the project's dissemination strategy and this is a strong component of current interactions with USA Department of Homeland Security and the USDA.

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Exploring community ecology and interactions of micro- and macroparasite infections in Ethiopian village chickens

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Implications Interactions between several infections are apparent in Ethiopian village chickens, and have potential implications both for bird health and for interventions aimed at improving flock health and productivity in this system.

Introduction Improving chicken production to offer people a route out of poverty is a current focus of development programmes worldwide. Chickens raised under village production systems are exposed to a wide variety of pathogens, some of which are known to alter the manifestation of other infections under experimental conditions. Studies in mammals indicate existing infections can alter the risk of further infections, and multiple coincident infections may impact host survival. This study aimed to investigate the epidemiology and ecology of a range of micro- and macroparasites in Ethiopian village chickens to (i) assess patterns of co-infection and (ii) identify common risk factors for co-infections.

Materials and methods Four cross-sectional surveys at 6-month intervals were conducted in 8 villages within two regions of Ethiopia. Questionnaire interviews were held with randomly-selected farmers, and two adult indigenous chickens per household underwent clinical examinations and had blood and faeces taken. Birds were scored for lice, using a timed count (Clayton and Drown, 2001) and graded for hyperkeratosis (none, mild or severe) of the feet and shanks, indicative of *C. mutans* infestation. Serum antibodies to Newcastle disease virus (NDV) were measured by haemagglutination inhibition (HAI) assays (O.I.E., 2009). ELISAs were developed in-house to measure antibody levels to *Pasteurella multocida* and *Salmonella* (Beal, 2004), and to Marek's disease (MD) virus (Zelnik *et al*., 2004); whilst a commercial ELISA was used to measure antibodies to infectious bursal disease (Flockscreen, x-OvO, UK). The concentration McMaster technique (Permin and Hansen, 1998) was used to quantify eggs of *Ascaridida* nematodes and *Eimeria* protozoa in faecal samples.

Statistical analyses Principal Components Analysis was used to investigate the patterns of distribution of pathogens amongst chickens across the range of samples. Redundancy Analysis (RDA) was used to investigate the extent to which putative explanatory variables, including bird production status (in lay, not in lay, brooding, rearing and male), explained variation in the patterns of pathogens observed. To account for differences in detection techniques a matrix of scaled response variables for 1056 birds was constructed from the ELISA results for four of the viral and bacterial pathogens; counts of three parasites and the hyperkeratosis grading. The adjusted R^2 value and permutation tests were used to test the significance of the explanatory variables and the canonical axes. Analyses were undertaken in R using the vegan package (R Development Core Team, 2008).

Results All birds were found to be in reasonable health at the time of sampling. Only 9 birds over all four seasons of testing were found to be serologically positive for NDV antibodies (HAI titre of 16 or greater); therefore this disease was not included in the ordination. Production status, weight, the use of chemical sprays for parasite control; and recent outbreaks of disease in chicks or growing birds all contributed significantly to variation in disease measurements. However, the measured explanatory variables accounted for very little of the overall variation (adjusted R-squared value 0.030). *Salmonella* and *Pasteurella* showed a positive correlation, even after accounting for the measured explanatory variables in common, and higher titres were associated with female birds, higher *Pasteurella* titres being particularly associated with females rearing chicks. Lice, MD and scaly leg were also positively correlated, but higher measurements were seen in male birds.

Conclusion Multiple pathogens were found to be circulating in the apparently healthy adult chicken population in Ethiopia. Ordination techniques were found to be a useful method for exploring this pathogen community and for generating hypotheses for future testing. The infection correlations suggest differences in bird immune response, and have implications for selective breeding programmes in Ethiopia, as protective immune traits should be retained in the population.

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Owner-reporting of adverse health events provides richer data than traditional veterinary-focussed data collection

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Implications Studies of canine health typically overlook data regarding signs of illnesses not presented to veterinarians. If collected, these data offer a new means for investigating canine disease.

Introduction Canine health studies normally focus on animals presenting at first and second opinion practices. Presentation relies on owner perception of the health event, meaning that many events go un-reported and un-investigated. The aim of this study was to collect data on owner-reported health events to assess what triggers a veterinary visit.

Material and methods Dogslife (Clements *et al*. 2013) began recruiting UK-based Kennel Club registered Labrador Retrievers in July 2010 with over 4,200 dogs recruited to date. Owners are asked to complete an online questionnaire monthly for the first year of their dog's life and quarterly thereafter. The questionnaire addresses potential presenting signs such as coughing, scratching, vomiting, diarrhoea and limping or lameness. The owner is asked for the duration of the presenting sign and whether they took the dog to the vet.

Results Over 6,700 presenting signs have been reported to Dogslife in nearly 3.5 years. Veterinary visits were associated with 47.6% of reported signs. Over 26% of signs were 'on-going' at the time of reporting and a further 4% have no reported event duration. The distribution of signs involving veterinary visits is shown according to type of presenting sign in Table 1.

Table 1 Distribution of presenting signs by type

		Diarrhoea	Vomiting	Scratching	Lameness	Coughing	Other
Visited Vet	No	1030	1179	671	268	89	267
	Yes	604	401	367	600	244	1185
	Yes (%)	37.0	25.4	35.4	69.1	73.3	81.6

The persistence of signs according to their type is shown for those lasting no more than one week in Figure 1. The lower portion of each bar (below the white line) represents signs associated with veterinary visits.

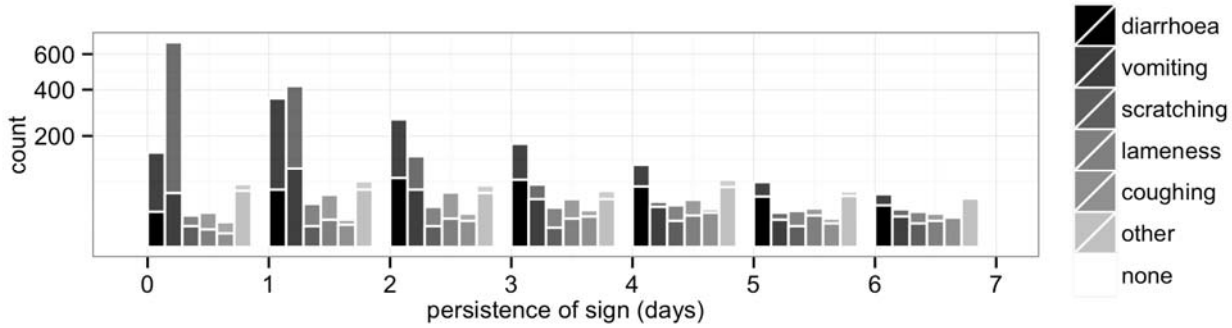

Figure 1 Persistence of presenting signs by type

Conclusion It is clear that all the presenting signs addressed in the study can occur without triggering a dog's owner to arrange a veterinary visit. These signs may represent potential illness instances that are omitted from traditional epidemiological studies. The decision to visit a veterinarian was affected by type and duration of the presenting sign. Owners were apparently quite concerned about coughing in their dog (73% resulting in a veterinary visit) but diarrhoea (37%) and vomiting (25%) were much less likely to involve a veterinary assessment, particularly when lasting under two days. Thus traditional studies of canine gastrointestinal health are potentially missing the majority of adverse health events. These data are a unique resource for future investigations of the impact of low-level illness on canine health.

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BVD testing strategies in neonatal calves: serial application of ELISA and real time RTPCR to address the potential "diagnostic gap"

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Implications BVD is a significant pathogen of cattle. It is appropriate that BVD control programmes ensure that the viral status of all age groups is as accurately assessed as possible while maintaining value-for-money. Serial application of antigen ELISA and real time rtPCR provides a cost effective means of addressing the "diagnostic gap" when testing neonatal blood samples.

Introduction Bovine Viral Diarrhoea (BVD) infection in-utero can lead to a congenital, lifelong, persistent infection in cattle. Currently all Irish calves are tested for BVD virus as part of a national eradication programme using a modified tag which collects an ear notch tissue sample.
Where an initial positive or inconclusive result is obtained, a confirmatory test on a blood sample taken at least 3 weeks later may be used to distinguish between persistent and transient infections. However testing such samples must take into account the potential of false negative results due to the presence of maternally derived antibodies (diagnostic gap) when using antigen capture ELISAs. On the other hand, testing samples individually by real time rtPCR is expensive. Therefore samples are initially screened by ELISA, with those that test negative being further tested by real time rtPCR. The results from this testing in 2013 were analysed to investigate the performance of both tests with particular emphasis on the diagnostic gap.

Material and methods A commercial (IDEXX) BVD Ag ELISA was performed on serum samples according to manufacturer's instructions and absorbance read in a Tecan Sunrise™ microplate reader (**TECAN** Austria GmbH, Salzburg, Austria). BVD ELISA negative samples were sent for real-time rtPCR. BVD RNA was extracted using ROCHE MagNa Pure LC automated extraction robot and detected using A11 and A14 primers (McGoldrick *et al*, 1999). Positive real-time PCR were given a cycle threshold (CT value) indicating the number of cycles required to amplify to pre-set cut-off limits. An in-house BVD Serum Neutralisation Test (SNT) was performed to obtain BVD antibody titre on a subset of serum samples. Briefly, samples were inactivated at 56˚C for 30min in a water bath. 50µl of medium (MEM 10% foetal calf serum (FCS)) was added to all wells in a 96 well plate. 50µl of sample was added to first well in plate and serial twofold dilutions were performed up to 1/4096. 50µl of working virus (cytopathogenic (CP) strain of BVD), calculated to provide 100-200 TCID50, was added across the plate. A back titration (up to 10^7) was included to check virus potency. Plates were incubated at 37˚C, 5% CO_2 for 30min. Foetal Bovine Kidney cells were trypsinised (after 3 days growth at 37˚C) and cell concentration adjusted to 2.5x10^5cells/ml. 50µl of cells were added to all wells. Plates were incubated at 37˚C, 5% CO_2 for 3 days and then examined for CP effect using back titration to check validity of test (permissible range of 30-300 TCID50).

Results Of the 6947 calf samples sent for confirmation of BVD status, 1921 (28%) were found to be negative on Ag ELISA and were retested using BVD rt-PCR. 285 of these proved ELISA negative/PCR positive (ENPP) (which estimates the diagnostic gap as 4% of the 6947). Both the age at blood sampling and the period between ear notch and confirmation sampling were significantly lower in this ENPP group at 46.1 and 35.7 days respectively, compared to 62.0 and 49.1 days respectively for confirmation calf samples testing positive on the ELISA alone. The rtPCR results for this group showed a range of CT values, with 17% having CT>34 and 4% having CT<25. The serology results for this group showed a range of titres with 44% of samples tested with titres >1/4096 and 5% of samples with titres < 1/8. There appears to be no quantitative association between rtPCR CT value or BVD antibody neutralisation titre in the confirmation blood sample and the initial ear notch testing result.

Conclusion The BVD antigen ELISA is a robust assay well suited to disease eradication programmes. It is however sensitive to interference arising due to lower levels of virus, higher levels of BVD specific antibody or combinations of both in serum. Reliance on ELISA testing alone would result in 4% false negatives, highlighting the importance of further testing of calf ELISA negative samples by rtPCR to individual farms and control programme alike.

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Tuberculosis in domestic pigs in Great Britain

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Implications *Mycobacterium bovis* infection in British domestic pigs occurs in regions where the incidence of bovine tuberculosis (bTB) is highest in cattle. However, infection may be attributable to contact with wildlife, rather than cattle.

Introduction *M. bovis*, the causative agent of bTB, is a gram positive, acid-fast bacillus, which infects a wide range of wild and domestic mammals. Tuberculous lesions ('tubercles') associated with mycobacterial infection may be identified in the carcase during the meat inspection process. Despite a control programme spanning many years, *M. bovis* infection levels in British cattle have continued to rise; in 2011 over £90M was spent trying to control the disease in the UK. It is generally believed that TB in British pigs is usually due to *M. avium* and that the disease remains localised to the lymph nodes of the head and neck. However, given that the increased incidence of *M. bovis* infection in cattle may be linked to that in swine, we created a study to investigate the cause of TB-suspect lesions identified in British pigs at slaughter.

Material and methods The sample population was all pigs slaughtered in British abattoirs between 2007 and 2011 (40M pigs). A total of 874 TB-suspect lesions were detected by the routine meat inspection process (EEC directive 854/2004). Any TB-suspect lesions submitted to AHVLA were cultured to identify the causative agent (van der Burgt *et al*, 2013). The genotype of any mycobacterial isolates was confirmed using a combination of spoligo- and Variable Number Tandem Repeat typing (Smith *et al*, 2006). Confirmed isolates of *M. bovis* were investigated by a Veterinary Officer who usually completed a tuberculosis disease report (TDR) form. Data from TDR forms were collated regarding the age of the affected pigs, number of affected pigs, herd size, the anatomical location of lesions, any contact with wildlife or cattle and the biosecurity of pens. We also investigated the geographical distribution of *M. bovis* and *M. avium* cases. Additionally, all cases of each individual genotype of *M. bovis* identified in pigs were mapped against the same genotype in cattle.

Results The results in Table 1 suggest that, nationwide, TB lesions were as likely to be caused by *M. avium* as *M. bovis*. However, mapping data indicates that almost all *M. bovis*-infected pigs originated from farms in the South-West (SW) and West-Midland (WM) regions of England. Data compiled from TDR forms suggests that pigs raised outdoors or on holdings with poor biosecurity may be more vulnerable to infection with *M. bovis*. In the majority of cases, the same strains of *M. bovis* were found in pigs as in cattle, despite that fact that opportunities for direct contact between these species were rarely observed. *M. bovis* lesions were often found to be more widely distributed in the carcase than previously suggested.

Table 1 Results from the culture of TB-suspect lesions:

	2007	2008	2009	2010	2011	Total
M. bovis	5	8	24	31	44	112
% of all submissions	7.4	12.5	20.7	9.1	15.4	12.8
M. avium	5	3	15	43	36	102
% of all submissions	7.4	4.7	12.9	12.6	12.6	11.7
Unclassified/ other	4	6	35	134	60	239
% of all submissions	5.9	9.4	30.2	39.3	21.1	27.3
Negative	54	47	42	133	145	421
% of all submissions	79.4	73.4	36.2	39.0	50.9	48.2
Number of submissions	68	64	116	341	285	874

Conclusion This study suggests that, nationwide, TB lesions in pigs are as likely to be caused by *M. avium* as *M. bovis*. However, in the SW and WM regions of England, *M. bovis* is more prevalent that *M. avium*. Although the same strains of *M. bovis* were found to circulate in cattle and pigs, opportunities for direct contact between these species are rarely reported. The more generalised lesions present in pigs raises the question of their possible role in transmission of infection, however further studies are needed to investigate this fully. Finally, it is important to acknowledge the limitations in this dataset: The relatively insensitive detection process (routine meat inspection (Corner *et al*, 1990)), sample population (only slaughter pigs) and government limitations placed on the number of samples cultured per farm, suggest that these data may in fact underestimate the true prevalence of *M. bovis* within the domestic pig population.

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Prevalence, risk factors and vaccination efficacy of contagious ovine ecthyma (orf) in England

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Implications Orf is endemic both to England and other parts of the world, affecting sheep production. The disease has health and welfare limiting consequences with a huge economic burden to the English sheep farming industry. Research has shown that up to 30% of people in the farming community may contact orf during their life time which may result in absence from work and in worst cases may result in hospitalisation.

Introduction Orf is a common viral disease of sheep in England which causes economic losses (Lovett *et al*., 2012). The disease is caused by an epitheliotropic (has affinity for the skin) parapoxvirus of the family poxviridae (Hosamani *et al*., 2009). Most studies have looked into virology (Billinis *et al*., 2012) while those focusing on epidemiological aspects in English sheep farms are very limited. It was therefore the aim of this study to investigate 1) the prevalence of orf 2) vaccination efficacy 3) potential risk factors for orf across England.

Material and methods 3000 farms, registered with the English Beef and Lamb Executive (EBLEX) were targeted for collection of data by survey with 762 complete responses received.
The following variables were included: number of ewes and lambs; biosecurity measures; presence of weeds (thistle, nettles, docks and ragwort); lambing start and end month; lambing management (indoors, outdoors); presence of orphan lambs and orf prevalence (%); vaccination of ewes and / or lambs against orf, month of vaccination; percentage of lambs and ewes in the flock affected with orf; numbers of new stock and type of farming. Number of animals was used to calculate stocking rates; the degree of weed infestation on the farm (with ratings of 0 to 5 used for nettles, thistle, docks and ragwort) was used to derive an overall rate for weeds (0 to 15), with ragwort being discarded as preliminary analysis found it was not a significant risk factor; lambing start and end month was used to create the variable lambing season (warm for month 6 to 9 and cool for the others), and lambing season duration; the other variables were left as originally collected. Analysis was conducted using both regression and generalised linear models routine and employed both IBM® SPSS® Statistics 21 software.

Results Disease relative risk (RR) was 2.04 for ewes and 0.75 for lambs, and therefore the vaccine is effective in the control of the disease when given to lambs (RR < 1) but not when given to ewes (RR > 1). Multivariate regression analysis demonstrated that disease prevalence in lambs and in ewes ($F = 20.59, 2, P < 0.001$) and ewes ($F = 29.15, 2, P < 0.001$) was influenced by degree of weed infestation. Risk factors associated with orf were weeds (thistle, nettles, docks and ragwort), orphan lambs and long lambing season. Probability modelling equations are available on the full paper.

Conclusion Weed infestation, long lambing season and high numbers of orphan lambs were identified as risk factors for orf in this study. From the findings it can be concluded that the prevalence of orf in both ewes and lambs affect each other, though the impact is higher in lambs with increased prevalence in ewes. A short lambing season lowers the probability of a farm experiencing cases of orf since tasks such as vaccination can all be concentrated within a short time. Vaccination is effective in lambs but not necessarily in ewes, even though, lambs benefit when ewes are vaccinated. Vaccination should be prioritised in lambs especially when there is vaccine shortage.

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Bovine tuberculosis test reactors: *seek, and ye shall find!*

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Implications Especially in national disease eradication programmes, if test protocols are suboptimal, costs associated with control efforts may be disproportionally large. Any needless protocol-associated loss of healthy animals, and chronic distress to farmers, will add to spending fatigue and therefore hamper control efforts in the longer term.

Introduction When attempting to eradicate disease from livestock populations, we need to find the optimum in a trade-off between removing enough diseased animals to halt transmission and removing too many healthy individuals as part of the process. Despite sustained test-and-remove efforts for bovine tuberculosis (BTB), disease incidence has been rising for over 25 years[1] and this tempts us to keep intensifying our efforts. However, as ever more BTB tests are carried out on the UK cattle herd, and each positive herd test triggers more short-interval testing, the question arises to which degree 'false positive' results contribute to our measured BTB prevalence. As the specificity of the tests available is less than unity[2], inevitably, false positive animals will be identified. But, especially as our current focus appears to be entirely on the application of more sensitive, and therefore less specific, tests, it is high time we also start to analyse the costs: the proportion of healthy animals slaughtered, alongside their economic and psychological effects. This paper initiates this process, aiming to quantify, at various prevalence levels and herd sizes, the probability of BTB tests being false negative or false positive and to use these probabilities to determine their prevalence-dependent optimum use.

Material and methods Simple probabilistic models of test behaviour were populated with Skin Test (SICCT) and Gamma Interferon (γIFN) test sensitivity (*Se*) and specificity (*Sp*) estimates taken from the literature. First, probabilities of individual animals testing false negative (FN) or false positive (FP) were calculated at various prevalence levels. Second, the binomial probability densities of the number of animals to test false positive were explored, at DEFRA-published *Sp* estimates, as a function of herd size. Third, the results of three published herd tests were SICCT and γ IFN had been applied at the same time were analysed. At prevalence levels indicated by the test results, the binomial probability densities of the number on animals predicted to have tested FN and FP were drawn and tested for overlap using Markov Chain Monte Carlo simulation.

Results When SICCT is applied to the average UK cattle herd, the estimated proportion of test-associated false positive new outbreaks is highly sensitive to small fluctuations the *Sp* of this screening test. However, precise and current SICCT *Sp* estimations are lacking. Once outbreaks have been confirmed in screening-test positive herds, the following rounds of intensive testing with more sensitive, albeit less specific, tests, such as γIFN, are likely to remove highly significantly larger numbers of FP animals. Despite this, up to a herd prevalence approximating 0.20 (i.e. one in five animals infected), it is unlikely that significantly more truly infected animals are removed. In lower-prevalence herds, sacrificing *Sp* for *Se* is likely to lead to significant extra loss of life without benefits in terms of removing disease more rapidly.

Conclusion
A frequent application of less specific tests, such as severe interpretation SICCT and γIFN, is likely to contribute substantially to the perceived UK BTB problem, triggering ever more intensive testing while removing large numbers of healthy animals from the national herd. Work on BTB test cost-benefit analyses, taking into account trade-offs in *Se* and *Sp*, is urgently needed. In order to prevent the needless slaughter of large numbers of healthy animals, BTB test protocols should become more fluid, based on quantified risk and targeted towards likely prevalence levels on individual farms.

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Further reading:
van Dijk J 2013. Towards Risk-Based Test Protocols: Estimating the Contribution of Intensive Testing to the UK Bovine Tuberculosis Problem. PLoS ONE 8(5), e63961. doi:10.1371/journal.pone.0063961 (freely downloadable online)

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Prevalence of gastrointestinal parasites among pigs in the Ejisu Municipality in Ghana

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Implications Parasitic infections in pigs are accompanied by delayed achievement of market weight, and information on their epidemiology would help in developing preventive measures and thus provide wholesome food to the populace.

Introduction The world food economy is increasingly driven by the shift in diet and food consumption patterns towards livestock products (FAO, 2010). Pig production is becoming one of the fast growing industries in the world and forms an integral part of the rural economy in many parts of the world (Seid and Abebaw, 2008). Pigs are susceptible to a number of diseases including internal and external parasites, which reduce the efficiency of production (Hale *et al*. 1986). The study seeks to investigate gastrointestinal parasitic infections among pigs in the Ejisu Municipality and to determine the types of ova present in faecal samples from pigs and the worm burden/egg per gram and thus give informed recommendations / solutions to the farmers.

Material and methods The study was carried out in the Ejisu Municipality near Kumasi in the Ashanti Region of Ghana. Ten pig farmers were selected in six communities in the Municipality. On each farm, a questionnaire was administered to the farmer and faecal samples taken aseptically per rectum from twenty randomly selected pigs belonging to different age groups for parasitological examination at the Regional Veterinary Laboratory in Kumasi. The flotation technique in a super saturated solution of sodium chloride was used in processing the faecal samples and parasite eggs were identified on a microscope based on keys listed by (Thienpont *et al*., 1995). The McMaster technique was then used to count eggs per gram (EPG) in positive samples. The total number of eggs in the grid of the chamber was multiplied by 100 to obtain the EPG. Data was processed and analysed by Microsoft Excel.

Results 43% (n = 86) of the sampled pigs were found to be infected with gastrointestinal parasites. Five parasites of veterinary importance were identified. These were Strongyle Spp (n=22), Eimeria Spp (n=29), Ascaris Spp (n=4), Flagellate Spp (n=9) and Trichuris Spp (n=1). These findings are similar to those by Obonyo *et al.* (2012) in the Homabay district of Kenya and Marufu *et al*. (2008) in Zimbabwe. The similarity may be as a result of similar husbandry practices on these farms. The mean EPG for these parasites was from 186 to 327. These values appear to be lower than those reported in Burkina Faso by Tamboura *et al.* (2006) and in Kenya by Kagira *et al.* (2002). The differences may be due to the fact that the pigs in these locations were scavenging pigs, which were more susceptible to worm infection.

Table 1 Frequency of parasites and their EPG

Parasite	Frequency	% Infection	Mean EPG	Range
Eimeria spp	29	14.5	186	100 – 1000
Strongyle spp	22	11	327	100 – 1400
Flagellate spp	9	4.5	189	100 – 300
Ascaris spp	4	2	250	100 – 700
Trichuris spp	1	0.5	200	200

Conclusion From the study five parasites were identified and the mean egg per gram ranged from 186 to 327. Less than half the pig population was infected and 32.5 % of this was through single worm infestation.

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The efficacy of the paratuberculosis vaccine in cattle and sheep: a meta-analysis of case-control trials

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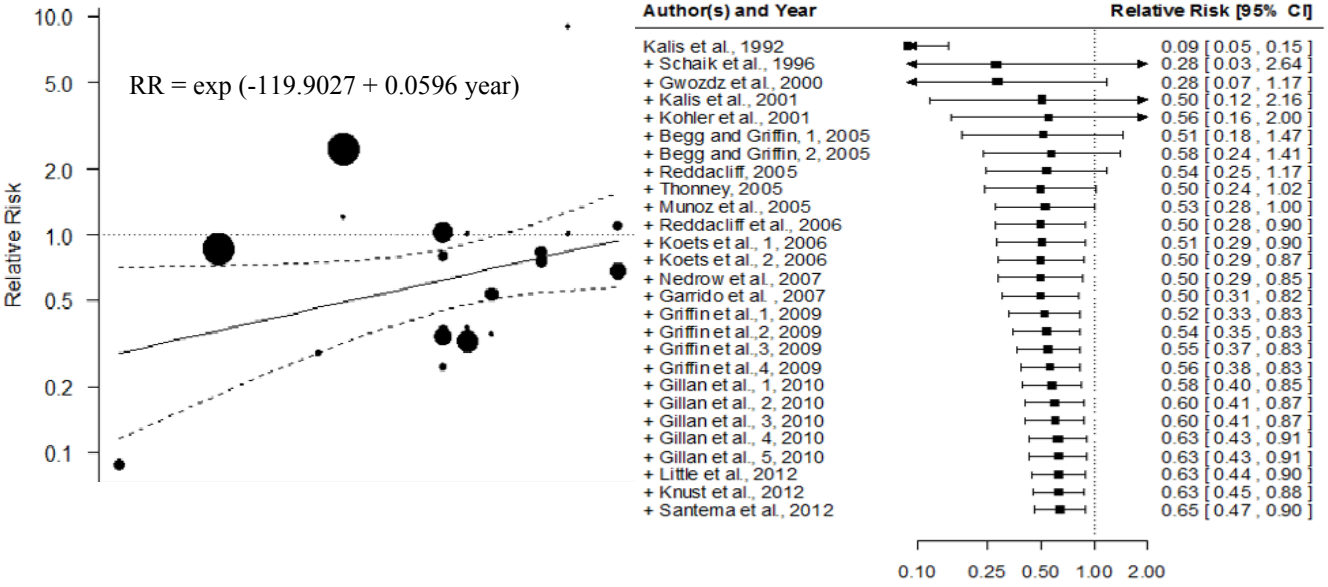
Implications The paratuberculosis (P) vaccine appears to have lost efficiency in recent years, but it has shown still a small advantage in its use. Better diagnostic techniques or new strains may be the explanation behind this fact. The vaccine should be used in a wider programme that includes biosecurity measures and cannot be seen as a sole solution.

Introduction P is a slow developing, chronic disease affecting several animals. In cattle and sheep has been characterised by chronic enteritis in the rectum. *Mycobacterium avium ssp. paratuberculosis* is the etiologic agent and once infected, animals can be asymptomatic for 2-5 yrs. Four clinical stages have been defined, the later showing severe weight loss, diarrhoea and infertility, causing economic losses to the livestock industry. Strategies to prevent P include biosecurity and diagnostic tests. Vaccination has been a mitigation tool, with studies showing reduction in disease incidence but not eradication. Vaccines have reduced mortality, faecal shedding and the appearance of clinical cases showing viability. Our aim was to investigate variability in reported P vaccination effectiveness, through a meta-analysis of case control trials.

Material and methods The literature search was executed using Google scholar and Science Direct with the key words: Vaccination, immunisation, paratuberculosis, Johne's disease, cattle, and sheep. The logarithm of the risk ratio (RR) was the outcome measure considered. The homogeneity of the data was tested via Cochran's Q-test and showed to be heterogeneous ($P<0.05$) and a mixed effects model was implemented. The moderators used were "species (cattle, sheep)", "vaccine type (dead, alive)", "number of boosters" and "year of publication". This last covariate was the only moderator found significant ($P<0.05$) and therefore a cumulative meta-analysis and meta regression were implemented. The publication bias was tested via regression test and found non-existent ($P>0.05$). The analysis was performed using the package "metafor" of R 2.15.2.

Results RR is persistently shown below 1, indicating efficiency of the vaccine, but as the years advance RR increases 0.0596 (95% CI [0.001; 0.121]) units per year. The figures below are illustrative of this process, with a meta-regression in the left and a cumulative forest plot in the right, showing that as years pass the relevance of the studies is higher (higher number of animals); This leads to a higher accuracy of the analysis and therefore shorter confidence intervals.



Conclusion This study suggested that vaccines have shown efficacy in the control of P that has decreased over the years. Possible explanations for this fact are the increasing sensitivity over time of diagnostic techniques, including PCR, which now detect any eventually undetected cases in the past. Also the eventual mutations with differences between strains may have an influence. It becomes therefore evident that the protective effects of the vaccine do not discard strong biosecurity measures. The vaccine has potential to be used as part of a solution to control the disease but cannot be the sole solution.

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E-learning: fad or future?

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Introduction E-learning refers to the use of electronic media and information and communication technologies in education. The terms e-learning is an umbrella for all forms of educational technology in teaching and learning, such as multimedia learning, technology-enhanced learning, computer-assisted learning, online education, virtual education, virtual learning environments and digital education. These different names represent a particular aspect or delivery method. E-learning can take place in various ways; it can be synchronous or asynchronous, with or without tutor input, at distance or as part of face-to-face teaching, the latter often being referred to as blended learning.

E-learning for on-campus students The use of e-learning in higher education (HE) continues to grow, with more and more school leavers fluent in the use of technology-enhanced learning. Students expect to have access to e-learning resources and for technology to be used to enhance their learning experience. Indeed, many classroom sessions are being replaced with virtual sessions and many face-to-face courses are being fully delivered online. E-learning can also be used in the classroom setting; for example by using electronic voting systems to encourage active learning through interactive engagement (Bates *et al*., 2006). Active learning strategies assist in long-term retention of course material and are more effective in developing higher order thinking skills. Learner-authored questions are also becoming more popular in HE settings; for example PeerWise developed by Denny *et al*. (2009), with higher student achievements for those that showed a greater engagement with using PeerWise. E-learning tools can also be an effective way of providing personalised feedback with growing class sizes, with one minute of audio feedback equating to six minutes of writing (Lunt and Curran, 2010). From the students' perspective, reports suggest that students have a greater connection with their instructor and their feedback when it is provided in this form (Ice *et al*., 2007).

E-learning for distance educations students In higher education, distance education (DE) has increased worldwide. Many educational establishments have embraced on-line education, with on-line courses being delivered by a great number of institutions, ranging from community colleges to major universities world-wide. Online distance learners have the flexibility to study anytime, anywhere; however, the approach to studying differs from education in the traditional classroom setting in that learners study at a physical distance from each other and their teacher. E-learning has played a key role in the emergence of on-line DE by distorting the concept of distance between the learner and instructor; thus, enabling learners to access education at any time and from any place (Beldarrain, 2012). E-learning technologies used in DE can reduce the feeling of distance and isolation from peers and tutor, and provide opportunities for collaborative learning activities (Bates, 2005). Such media include asynchronous discussion boards, synchronous chat rooms (e.g. Skype), virtual classrooms (e.g. Collaborate) and virtual worlds (e.g. Second Life). These technologies can be used to promote communication in DE courses, since interaction is considered to be the cornerstone of effective DE practices.

It is important to state that using technology in education does not implicitly improve learning; in order to enhance learning and improve the student experience, technology must be employed in conjunction with appropriate pedagogical approaches. It is essential that pedagogy drives the use of technology, instead of the other way round.

Conclusion The use of e-learning in higher education is likely to continue to grow in use. E-learning technologies can provide solutions to enhancing the student experience; however, care must be taken to employ e-learning appropriately.

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Academic staff perceptions of the importance of generic skills for veterinary graduates and the contribution of the different components of the final phase of a veterinary curriculum

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Implications In this study, we assessed academic staff opinions on the importance of generic skills for veterinary graduates and how much they thought the different components of the final part of their veterinary training contributed to each of those skills**.**

Introduction Professional, non-veterinary specific skills are important to the veterinary graduate. However, little is known about how these are best taught and their teaching poses a considerable challenge in the traditionally content-heavy veterinary curriculum.

Material and methods This study surveyed 116 academic staff who are actively involved in teaching veterinary students. We asked the participants to rate the students' competence in generic skills and their perception of the contributions that the different components of the final year made to those generic skills.

Results The table below shows the importance of core skills (mean ± SD, scale from 1 to 4, 1=absolutely useless, 2= not important, important, 4=very important) and how much the different components in the final year have contributed to the listed generic skills (1=was detrimental, 2= has done nothing, 3=has contributed in a minor way, 4 = has contributed in a major way). In italics are the same data from students for comparison (Weller and May 2013).

General skills	Importance	Research project	Extramural studies	Intramural studies	Free study
Communication skills – writing	3.42±0.63	3.39 ± 0.94 *3.36 ± 0.63*	2.11 ± 1.08 *2.64 ± 0.75*	2.45 ± 1.19 *3.37 ± 0.72*	1.76 ± 0.96 *2.42 ± 0.71*
Communication skills – oral	3.95±0.37	2.68 ± 0.77 *2.50 ± 0.67*	3.15 ± 1.38 *3.84 ± 0.47*	3.15 ± 1.32 *3.82 ± 0.45*	1.68 ± 0.86 *2.18 ± 0.50*
Information gathering	3.48±0.37	3.86 ± 0.50 *3.57 ± 0.57*	2.36 ± 1.16 *2.90 ± 0.67*	2.68 ± 1.28 *3.35 ± 0.68*	2.52 ± 1.36 *3.05 ± 0.78*
Information evaluation	3.71±0.55	3.50 ± 0.66 *3.48 ± 0.64*	2.53 ± 1.17 *2.82 ± 0.70*	2.82 ± 1.25 *3.25 ± 0.71*	2.33 ± 1.32 *2.89 ± 0.73*
Statistics	2.45±0.73	3.33 ± 0.79 *3.39 ± 0.80*	1.71 ± 0.72 *2.05 ± 0.35*	1.80 ± 0.85 *2.10 ± 0.43*	1.71 ± 0.87 *2.13 ± 0.42*
Teamwork	3.79±0.51	2.42 ± 0.81 *2.22 ± 0.57*	2.91 ± 1.32 *3.59 ± 0.59*	3.20 ± 1.35 *3.83 ± 0.46*	1.62 ± 0.96 *2.24 ± 0.53*
Ability to work independently	3.70±0.66	3.47 ± 0.81 *3.40 ± 0.72*	2.45 ± 1.23 *3.10 ± 0.68*	2.55 ± 1.15 *3.38 ± 0.67*	2.74 ± 1.37 *3.40 ± 0.77*
Management skills	3.23±0.74	2.83 ± 0.92 *2.80 ± 0.81*	2.29 ± 1.23 *2.88 ± 0.71*	2.26 ± 1.14 *3.20 ± 0.76*	1.94 ± 1.14 *2.66 ± 0.80*
Time management	3.58±0.61	3.20 ± 0.88 *3.24 ± 0.74*	2.53 ± 1.15 *3.10 ± 0.76*	2.68 ± 1.34 *3.65 ± 0.56*	2.44 ± 1.31 *3.17 ± 0.81*
Problem-solving	3.83±0.51	3.24 ± 0.90 *2.82 ± 0.73*	2.71 ± 1.25 *3.34 ± 0.68*	2.88 ± 1.38 *3.66 ± 0.57*	2.21 ± 1.22 *2.87 ± 0.72*
Critical thinking	3.73±0.60	3.38 ± 0.89 *3.05 ± 0.71*	2.55 ± 1.17 *3.24 ± 0.70*	2.77 ± 1.30 *3.53 ± 0.62*	2.12 ± 1.14 *2.89 ± 0.69*
Designing experiments	2.23±0.68	3.33 ± 0.90 *3.32 ± 0.74*	1.89 ± 1.50 *2.14 ± 0.49*	1.82 ± 0.84 *2.15 ± 0.53*	1.67 ± 0.85 *2.17 ± 0.49*

Conclusion Different components of the curriculum contribute differently to these generic skills. Statistics and experimental design were thought to be the least relevant, which may reflect the clinical background of the participants. The embedded research project contributed the most to the majority of skills according to the surveyed staff.

Acknowledgements The authors would like to thank all participants, as well as Dr Anne Crook from the University of Reading, UK, for their permission to modify and use part of their Research Skills Questionnaire.

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Design and validation of a computer-aided learning program to enhance students' ability to recognize lameness in the horse

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Implications The ability to recognize lameness in the horse is an important skill for veterinary graduates; however, opportunities to develop this skill at the undergraduate level are limited. Use of a computer aided learning program (CAL) could aid this area of teaching.

Introduction Lameness is one of the most common clinical problems in horses (Egenvall *et al*.,2009) The ability to recognise lameness is key to diagnosis, investigation and management of equine lameness. Computer-aided learning programs (CALs) have been successful in supplementing practical skills teaching in other areas of veterinary medicine (Abutarbush *et al*., 2006). The aim of this study was to design and validate a CAL to supplement veterinary student teaching of equine lameness recognition skills.

Material and methods A bespoke computer aided learning programme (CAL1) was designed to highlight the cardinal signs of equine lameness. Footage was acquired specifically for use in the CAL which was edited to include slow motion clips, annotations and a voice over. A control CAL (CAL2) was created using archived footage to simulate teaching methods currently employed during clinical teaching. 61 student volunteers were randomly assigned to either CAL1 (n=27) or CAL2 (n=34). They were tested to establish their current ability to recognize lameness (pre- test) and then exposed to their allocated CAL. Retesting occurred both immediately following exposure (post- test) and 1 week later (recall test). Testing material contained video clips of six fore and six hind limb lame horses. At each test point, the number of correct responses for forelimb and hind limb cases was determined. Student confidence was assessed before and after CAL exposure, with previous opportunities to recognize lameness also taken into account. Differences in test results between CAL1 and CAL2 were analysed using Mann- Whitney U test. To compare test results within each CAL group, across the three test points, a Kruskal- Wallis test was used. This test was also used to compare test results between years of study. Changes in the feeling of confidence was assessed using Mann- Whitney U test. This study was approved by The Royal Veterinary College ethics and welfare committee.

Results The results are summarised in table 1.There was no significant difference between CAL1 and CAL2 test scores at the pre- test point (p=0.924). In the post- test the number of correct responses was significantly higher for CAL1 than for CAL2, both overall (p= 0.008) and for forelimb cases (p=0.02) but not for hind limb cases (p=0.1). At the recall test point, CAL1 performed significantly better overall (p=0.49) compared to the CAL2 but there was no significant difference in CAL group performance in forelimb cases (p=0.164) or hind limb cases (p=0.58).

Table 1 Median, interquartile range (IQR), and p values for pre- test, post-test and recall test points for forelimb, hind limb, and overall scores. Bolded text indicates a significant value.

	Pre- Test			Post- Test			Recall Test		
	CAL1	CAL2	*p* value	CAL1	CAL2	*P* value	CAL1	CAL2	*p* value
Forelimb	4(IQR=6)	4(IQR=6)	.819	5(IQR=1)	6(IQR=5)	**.022**	5.5(IQR=2)	5(IQR=5)	.164
Hindlimb	3(IQR=6)	3(IQR=6)	.733	5(IQR=3)	3.5(IQR=5)	.100	5(IQR=2)	3.5(IQR=2)	.580
Overall	6(IQR=6)	5(IQR=5)	.924	10(IQR=4)	3.5(IQR=5)	**.008**	10(IQR=2)	8(IQR=6)	**.049**

Between the pre-test and post-test, CAL1 students showed a significant improvement in their overall scores (p=0.004), scores for forelimb cases (p=0.003), and scores for hind limb cases (p =0.026). There were no significant differences in overall scores (p=0.341), forelimb scores (p=0 .687), or hind limb scores (p=0 .275) across the three testing periods in CAL2.

Student confidence and ability to recognize lameness were significantly improved following exposure to CAL1 ($p<$ 0.0001).

When considered as one category, students in years 4 and 5 performed significantly better than year 3 students (p=0.04). Gender did not significantly affect performance (p= 0.088).

Conclusion This study successfully validated a multimedia CAL (CAL1) as a suitable learning resource for supplementing the current lameness recognition skill teaching. The CAL is, however, limited in its ability to satisfy the requirement of offering practical experience in lameness recognition, especially in regards to hind limb lameness recognition.

Acknowledgements We would like to thank the Royal Veterinary Collage e-media team and Peter Nunn from the LIVE team for technical advice, the RCVS Trust for funding the equipment for this study, and all the students for their participation.

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Demographic influences on staff and student attitudes to veterinary student support requirements

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Implications Age had a significant effect on staff and student attitudes to veterinary student mental wellbeing and the requirement for support services. Being a second degree student or repeating a year also significantly affected attitudes.

Introduction Demographics of veterinary staff and students may influence perceptions of student mental wellbeing and their need for support services. Analysis of demographics may therefore identify particular cohorts of interest. The aim of this study was to investigate such demographic influences at a UK veterinary school.

Material and methods Web-based questionnaires designed to investigate attitudes towards student mental wellbeing and support services were completed by staff and students at a UK veterinary school. Demographic analysis of responses was performed using Kruskal Wallis, Jonckheere-Terpstra and Chi-square tests.

Results Questionnaire responses were received from 250 students and 90 staff (35 per cent and 52 per cent response rates, respectively). The respondents' demographic data were representative of staff and students at Edinburgh veterinary school. Female students perceived that veterinary students had a greater need for counselling than other undergraduates compared to male students (P=0.01). Age, being a second degree student and repeating one or more years significantly affected students wanting, but feeling unable, to attend counselling (P<0.001, P=0.03, P=0.02 respectively, Fig. 1). Age and being a second degree student also significantly increased the perceived value of student counselling (P=0.005, P=0.003) and likelihood of having attended counselling (P<0.001, P=0.02) respectively. Advancing year of study significantly affected student perceptions of veterinary students having poorer mental wellbeing than other undergraduates (P<0.001, Fig. 2).
A more senior age and position within the veterinary school had a significant effect on staff perception of veterinary students having worse mental wellbeing compared to other undergraduates (P=0.03, P= 0.01 respectively). Senior staff were more inclined to perceive student counselling of little value (P=0.04).

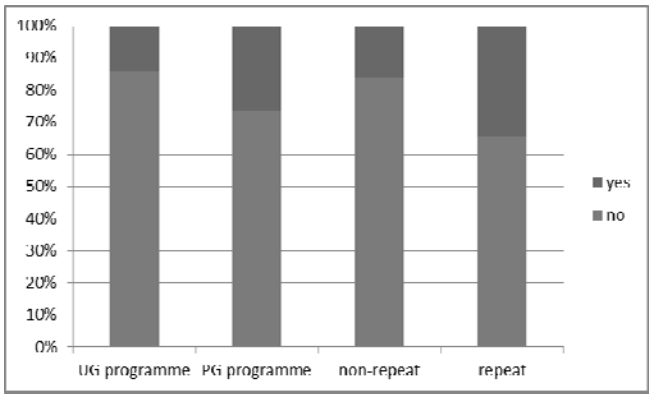

Figure 1 Percentage of veterinary students on the undergraduate (UG) and postgraduate (PG) programmes repeating one or more year that wished to have attended student counselling but felt unable to

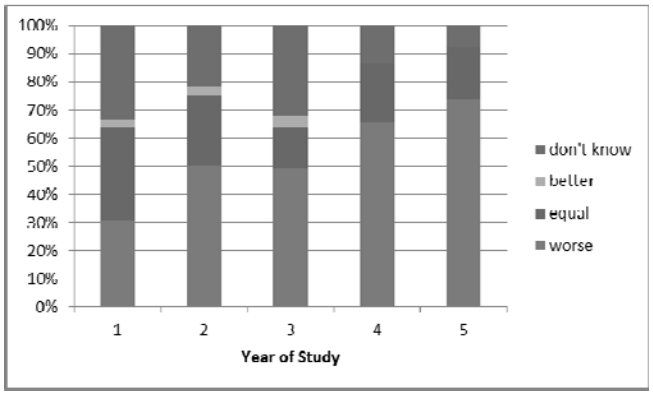

Figure 2 Veterinary student perceptions of the mental wellbeing of veterinary undergraduates compared to the general undergraduate student population.

Conclusion Older students, second degree students and students who repeat years may benefit from targeted encouragement to seek support.

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A novel method of educating students about zoonotic disease: bringing the vets and medics together

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Implications Educating veterinary and medical students together about identification and management of zoonotic diseases, enhances the learning experience and emphasises the importance of inter-professional collaboration.

Introduction Zoonotic disease education is an important part of the veterinary curriculum, and yet appreciation of the key role vets play in public health is often overlooked by students. Control of zoonotic disease outbreaks requires collaboration between infectious disease experts in both animal and human health, however current teaching programmes keep veterinary and medical education separate. Contact between the medical and veterinary professions is usually limited, and awareness of each other's knowledge or roles can be minimal. This limits the desire to collaborate and we sought to address this by highlighting the importance of veterinary public health to both medical and veterinary clinical students.

Material and methods A zoonoses workshop was designed to enable clinical medical and veterinary students to study and learn together about public health issues. The 2hr workshop involved an interactive quiz, small group discussions and a 'real-time' outbreak scenario, The workshop was facilitated by infectious diseases physicians, veterinarians and a Consultant in Communicable Disease Control.

Results A total of 32 students participated, 17 vets and 15 medics. Participating students showed an increase of 1.0 on a 5-point Likert scale for confidence in management of zoonoses. The workshop also achieved an increase of 0.9 for student understanding of the roles of medics and vets in zoonoses outbreaks. The most common suggested improvement was for the event to be longer, and 97% would recommend the event to their peers.

Conclusion Student understanding of zoonoses identification and management was significantly improved by the workshop. Excellent team-work was shown between medical and veterinary students, and students and facilitators alike were impressed by the level of knowledge shown by members of the alternative profession. Medical and veterinary students can be taught together to successfully learn about zoonotic disease and the importance of inter-professional collaboration.

Acknowledgements Workshop facilitators: Professor James Wood, Professor Ann Louise Kinmonth, Dr David Williams, Dr Mark Holmes, Dr Chris Williams, Dr Elinor Moore and Dr Sani Aliyu,
The event was kindly sponsored by Cambridge Infectious Diseases

The impact of maths support tutorials for higher education animal science students

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Implications Small-group tutorials improved mathematics confidence in first year animal science students and led to a higher mark in a first year statistics exam. Small-group tutorials are an effective method to support animal science students.

Introduction According to the 2010-2015 BBSRC Strategic Plan, there is an urgent need to raise the mathematical and computational skills of biologists at all levels due to the increasingly quantitative nature of the bioscience disciplines (BBSRC, 2012). Hodgen *et al.* (2010) reported that the UK has the lowest participation of students in post-16 maths out of 24 OECD countries. This leaves a gap between the knowledge and skills that are required for undergraduate bioscience degrees and the knowledge and skills with which new entrants to these degrees present, e.g. many entrants on a bioscience degree lack the skills that define a "numerate individual", even though most of them have at least a grade C in GCSE maths (Tariq *et al.*, 2002). According to most academic staff a lack of mathematics knowledge, skill or confidence is preventing postgraduate bioscientists from becoming involved in interdisciplinary research (Koenig, 2011). This paper investigates the efficacy of use of small-group mathematics tutorials as a method of improving both numeracy and mathematical confidence of first year undergraduate HE Animal Science students.

Material and methods A survey questioning students about their mathematics confidence and an analysis of academic performance in modules with a mathematical content were performed for a cohort of students (N = 105) enrolled on the first year of an animal science programme. The minimum level of mathematics for this cohort was grade C at GCSE level. In order to investigate student confidence in mathematics an online questionnaire was set up which included demographic information about the respondent and their previous academic qualifications, as well as sliding-scale questions and questions regarding feedback on the mathematics tutorial programme and reasons for participating. The effect of the mathematics tutorials on student performance was analysed by applying a diagnostic test (basic numeracy and simple algebra questions) at the beginning and the end of a 12 session mathematics tutorial programme delivered by an independent mathematics tutor. Thirty students followed the entire 12 session programme. Bivariate analysis of the survey data was performed using Fisher's exact test or Chi-square tests. Student performance was analysed using t-tests for matched groups of students based on previous mathematics experience and tutorial attendance. This project was approved by the Writtle College Ethics Committee on 18 April 2012.

Results The results (Fig. 1) indicate that student maths confidence was significantly increased (P<0.001) after attending tutorials to the same level of that of students who felt tutorials were not necessary. Students attending the tutorial programme achieved significantly higher marks for their year 1 statistics exam (P<0.05, Cohen's d = 0.61) compared with matched controls.

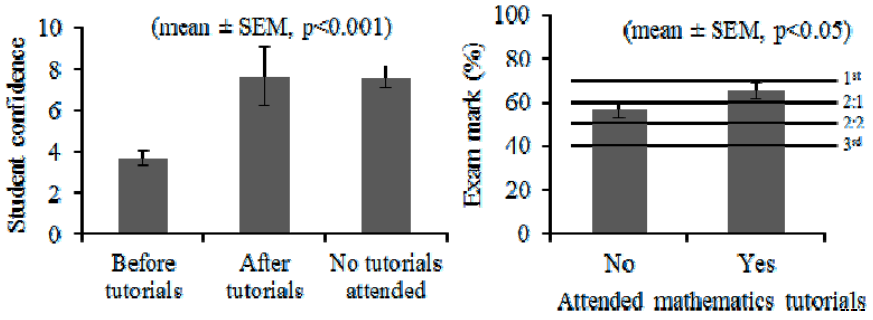

Figure 1 a) Student confidence levels on a scale of 0-10 (10 being highest) before and after attending maths tutorials. b) Effect of attending tutorials on first year statistics exam marks (%).

Conclusion The outcomes of this project demonstrate that students will make use of small-group math tutorials if these are provided and that these tutorials improve basic mathematics skills and student confidence. Small-group tutorials are therefore an appropriate student support strategy in bioscience education.

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On-line resources for teaching statistical methods to students of the animal sciences

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Implications Innovative uses of on-line resources can supplement and enhance traditional forms of learning and teaching.

Introduction Traditional approaches to the teaching of statistics using a combination of lectures and practical classes are difficult to maintain as class sizes increase. Experience indicates that many students find it difficult to follow the logical thread of lectures in statistics and need time to think and develop their own understanding and applied skills. Faced with an expansion of class size to approximately 400 students, we developed an on-line course that aimed to provide materials that would allow individuals to work at their own pace and would approximate one-to-one tuition as closely as possible.

Material and methods The course is presented as a series of learning units within the BlackBoard virtual learning environment. A series of learning units cover the basic principles of statistics and tuition in a range of parametric and non-parametric methods. The main features of a learning unit are shown in Figure 1 and include as introductory materials (a) a file (README) to outline the structure of the learning unit and the tasks required of the students, (b) short explanatory lectures (c) a demonstration of data analysis using SPSS software and a data set of generic interest to all students. This material is replicated in a handbook that summarises the principles of the analysis, provides screen shot instructions for the use of the software, and a guide to the interpretation of the outputs. Subsequently, students are required to repeat the analysis and complete quiz material that tests their understanding of principles, that the analysis has been completed correctly and that valid interpretations of the data have been made. Predictive feedback is given at the end of each quiz and students are not allowed to progress until they have achieved a pass mark of 75%. Subsequently, students are required to complete a further one or two formative assignments in this way before they are allowed to complete the summative assessment. Formative assignments use examples of experimental scenarios taken from published literature, and data sets were constructed to replicate their results. Separate assignments were created to meet the course interests of different students e.g. equine science, animal science or agriculture, with topics of generic interest used in the summative assignment. Further support is offered through a discussion board that allows both anonymous and named postings, and by the provision of face-to-face teaching to individuals and small groups in a workshop environment. Student attitudes to the course were evaluated over two cohorts using on-line surveys.

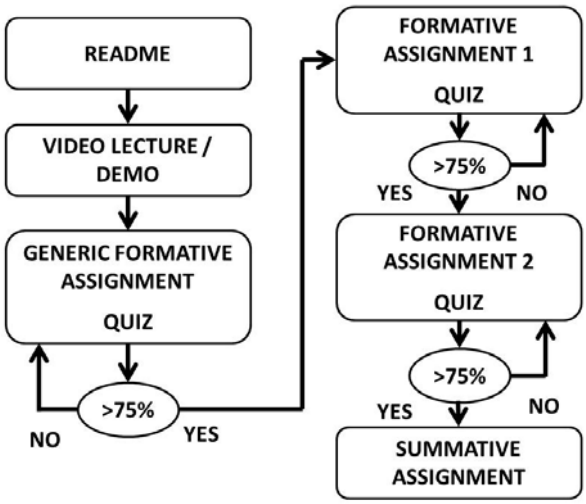

Figure 1 Structure of a learning unit

Results Surveys were completed by 88/138 and 85/383 students. Sixty-eight and 72% of respondents agreed that this was a satisfactory method of studying the subject, with 21% and 7% giving a neutral response. Students valued the flexibility of work time and location that on-line materials allow and also the ability to replay videos and engage with the handbook and immediate feedback materials when working at their own pace. However, 10% and 21% of respondents disliked this method of study and need to be encouraged to attend workshops where they can receive face to face tuition. Technical problems with the use of software off-campus were reported as the major difficulty (48%) and difficulties of accessing staff (11%). Despite this, workshops were poorly attended and students reported that course materials sufficed (67%) or their problems were solved in group discussion, by communication by e-mail or discussion board (24%).

Conclusion On-line resources have proved an acceptable delivery method for the teaching and learning of statistical methods. The use of a mix of resources and their presentation within a virtual learning environment allows for relatively close control of the learning process and increased provision of formative work, whilst giving students flexibility in time and place of learning and a useful resource for future reference.

Acknowledgements Technical support by Kate Wright, Johanna Westwood and Mary Jacob.

Academic staff perceptions of the usefulness of an embedded research project in the final phase of a clinical veterinary programme

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Implications This study shows that attitudes and opinions vary widely between academic supervisors on the usefulness of an embedded research project. Since we have previously shown that the supervisor's attitude is one of the main factors influencing a student's attitude towards and their experience of research (Weller and May 2013), it is important to identify and optimise factors that will improve the staff, and thus the student, experience of student research.

Introduction Veterinary training has to equip the future clinician with the skills and knowledge to practise evidence-based veterinary medicine and the future researcher with the background to embark on a research career path. Different schools have adopted different ways to achieve this. At the authors' institution, the research training strategy includes didactic teaching as well as two embedded projects, the second of which encompasses an eight week long, hypothesis driven project conducted by the students, towards the end of their clinical veterinary programme, under the supervision of an academic member of staff.
In this study, we assessed supervisor attitudes and their perception of student attitudes towards this research project. We hypothesised that staff attitudes and their perception of student attitudes were related to their own research experience.

Material and methods This study surveyed 116 academic staff, who have acted as supervisors for student projects. The survey was conducted online and included 30 questions in Likert, yes/no and free text format. Participation was voluntary and anonymous, and ethical approval for the study was granted by the institution's Ethics and Welfare Committee. Data were analysed using regression models.

Results The 116 participants had 1-24 years (mean±SD 5.6±5.4) supervisory experienced, and had each supervised between 1-82 projects (mean±SD 12.9±16.4). The majority of participants thought that the embedded research project was a very useful (28%) or useful (57%) part of the veterinary curriculum. 7.8% thought it was not useful and the remainder had no opinion. The professional role of the participant significantly influenced this, with full time clinicians finding the project significantly less useful than clinician/researchers or researchers. 16.4% of participants were of the opinion that the eight weeks allocated to the project would be better spent on something else, mainly on extramural and intramural studies. The majority of participants did not think it would be a good idea to extend the research time to offer a Masters degree, they also did not think the students would welcome this opportunity, directly contradicting the student view (Weller and May 2013).
The majority of participants spent between 1-3 hours supervising project students during their research block and responded within 24 hours to e-mails from their research students. 28% of participants would prefer to spend more time on supervision of student projects, and 7.8% less time. The remainder judged the time to be just right. The total number of projects supervised by the participants by the study date was 902, and the total number of publications was 181 (20.0%). Out of these, 68 (7.5%) were based on a single project, 31 (3.4%) were based on more than 1 project, and in 82 (9.1%) the student project contributed data to publication. The cumulative number of students supervised positively correlated with the number of publications from combined projects. In addition, experience with PhD student supervision and the professional role of supervisor were related to the number of publications, with the clinician/researcher being more likely to publish student projects.

Conclusion Staff opinion on the usefulness of student research varies, but the majority of the participants agreed that it is useful, while a minority finds it not useful. Clinician/researchers found it the most useful and also had the most publication success, whereas full-time clinicians found it less useful and were less successful in terms of publications. This may be due to the lack of a research background or lack of research time on the part of the full-time clinician, and these factors should be considered before allocating project supervisors.

Acknowledgements The authors would like to thanks all the participants

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R Weller, SA May. 2013. Factors Influencing Clinical Students' Perceptions of an Embedded Research Project and Associated Publication Output Journal of veterinary medical education 40 (2), 119-127.

Analysis of the success of two live-animal assessment aids in determining readiness for slaughter in beef cattle

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Implications Farmers are significantly more successful at getting cattle to meet desired carcase classification after attending an EBLEX Live-to-Dead day. There was no relationship between age and education level and attending a Live-to-Dead day.

Introduction It was reported by the English Beef and Lamb Executive (EBLEX) that in 2011 up to 51% of all bovine carcases failed to meet UK premium classifications through being too fat, lean, poorly conformed or combinations thereof (Brown and Powdrill, 2012). Lambe *et al.* (2010) had previously reported that significant financial losses are seen when sending unfit animals to slaughter. A number of live-animal assessment aids have come about in recent years to try and combat this continued failure to meet desired classification, including the Better Returns Programme (BRP) and Live-to-Dead (LtD) days. The aim of this study was to examine usage and efficacy of these two assessment aids in relation to successful carcase classification.

Material and methods A voluntary postal survey of 200 randomly selected beef producers in England was undertaken and alongside conventional questions relating to herd size, age and education level, it focused on live-animal assessment methods and use of available assessment aids as well as desired and actual carcase classifications achieved. Data from the survey was analysed using Pearson's Chi-Square statistical test for comparisons between categorical factors, and was corrected for through the Fisher's Exact Test. Further analysis of use of the BRP and attendance to the Live to Dead days was conducted using Logistical Regression Modelling for binomial proportions (n successes for premium grades) with binary explanatory variables for utilisation of BRP and attendance at Live-to-Dead events (0 = no, 1 = yes). The model was stipulated thus:

$$\log(y) = \beta_0 + BRP\ x + L\ to\ D\ x + \varepsilon$$

Where BRP and LtD are the binary variables.

Results Nearly all of the farmers had heard of the BRP; but only 49% chose to use it to aid them in live animal assessment. Farmers under the age of 50 are significantly more likely to make use of the tools provided by the BRP than those over the age of 50 (X^2= 4.846, P = 0.028). Interestingly, those farmers who did not use the BRP had a slightly increased chance of meeting desired classification. 38% of farmers questioned had attended a LtD day. There was no significant relationship between age and attendance. Education level played no significant role in utilisation or attendance of either of the aids.

Table 1 Output from the Logistic Regression Model

Parameter	B estimate	Significance	Odds ratio (antilog)
Use BRP	-0.6966	<.001	0.4983
Attended LtD	0.7613	<.001	2.141
BRP and LtD	0.4859	<.001	1.626

The outputs shows that both using the Better Returns Programme and attending a Live-to-Dead day were statistically significant variables. However, the odds ratio for use of the BRP is 0.5, displaying a decrease in the percentage of cattle attaining the desired class in those farms using the BRP alone. Attendance to a LtD day alone showed an increase in the percentage of cattle attaining the desired class with an odds ratio of 2.14. The result from farms using both aids was also shown to be significant with an increase in the percentage of cattle reaching desired classes. However, the odds ratio of 1.63 indicates that use of both assessment aids together was not as successful as attending a Live-to-Dead day alone.

Conclusion Experiential learning plays an important role in advising and aiding farmers in pre-slaughter assessment of live animals. With this obvious success in utilising Live-to-Dead days to achieve desired carcase classification, greater emphasis should be placed on farmers to attend these days, with future research looking into combining this aid with more traditional methods of pre-slaughter selection to improve live-animal assessment across the UK beef sector.

Acknowledgements The authors would like to gratefully acknowledge the team at EBLEX for helping to fund this study.

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Current methods used by English farmers to ascertain prime slaughter condition in beef cattle

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Implications Weight recording is utilised significantly more by younger farmers, although the older generation has a better grasp on how to select animals for slaughter. Education level plays no significant role in choice of selection method.

Introduction With less than 50% of all bovine carcases failing to meet UK premium classifications in 2011 through poor conformation and fat class (Brown and Powdrill, 2012) and thus causing significant financial losses to the UK beef industry (Lambe *et al.,* 2010), the methods that farmers use to select animals for slaughter need to be analysed. The aim of this study is to therefore determine what methods of pre-slaughter assessment UK beef farmers are currently utilising to determine suitability of their beef cattle for slaughter; in respect of age and education of the person undertaking the assessments, to try a obtain a better understanding of why so many carcases are failing to meet the grade.

Material and methods A voluntary postal survey of 200 randomly selected beef producers in England was undertaken, with a total of 49 responses. Alongside conventional questions relating to herd size, age and education level (school only; further education; higher national diploma; higher education), it focused on finding out what methods farmers are currently using to assess their animals to determine prime slaughter condition (weight-recording/manual handling techniques/visual assessment or combinations thereof). The initial survey was piloted on local beef producers and final editing was undertaken by the researchers and EBLEX representatives. Data from the survey was analysed using Pearson's Chi-Square statistical evaluate associations between categorical factors and was corrected for through the Fisher's Exact Test.

Results In regards to weight recording (WR), it was found that older farmers (>50 years) used the method significantly less than farmers under 50 (X^2 = 8.081, P = 0.004). 17% of farmers over the age of 50 used weight recording, all of which used it alongside either handling techniques or visual assessment. Over half of all farmers under 50 used weight recording, again in conjunction with other live-animal assessment methods. A comparison between age and choice of assessment method is displayed in figure 1. Education level had no significant effect (P >0.05) on use of this method. Neither age nor education level had a significant effect on use of manual handling (MH) techniques with less than half of all farmers surveyed utilising this method. Those that did use this method focused on specific areas such as the ribs and loin rather than the entire animal. 97% of farmers used visual assessment (VA) as a means of live animal assessment and a high proportion of farmers under 50 (42%) assessed specific points of the animal, rather than focusing on the overall animal, the reverse is seen in older farmers. Figure 2 compares the age of farmer against their choice of focus – be it the entire animal or specific points.

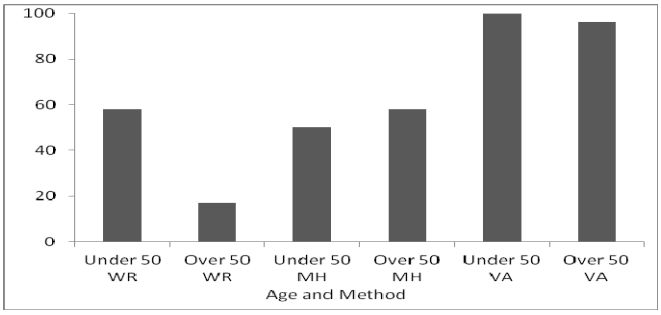

Figure 1 Age of farmer and choice of assessment method (%)

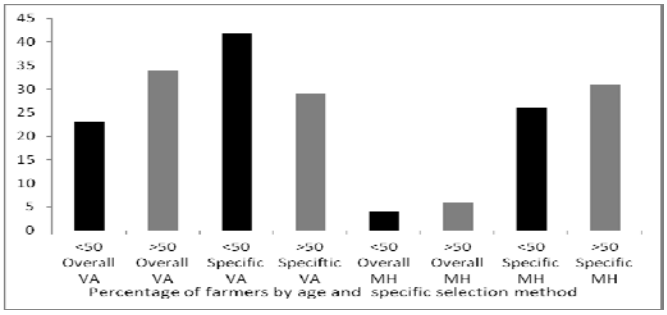

Figure 2 Specific points vs. overall animal in relation to age

Conclusion Traditional methods of assessment are being utilised more by younger farmers. Older farmers have a better grasp on how assess live cattle; looking at the animal as a whole, and then handling specific regions. Knowledge transfer schemes need to focus more on what a prime beef animal should look like with elder farmers, and more on use of selection methods with the younger generation. Emphasis should also be placed on the younger generation to continue with education, as this will help in the transfer of skills in terms of new methods of live-animal assessment, thus helping to ensure that more animals meet desired carcase classification in the future.

Acknowledgements The authors would like to gratefully acknowledge the team at EBLEX for helping to fund this study.

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Could we use the Wiki Vet approach to assist animal science/husbandry teaching and training?

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Implications A continued source of up-to-date information about animal science/husbandry for all interested users, including the general public.

Introduction Anyone teaching animal science and husbandry is well aware of the lack of suitable modern texts to support classes. Most books in our subject area are out of date and there is little chance that any publisher will be interested in reviving the market. In any case, the world has moved on and digital media are much more useful to the modern student and institution. The RVC has implemented a Wikipedia approach to underpin some of its basic teaching, called WikiVet, which has proved to be well used in the 7 years since its inception. The question addressed in this presentation is 'Could we use the WikiVet approach to develop material to support teaching and training in animal science/husbandry?'

Material and methods This project is potentially of interest to all colleges, universities and training institutions in UK and overseas who undertake teaching in animal science/husbandry. Its objective is to provide free access to material dealing with all subject areas of interest to the target community. There are funds available to develop this type of material from a number of sources, both public and private. Following the WikiVet model it is intended to bring relevant material together in structured way. The intention is to provide material at multiple levels for use in a range of institutions. As with other Wiki models, registered contributors will add material to fill in a framework arranged by the originators of the site. It will be curated lightly but in line with good practice in the field. Management of the new Wiki system will be overseen by a board of interested collaborators and managed on a day-to-day basis at the host institution.

Results This approach should provide a flexible system which supports many levels of teaching/training. It should be easily modernised and updated since teachers know what is required and will contribute relevant material. After all many contributors spread the burden of developing the material. Of greatest importance is the fact that experts will contribute in their area of knowledge for the benefit of the whole community. The provision of animal science content in this format in no-way impinges on an institution's role to validate the learning experience of the student/trainee. Each institution will remain in total control of their degrees, diplomas, certificates etc. The Wiki approach should be seen as a potential library substitute, not an institution substitute.

Conclusion A happy animal science/husbandry community with access to the latest material for courses *etc* but only if you think it is a useful idea and are prepared to contribute material. What do you think?

Acknowledgements You as potential contributors and users.

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http://en.wikivet.net/Veterinary_Education_Online

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Predicted energy balance for dairy cows based on National herd data

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Implications This research explores the development and application of prediction equations derived from resource population data to provide farmers with a quick, inexpensive and reliable estimate of the energy status of their dairy cows as part of routine milk-recording operations.

Introduction During early lactation dairy cows may experience negative energy balance (NEB); a situation in which the food intake doesn't meet the energy demands of lactation and requires the mobilisation of body fat (Coffey *et al.,* 2004). The duration of time that a cow spends in NEB can be highly detrimental to its health and fertility (McParland *et al.,* 2011). Combining experimental data from our diary research herd in Dumfries and building upon McParland *et al.* (2011), prediction equations have been developed based on routinely available data from the high-throughput mid-infrared (MIR) analysis of milk, due to its proposed relationship with energy balance (McParland *et al.,* 2011; Friggens *et al.,* 2007). The objective of this research is to use these prediction equations to provide a quick and robust estimation of the energy status in dairy cows which could be directly incorporated into future selection indices to facilitate selection of animals (genotypes) with appropriate energy profiles to optimise profit and animal welfare (Coffey *et al.,* 2004; McParland *et al.* 2011).

Material and methods Using a non-random selection of animals (dataset 1; c. 3305 records on 500 cows) we have calculated all of the following energy balance traits across lactation (Table 1) (Banos and Coffey, 2010; McParland *et al.* 2011); (i) energy balance (EB; MJ/d), a function of milk yield, fat and protein content, DMI, BW, and BCS; (ii) body energy content (BEC; MJ), a function of body weight and condition score, predicting body lipid and protein weight and (iii) effective energy intake (EEI) per day (MJ/d; Banos and Coffey, 2010). Concurrent MIR spectral records and milk production data were also available for these animals. For national cows (dataset 2; approx. 5,000 cows with 3-6 monthly milk tests) a dataset compromising MIR spectra and monthly milk production test results only was extracted from National Milk Records database. These datasets were combined such that equation for predicting energy balance traits was derived on the smaller complete dataset (1) and then applied to the larger dataset (2) of animals for which we only have the spectral and milk yield data. Analysis initially involved performing principal component analysis (PCA) on transformed spectral data in order to align the spectral data from the national data to that represented in the prediction dataset. Subsequently, a partial least squares (PLS) analysis was performed on the spectra to generate the predicted energy balance for those animals for which this parameter is unknown.

Results The precision of the prediction energy balance calculated to date has been shown to be high and similar to the best estimates of McParland *et al.* (2011) with an R of 0.64 - 0.77 for the different traits across lactation. This precision deems these predictions suitable for genetic evaluations and for translation into a sensible reporting trait. Research is currently underway to convert the prediction equations into programming language in a live database, which would help automate energy balance prediction during milk-recording.

Table 1 Energy balance traits across lactation for 3305 records (dataset 1)

Average parameter estimates	Lactation Number			
(± standard deviation)	1	2	3	4
Direct Energy balance (MJ)	0.18 ± 29.89	-4.66 ± 35.87	-5.04 ± 40.63	-8.08 ± 46.34
Energy content (MJ)	4702.12 ± 798.29	5323.26 ± 921.93	5615.54 ± 929.30	5757.01 ± 969.27
Effective energy intake (MJ)	146.72 ± 37.44	171.01 ± 41.65	185.56 ± 42.85	177.91 ± 45.5
Fat-to-protein ratio	1.17 ± 0.12	1.15 ± 0.11	1.16 ± 0.12	1.19 ± 0.10
Total yield	23.96 ± 7.79	29.56 ± 9.61	31.81 ± 11.12	31.34 ± 12.07

Conclusion This work demonstrates the development of prediction equation that can be directly applied to the national spectral data to give a robust and accurate indication of energy balance.

Acknowledgements The authors gratefully acknowledge collaboration with NMR and Marks and Spencer, funding from TSB and BBSRC, and cooperation with the participating farms.

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Genetic parameters of mastitis related traits in dairy sheep

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Implications The results can contribute towards defining a breeding strategy for mastitis resistance in dairy ewes.

Introduction Mastitis is a costly disease in dairy sheep and one of the main reasons for culling. Improvement of resistance to mastitis can be achieved directly by selecting on incidence of clinical mastitis (CM) or indirectly by selecting for a correlated trait. Milk somatic cell count (SCC) has been widely considered as a selection criterion to improve mastitis resistance (Gonzalo *et al*., 2002). Other traits that can be used are total viable bacterial count (TVC) and California Mastitis Test (CMT). Few genetic studies have been conducted on Lacaune and Valle del Belice sheep breeds. The objective here was to estimate genetic parameters of mastitis related traits in intensively reared ewes of the Chios dairy breed in Greece.

Material and methods The study was carried out in four commercial farms of purebred Chios dairy ewes. A total of 609 dairy ewes in 1st or 2nd lactation were used. Milk samples from individual ewes were collected at monthly intervals for the first five months of the milking period. Each sample was subjected to cytological examination using CMT. Tests were also performed in the laboratory to assess SCC and TVC. Test-day milk yields and incidence of CM were also recorded monthly. Total number of repeated records amounted to 2,436. Each trait was analysed with a univariate random regression model, including the fixed effect of farm, year-season of lambing, parity, age at lambing and week of lactation, and the random regressions on week from lambing associated with the additive genetic effect and the permanent environment effect of ewe. Estimates of (co)variance components from this model were used to calculate weekly heritabilities for each trait. The ASREML software was used for all statistical analysis (Gilmour *et al*., 2006).

Results Heritability estimates for SCC, TVC and CMT are shown in Figure 1 and were always greater than zero (P<0.05). Average estimates of heritability were 0.17±0.04, 0.16±0.03 and 0.18±0.05 for SCC, TVC and CMT, respectively. Heritability for CM was no different from zero. Consistent with Figure 1, heritability estimates were decreased in the first ten weeks of lactation and increased as lactation progressed. Repeatability estimates for SCC, CM, TVC and CMT are in Figure 2. Average estimates of repeatability for those traits were 0.62±0.04, 0.41±0.04, 0.33±0.03 and 0.67±0.03, respectively. Animal correlations between milk yield and SCC ranged from -0.28 to -0.32 (P<0.05) throughout lactation. Phenotypic correlations were from -0.23 to -0.16 (P<0.05) for the same period.

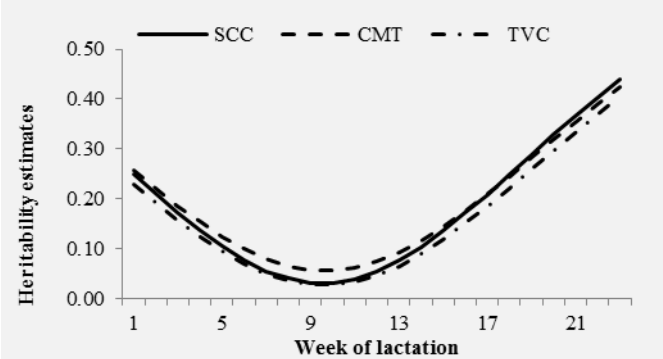

Figure 1 Heritability estimates

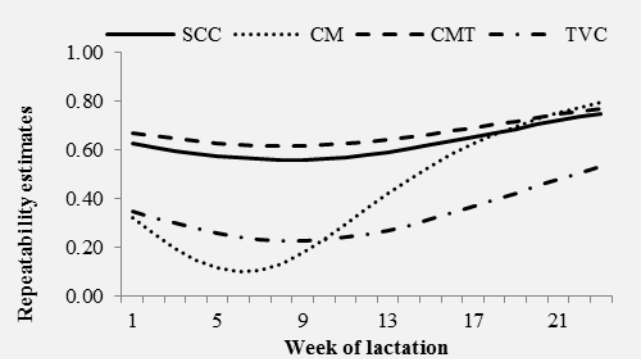

Figure 2 Repeatability estimates

Conclusion The results showed that mastitis related traits are heritable. Selection against mastitis may be achieved using primarily indirect traits. Besides SCC, other indirect traits, such as TVC and CMT, can be used as selection criteria for mastitis resistance in Chios dairy ewes.

Acknowledgements This work was partly funded by the European Commission 3SR Project (http://www.3srbreeding.eu)

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Validation of previously discovered markers for mastitis resistance in an independent population of dairy sheep

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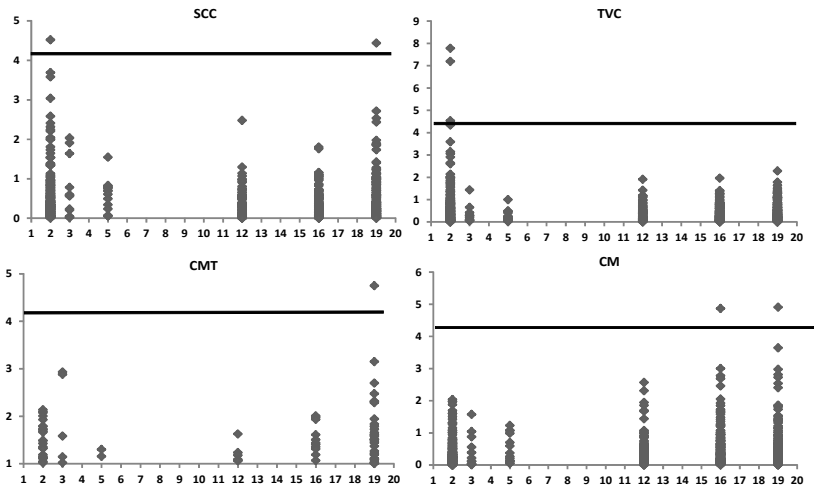
Implications The same SNP were found to affect mastitis resistance in different sheep breeds thereby facilitating genetic improvement programmes across breed.

Introduction Mastitis in dairy sheep is a very important issue from an economical, hygienic and legal point of view (Bergonier *et al*., 2003). Good farm management plays a key role in the reduction of mastitis incidence. A complementary approach to control the disease is breeding for enhanced mastitis resistance, since there is evidence for relevant genetic variation. Moreover, identification of selectable genetic markers suitable for use across breeds would be a very useful and cost effective tool in this regard. Putative polymorphisms for mastitis resistance have been identified in French (Lacaune), Italian (Sarda) and Spanish (Churra) sheep breeds (Rupp *et al*., 2013; Sechi *et al*., 2013) leading to the development of a 960 Single Nucleotide Polymorphisms (SNP) custom-made DNA array. The objective of this study was to validate the effects of these SNP on mastitis resistance in an independent population of Greek Chios dairy sheep.

Material and methods Six hundred and nine purebred Chios ewes raised in four different commercial flocks in Greece were used. All ewes were genotyped with the 960 SNP array. Four monthly records per animal were collected for milk yield and four traits related to mastitis: milk somatic cell count (SCC), total viable bacterial count in milk (TVC), California mastitis test (CMT) and presence of clinical mastitis (CM). A total of 2,362 records were collected. Records were adjusted for the fixed effects of flock, year-season of lambing, age at lambing, lactation number and week of lactation. These analyses yielded four residuals per animal. A further analysis including the animal in the model was performed, which yielded estimates of the animal effect. Residuals and de-regressed estimates of the animal effects were used as phenotypes in genome wide association studies (GWAS) that included the genomic relationship matrix between animals. Individual SNP found significant at genome-wide level (post a Bonferroni correction) in GWAS were tested using a mixed model analysis based on an allele substitution mode to verify their significance and assess the magnitude of their effect.



Results GWAS revealed 22 SNP markers located on chromosomes 2, 5, 16 and 19 with a genome-wide significant association with at least one of the mastitis related traits. A total of 18 SNP were verified by the mixed model analysis. Figure 1 shows four indicative Manhattan plots with the most pronounced effects on each trait. The horizontal line shows the genome wide significant threshold. High peaks were detected on chromosome 2 for SCC and TVC, chromosome 19 for SCC, CMT and CM, and chromosome 16 for CM. Collectively the SNP accounted for 5-16% of the phenotypic variance. None of these SNP was associated with a significant effect on milk yield.

Figure 1 Manhattan plots for four mastitis related trait; X-axis is chromosome number; Y-axis is -log (P-value).

Conclusion Selectable markers associated with mastitis resistance found in previous studies were validated in the present study using an independent population. These markers can be used in across breed selection programmes. Importantly, none of the markers related to mastitis resistance was associated with milk production, implying that this information can be directly used to genetically control mastitis without compromising the productivity of the animals.

Acknowledgements
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Estimation of genomic breeding values for milk yield in UK dairy goats

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Implications Single step GBLUP provides an opportunity to increase the accuracy of genomic predictions in breeding programs with small reference populations containing a mixture of males and females.

Introduction Recent availability of the Illumina caprine 50K chip (Illumina Inc., San Diego, CA; Tosser-Klopp *et al*. 2012) allows for the implementation of genomic selection in dairy goats. However due to the smaller scale of the goat breeding industry compared to cattle or poultry it is important to select an optimal method that can accommodate the structure (mixture of dams and sires), and small size of the reference population. Females are usually not included in the reference population in dairy cattle as there are thousands of bulls with accurate proofs. However, smaller populations have to supplement the reference population with females to increase prediction accuracy. The objective of this study was to evaluate two different methods for the estimation of genomic breeding values in dairy goats with a small reference population.

Material and methods The research was based on data provided from two commercial goat farms in the UK. The dataset comprised 566,641 milk yield records on 14,434 mixed-breed dairy goats (Saanen, Alpine, Toggenburg). The pedigree file contained 32,196 individuals, out of which 2,414 were founders. In total 1,960 animals were genotyped with a 50K Illumina caprine chip. After standard quality assurance 1,900 animals and 47,351 SNP remained. Two methods for estimation of genomic breeding value were compared. First method was BLUP-SNP, where de-regressed sire and dam proofs were used as phenotypes. SNP effects were estimated with the following statistical model:

$$y_i = \mu + v_i + \sum_{j=1}^{m} z_{ij} u_j + e_i$$

where: y_i is the deregressed proof, μ is the overall mean, v_i is the residual polygenic effect (10%) of i-th goat, z_{ij} is the genotype value, u_j is the random regression coefficient for j-th SNP, and e_i is the residual effect. The second method was based on the single step GBLUP approach (Misztal *et al*. 2009). A random regression animal model with **H** matrix was used. The relationship matrix **H** was constructed by combining the genomic relationship matrix **G** with pedigree relationship matrix **A**:

$$\mathbf{H} = \mathbf{A} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{G} - \mathbf{A}_{22} \end{bmatrix} \qquad \mathbf{G} = 0.95 \frac{\mathbf{SS}'}{2\sum_{i=1}^{n} p_i (1 - p_i)} + 0.05\mathbf{A}$$

where **S** is a centered incidence matrix of SNP genotypes, n is the number of SNP markers, p_i is allele frequency of marker i, and A_{22} is a pedigree relationship matrix for the genotyped animals.

Results Accuracy of genomic breeding values was 0.21 and 0.44 with BLUP-SNP and single step GBLUP, respectively. The result of a single step GBLUP is comparable with the accuracy for milk yield that has already been reported for French dairy goats, which reached 0.39 (Carillier *et al*. 2013).

Conclusion The single-step approach resulted in higher accuracy of genomic breeding values in comparison with BLUP-SNP for a small reference population. This method can be recommended for breeding programs with small reference populations with both males and females genotyped.

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Can limited molecular data be useful to improve a rare breed of cattle?

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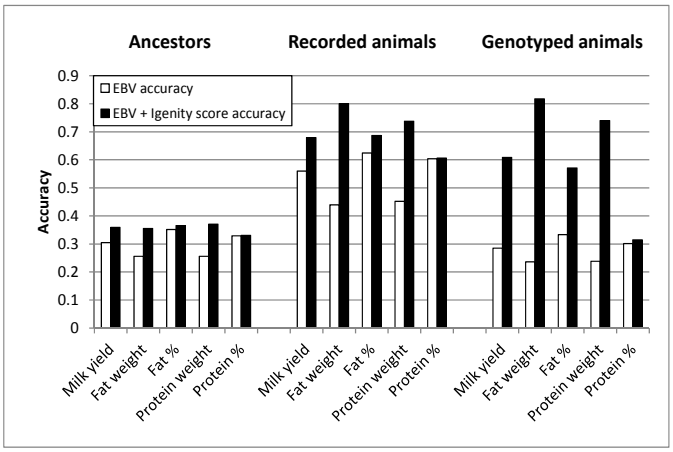
Implications Scores based on limited genetic data can be used to improve the accuracy of genetic merit for most dairy traits in a rare breed of cattle.

Introduction Rare breeds, by their very nature, suffer from a number of issues which may be a drawback when trying to implement genetic improvement programmes. The molecular genetic revolution is changing the way mainstream breeds are selected and it is of interest to investigate how such methods might help owners of rare breeds to improve their animals. This study investigated the use of a small set of SNPs used by Merial to derive Igenity scores for a range of traits on cattle (Merial, 2008). These scores, along with other more traditional genetic evaluation methods, were studied as an aide to determining the genetic potential of a rare breed of cattle, i.e. Gloucesters.

Material and methods The dairy production data used in this study were collected from the milk records kept on 20 farms and follow the trait descriptors commonly used by organisations such as National Milk Records Ltd. (Harrogate, North Yorkshire, UK). Information on herd, calving date and lactation number were collected for 176 lactations from 82 cows. Starting in 2009, the Gloucester Cattle Society obtained DNA from 199 animals: 37 males and 162 females. These samples were used to obtain profiles for each individual of their predicted dairy characteristics based on an Igenity score. This was a system developed by Merial which produced scores (on a 1-10 scale) for each animal for 12 dairy traits based on 123 SNP genotypes. This scoring system was produced using evidence of associations between the DNA profile (genotype) and the actual measured trait (phenotype) from Holsteins in the US. The production data was used to derive estimated breeding values (EBV) and their accuracies for 5 dairy traits using an appropriate mixed model in ASReml (Gilmour *et al*., 2009) using a database of 6,527 pedigrees. These traits were reanalysed using a bivariate analysis including the Igenity scores for the same trait. Accuracies for both runs were summarised for 3 groups of animals; genotyped, recorded and ancestors back 3 generations. Recorded animals were not genotyped and *vice versa*.

Results

Figure 1 summarises the accuracies of the EBV for 5 dairy traits and the 3 groups of animals. The addition of Igenity score in the genetic evaluation increased the accuracy of all traits in all groups of animals, except protein %. The largest improvements in accuracy were seen in the genotyped animals. These were not recorded but received their EBV via recorded close relatives. The greatest gain in accuracy was seen for fat % for all groups of animals and the least in protein %. The accuracy of ancestors, with neither records nor genotypes, remained relatively low in both the uni- and bi-variate analyses.



Conclusion The use of a limited amount of SNP information, converted to Igenity scores, can improve the accuracy of most milk traits in a rare breed of cattle. The exact balance between the level of recording and genotyping needs further investigation to optimise the system.

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Analysis of candidate innate immune genes and signalling pathways associated with resistance to mastitis in dairy cows

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Implications The ability to resist bacterial infection differs between cow breeds. To a large extent, disease resistance is due to discrepant innate immunity between genetic variants. Identification of the genetic base and mechanisms of these differences will benefit breed selection for increased disease resistance.

Introduction In recent years, genetic selection has produced modern high yielding dairy cows. However, this seems to have resulted in the apparent associated decline in disease resistance (Oltenacu & Algers 2005). Poor health reduces longevity and causes serious economic losses. In this desk study, we investigated the innate immune responses to bacterial infections in the mammary gland to identify candidate genes and common signalling pathways associated with disease resistance in dairy cows.

Material and methods We reviewed the literature published from January 2003 to November 2013 in PubMed to identify candidate genes potentially conferring genetic resistance to mastitis in cows. Genes were collected based on: 1) genes with expression profiles associated with mastitis; 2) genes with sequence variations showing specific allele-phenotype interactions associated with mastitis, and 3) genes involved in the innate immunity responses to bacterial infection in the mammary gland. All the genes collected above were compared with the gene classification in Gene Ontology (GO) biological process (GO: 0045087 innate immune response, with species included *Bos taurus*, *Homo sapiens* and *Mus musculus*) to obtain a list of genes related to innate immunity and inflammation responses. Further, we selected the genes with the same expression trend in mammary glands challenged with *Escherichia coli* and *Streptococcus uberis* at 20-24 h using the data from Gene Expression Omnibus datasets (GEO) series files of GSE15025 and GSE15344. The selected genes were analyzed using Ingenuity Pathways Analysis software (IPA, Ingenuity® Systems, www.ingenuity.com) with gene symbol as IDs, fold change and P values as observations. Networks and canonical pathways of these genes were generated according to their known connectivity and statistical P values.

Results The initial list of genes identified from the literature included 2,863 genes. Expression of 307 genes was significantly changed in both infection with *Escherichia coli* and *Streptococcus uberis*, including 276 genes with similar trends. Of these, 52 genes met all the above criteria and were selected for further investigation. These 52 genes were part of 5 significant common canonical pathways including *IL-6, IL-10* and *TREM1* signalling, *LXR/RXR* activation, and granulocyte adhesion and diapedesis. The receptors *TREM1* and *TLR4* were both significantly up-regulated in infection. The synergy between these two genes can lead to neutrophil degranulation, phagocytosis and the respiratory burst in addition to production of pro-inflammatory cytokines. Pathway analysis revealed the gene functions included inflammatory response, cellular function and maintenance, haematological system development and function, cellular movement and immune cell trafficking, *etc*. A number of genes, such as *RelA, NFKBIA, NFKB1* and *MEOX2* were predicted as important upstream regulators. *RelA* is positively related to 21 genes associated with many biological processes, such as cell movement of neutrophils (e.g. *IL6, CXCL8, PTGS2, FOS, CXCL2*). *MEOX2*, which inactivates 9 of the 52 selected genes (e.g. *PTX3, ITGAV, IL6, CXCL8, CCL2, CXCL6*) was itself inhibited during infection according to the prediction. Then we analyzed the canonical pathway of 9 genes down-regulated by *MEOX2* with IPA software, and found it was most related to the roles of *IL-17F* in allergic inflammatory airway diseases and *IL-17A* in arthritis.

Conclusion When dairy cows develop an infection, some innate immune pathways (e.g. the *TREM1* signalling pathway) respond to invasion with different pathogens with similar patterns of gene expression. This suggests that these probably represent some common resistance mechanisms. We also found that *RelA*, *NFKB1* and *NFKBIA* can regulate most of the innate immune genes, indicating that the *NFKB/Rel* family of transcription factors are important regulators of innate immunity to disease. The present study suggests that pathway analysis may benefit selection of disease resistance genes according to their common responses to different pathogen challenges.

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Diners' attitudes towards broiler chicken welfare

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Implications Diners' attitudes towards broiler chicken welfare have not been previously researched. The study provided an indication on what cues might help consumers and restaurateurs make more animal-welfare friendly choices and also establish a dialogue between them.

Introduction In 2011 more than 58 billion chickens were slaughtered for meat worldwide (FAO, 2013). Welfare issues in broiler production are one of the most serious in the modern livestock industry and Europeans identify broiler chickens as a farm animal species that requires most protection and animal welfare improvements (European Commission, 2005). Several reports show animal welfare is an important determinant while making shopping decisions and shoppers are ready to pay extra for it (Defra, 2011; IGD 2011). Whilst every bigger supermarket chain offers its own higher welfare chicken label, the food service industry appears to be lagging behind. This study determined whether people that consider animal welfare when shopping do the same when they are eating out. The study also investigated restaurateurs' views on broiler chicken welfare and explored whether there is agreement between customers' and restaurateurs' attitudes.

Material and methods A web survey among 162 British diners and interviews with 15 restaurateurs were carried out. Respondents were recruited electronically by sending out email invitations to individuals that were subscribed to the mailing list of the Sustainable Restaurant Association. The questionnaire for customers consisted of three parts on attitudes when eating out, when shopping and general attitudes towards farm animal welfare. Category questions were analysed using chi squared tests (α=0.05). The objective of the survey into restaurateurs' attitudes was to obtain a preliminary, qualitative understanding of restaurateurs' attitudes and perceptions of their customers' attitudes. Data were collected from 1st August to 21th September 2013. The study was approved by the Writtle College Ethics Committee.

Results The sample was dominated by females (111:51), biased to higher education (66%), and almost a quarter (24%) of respondents had annual income of over £50.000. Many respondents did not know what type of chicken is sourced by food service places (Table 1). Behaviour of having a free-range chicken meal when eating out was associated with thinking about the wellbeing of chickens (chi-squared, $p = 0.018$) and buying higher welfare chicken meat products in supermarkets (chi-squared, $p < 0.001$). Seven restaurateurs out of 15 said that price was driving their customers' attitudes. Lack of available local supply was identified by restaurateurs as an obstacle for sourcing higher welfare chicken meat products.

Table 1 Respondents' opinions and knowledge on seven well-known Food Service Providers (FSP)

	Respondents' opinion on FSP (%)			
Food Service Provider	Serves free-range chicken	Does not serve free-range chicken	I don't know	Type of chicken meat actually served
KFC	0.6	56.8	42.6	minimum welfare standards
Subway	1.2	42.6	56.2	minimum welfare standards
Chipotle Mexican Grill	4.3	26.5	69.1	RSPCA Freedom Food indoor
Nando's	7.4	38.3	54.3	minimum welfare standards
McDonalds	8.0	45.7	46.3	minimum welfare standards
Pret A Manger	25.3	21.6	53.1	higher welfare indoor
Jamie Oliver restaurants	43.2	6.8	50.0	free-range

Conclusion The study demonstrated that consumers held similar attitudes when eating out and when shopping. Inability to find food service places that source higher welfare chicken meat, poor public knowledge on farm animal welfare, perception of price and lack of local supply of higher welfare chicken meat for food service places were identified as main challenges that need to be addressed for development of animal welfare orientated food service places.

Acknowledgements for Sophie Elwes, Sustainable Restaurant Association and the participating restaurateurs.

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The long chain n-3 polyunsaturated fatty acid content of chicken breast muscle is increased by supplementation of the birds' diet with linseed oil but not folic acid

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Implications Skinless chicken breast meat can be enriched with long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) using sustainable sources (linseed oil). Adding additional folic acid to the diet does not increase the accumulation of LC n-3 PUFA. Over 40% of the LC n-3 PUFA in chicken meat is present as DPA (C22:5 n-3) and the health effects of DPA need to be established.

Introduction Birds fed C18:3 (LNA, a fatty acid precursor to long chain, C≥20, n-3 polyunsaturated fatty acids, LC n-3 PUFA) usually produce meat with low concentrations of LC n-3 PUFA (Rymer *et al*., 2005). However, the LC n-3 PUFA reported are usually only EPA (C20:5 n-3) and DHA (C22:6 n-3) but poultry accumulate LC n-3 PUFA in the form of EPA, DHA and DPA (C22:5 n-3, Howe *et al*., 2006). Including DPA in the calculation of poultry meat LC n-3 PUFA content could enhance the perceived nutritional value of enriched meat. Poultry meat LC n-3 PUFA content might be further enhanced if the efficiency of conversion of LNA to LC n-3 PUFA by the bird could be increased. Devlin *et al*. (2007) observed that folate deficiency reduced transcription of Δ6 desaturase, Δ6 desaturase activity and liver concentrations of LC n-3 PUFA in the mouse. The objective of this experiment was therefore to determine the effect of folic acid (FOL) supplementation on the efficiency of conversion of dietary LNA to LC n-3 PUFA in the breast muscle of broiler chickens.

Material and methods Ross 308 chicks (72) were randomly allocated (at 15 d) to one of six treatments (four replicate pens per treatment, three birds per pen). They were fed diets supplemented with 0, 20 or 40 g/kg linseed oil (LO, source of LNA, 0L, 20L, 40L respectively). Diets were balanced with palm oil so that all diets were supplemented with a total of 40 g/kg oil. Diets were also supplemented with either 2 (B1) or 20 (B10) mg/kg FOL. Feed intake per pen was recorded. At 42 d, birds were slaughtered, and the mass of breast muscle in each bird recorded. Feed and tissue were analysed for fatty acid composition. Intake (15-42 d) of LNA and the amount of LC n-3 PUFA in the breast muscle was calculated. The apparent efficiency of conversion of LNA to accumulated LC n-3 PUFA in the breast muscle was then determined. The effect of inclusion rate of LO and FOL, and their interactions, on the concentrations of LC n-3 PUFA, and apparent efficiency of conversion and accumulation of LC n-3 PUFA in the breast muscle were determined by analysis of variance.

Results LC n-3 PUFA were deposited in breast muscle as EPA, and (to a greater extent) DPA and DHA (Table 1). LO supplementation increased LC n-3 PUFA content of breast muscle significantly, but FOL had no effect. The apparent efficiency of conversion and accumulation of EPA was increased, but of DHA was decreased, by supplementation with LO (Table 2).

Table 1 Effect of linseed oil and folic acid supplementation on the concentration (mg/100 g tissue) of LC n-3 PUFA in breast muscle.

Fatty	Diet fed to broilers						SEM	P		
acid	0LB1	20LB1	40LB1	0LB10	20LB10	40LB10		LO	FOL	LOxFOL
EPA	5.24	16.7	22.2	4.40	13.9	28.9	2.520	<0.001	0.627	0.159
DPA	15.1	38.1	35.4	18.5	28.1	42.6	4.32	<0.001	0.954	0.131
DHA	15.2	34.2	22.5	18.3	24.8	33.5	4.31	0.010	0.669	0.075
LC n-3 PUFA	35.6	59.0	80.1	41.2	66.8	104	10.30	<0.001	0.747	0.089

Table 2 Apparent efficiency of conversion and accumulation (%) of LNA to different LC n-3 PUFA

Fatty	Diet fed to broilers						SEM	P		
acid	0LB1	20LB1	40LB1	0LB10	20LB10	40LB10		LO	FOL	LOxFOL
EPA	0.58	0.75	1.10	0.46	0.53	1.23	0.152	0.001	0.577	0.502
DPA	1.68	1.71	1.75	1.93	1.07	1.84	0.244	0.178	0.629	0.173
DHA	1.69	1.54	1.10	1.92	0.94	1.48	0.243	0.059	0.970	0.126
LC n-3 PUFA	3.94	4.00	3.95	4.32	2.54	4.55	0.580	0.209	0.738	0.179

Conclusion The apparent efficiency of conversion and accumulation of LNA to LC n-3 PUFA in the skinless breast meat of poultry was low and not affected by dietary FOL. Birds fed diets containing 40 g/kg LO produced skinless breast meat (100 g portion) with an LC n-3 PUFA content that is 20% of the UK's daily recommended intake of 450 mg (SACN/COT 2004) and could be labelled a source of omega-3 fatty acids. The health effects of DPA compared with EPA and DHA need to be established (Howe *et al*., 2006).

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Meta-analysis of factors that affect nutrient requirements in laying period of broiler breeder hens. Part 1: Developing nutrient requirement models

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Implications Modelling for precision nutrition and nutrient management according to environmental variables is discussed in order to properly demonstrating biological phenomena in prediction equations.

Introduction Emmans (1995) identified that potential growth rate is a function of genotype and state (growing or laying stages) of bird. Chwalibog (1992) explained the effect of non-genetic items (environmental conditions, methodological limitation and experimental design) beside genetic effects as the reason that can cause the variation of estimated coefficients in the literature. Current meta-analysis was conducted to develop nutrient (energy, crude protein, lysine, methionine and cystine) requirement models by considering environmental variables and management conditions for breeding period of Ross 308 broiler breeder hens.

Material and methods Initially, by using Web of Science, Scopus and Magiran, a total of 1608 peer-reviewed literature published in the past 47 y between 1966 and 31 December 2012 were retained in database. After the initial search, by reviewing the titles, abstracts and full texts, repeated studies and irrelevant ones were excluded. Eleven investigation articles matching the following criteria were included in final meta-analysis: (1) intervention study with broiler breeder hens or both sexes of broiler parent stock of Ross 308; (2) dietary nutrients (at least metabolizable energy and crude protein) were reported; (3) body weight (BW), average daily gain (ADG) and egg mass (EM) were reported or enough original data were available to calculate these values; (4) study was performed between 24 and 64 wk of age in windowless barn with litter-based housing system. Analyses were performed with metareg command of Stata Statistical Software by using the restricted maximum likelihood method. If the equation I.D. named as full, it means that the effectiveness of all environmental and management variables, instead of the ones which were dropped because of collinearity, were investigated in related to nutrient (E, CP, TL, TM, TMC) requirement. In cases that equation I.D. named as final, it means that just the effect of significant ($P < 0.05$) variables were estimated in equation. Goodness of fit of the equations was detected according to RSS and R^2.

Results Results of meta-regression which was conducted separately for each period of five different nutritional management programs of laying period indicated that prediction equations of available data set for our candidate phases (24-to-28 wk, 29-to-33 wk, 34-to-47 wk, 48-to-64 wk, 24-to-26 wk, 27-to-36 wk, 37-to-66 wk, 22-to-34 wk, 35-to-54 wk, 55-to-64 wk, 24-to-34 wk, 35-to-60 wk, 24-to-64 wk) was neither accurate nor precise (results not shown). By current available data set, just the meta-regression of response variables of interest during 32-to-52 wk of age resulted in much better prediction of nutrient requirement (Table 1). Metabolic BW, EM, ADG and Lgn, respectively, were the most important factors affecting daily ME requirement. Metabolic BW, EM and ADG, had the most effect on prediction of daily CP requirement. Both TM and TMC best fitted models revealed that metabolic BW, ADG and EM were the key factors for these equations.

Table 1 Prediction equation for nutrients requirement of Ross308 broiler breeder hen during 32 to 52 wk of age

Nutrient	Equation I.D.	Adj-R^2	RSS[1]	Equation[2]
E	Full	0.22	266540	$E= -1731+ 10ADG + 569.7BW^{0.75} + 10.9EM + 0.01Alt + 0.1Lgn$
	Final	0.24	327884	$E= -1775 + 10ADG + 579.7BW^{0.75} + 11.4EM$
CP	Full	0.23	840	$CP= -183.45 + 0.75ADG + 53.17BW^{0.75} + 1.3EM -0.12Lgn + 0.002Alt$
	Final	0.24	785	$CP= -168.08 + 0.74ADG + 49.84BW^{0.75} + 1.1EM$
TL	Full	0.48	1.04	$TL= -4.05 + 0.04ADG + 1.27BW^{0.75} + 0.03EM - 0.000008Alt$
	Final	0.48	0.97	$TL= -4.08 + 0.04ADG + 1.28BW^{0.75} + 0.03EM$
TM	Full	0.46	0.11	$TM= -2.22 + 0.02ADG + 0.68BW^{0.75} + 0.02EM - 0.00002Alt$
	Final	0.46	0.02	$TM= -2.34 + 0.02ADG + 0.70BW^{0.75} + 0.02EM$
TMC	Full	0.47	0.28	$TMC= -3.90 + 0.04ADG + 1.17BW^{0.75} + 0.03EM + 0.00007Alt$
	Final	0.47	0.04	$TMC= -3.59 + 0.04ADG + 1.15BW^{0.75} + 0.03EM$

[1] Residual sum of square. [2] In each equation: E= metabolizable energy (kcal/b/d); CP= crude protein (g/d); TL= total lysine (g/d); TM= total methionine (g/d), TMC= total methionine+ cystine (g/d); $BW^{0.75}$= metabolic body weight (kg); ADG= average daily gain (g/d); EM= egg mass (g/d/b); Alt= altitude (m); Lgn= longitude digit (degree).

Conclusion In available publications the effects of environmental variables other than temperature on nutrient requirement are less evident from the literature and warrant more study. According to the findings of current study, more primary researches in field of nutrient requirement during 24 to 31 and 53 to 64 wk of age for Ross 308 broiler breeder hens in future would benefit meta-analysis for predicting nutrient requirement of mentioned periods of that strain.

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Meta-analysis of factors that affect nutrient requirements in laying period of broiler breeder hens. Part 2: Developing Performance prediction models

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Implications Predicting the effect of environmental variables and management conditions on performance of broiler breeder flocks is a hard challenge because the conversion of feed into breeder's product has large influence on the cost of production.

Introduction Sauvant *et al*. (2008) explained the importance of periodically requiring quantitative summarizations of animal nutrition literature data by processing and modelling of databases because in animal sciences especially in the field of nutrition the number of publications in each filed of research is increasing**.** Current meta-analysis was conducted to develop performance prediction models according to dietary nutrients intake and considering environmental variables and management conditions for breeding period of Ross 308 broiler breeder hens.

Material and methods Initially, by using Web of Science, Scopus and Magiran, a total of 1608 peer-reviewed literature published in the past 47 y between 1966 and 31 December 2012 were retained in database. After the initial search, by reviewing the titles, abstracts and full texts, repeated studies and irrelevant papers were excluded. Eleven studies matching the following criteria were included in final meta-analysis: (1) intervention study with broiler breeder hens or both sexes of broiler parent stock of Ross 308; (2) dietary nutrients (at least energy and crude protein) were reported; (3) body weight (BW), average daily gain (ADG) and egg mass (EM) were reported or enough original data were available to calculate these values; (4) study was performed between 24 to 64 weeks of age in windowless barn with litter-based housing system. Analyses were performed with metareg command of Stata Statistical Software by using the restricted maximum likelihood method. Goodness of fit of the equations was detected according to R^2 and tau^2 (tau-square is the estimate of between-study variance, to quantify heterogeneity).

Results Results of meta-regression which was conducted separately for each period of five nutritional management programs of breeding period indicated that prediction equations of available data set for our candidate phases (24-to-28 wk, 29-to-33 wk, 34-to-47 wk, 48-to-64 wk, 24-to-26 wk, 27-to-36 wk, 37-to-66 wk, 22-to-34 wk, 35-to-54 wk, 55-to-64 wk, 24-to-34 wk, 35-to-60 wk, 24-to-64 wk) was neither accurate nor precise (results not shown). By current available data set, just the meta-regression of response variables of interest during 32-to-52 wk of age resulted in much better development of performance prediction equations (Table 1).

Table 1 Prediction equation for metabolic body weight, average daily gain and egg mass of ROSS 308 broiler breeder hen during 32 to 52 wk of age

Nt[1]	NP[2]	Obs[3] (n)	Adj-R^2	tau^2	Equation[4]
E	$BW^{0.75}$	144	1.00	0.00	$BW^{0.75}$= 3.85 - 0.0001E - 0.002ADG -0.03EM + 0.01Lgn
	ADG	144	0.24	202.40	ADG= 177.48 + 0.1E - 57.97$BW^{0.75}$ - 1.14EM
	EM	144	0.88	5.26	EM= 95.68 - 0.01E - 20.87$BW^{0.7}$ - 0.03ADG + 0.30Lgn - 0.002Alt
CP	$BW^{0.75}$	144	1.00	0.00	$BW^{0.75}$= 3.69 + 0.004CP - 0.002ADG - 0.03EM + 0.005Lgn
	ADG	144	0.24	202.40	ADG= 226.91+ 1.35CP - 67.28$BW^{0.75}$ - 1.48EM
	EM	144	0.88	5.49	EM= 95.09+0.05CP - 22.60$BW^{0.75}$- 0.05ADG +0.28Lgn - 0.002Alt
TL	$BW^{0.75}$	132	1.00	0.00	$BW^{0.75}$= 3.98 + 0.09TL - 0.002ADG - 0.03EM -0.00002Alt
	ADG	132	0.48	21.74	ADG= 95.26 + 23.36TL - 29.81$BW^{0.75}$ - 0.70EM
	EM	132	0.81	4.22	EM= 112.57 + 1.32TL - 23.15$BW^{0.75}$ - 0.10ADG -0.003Alt
TM	$BW^{0.75}$	132	1.00	0.00	$BW^{0.75}$= 3.88 + 0.22TM - 0.002ADG - 0.03EM
	ADG	132	0.46	22.44	ADG= 102.72 + 43.99TM - 30.62$BW^{0.75}$ - 0.78EM
	EM	132	0.81	4.20	EM= 112.46 + 3.26TM - 23.31$BW^{0.75}$ - 0.10ADG -0.003Alt
TMC	$BW^{0.75}$	132	1.00	0.00	$BW^{0.75}$= 3.99 + 0.10TMC - 0.002ADG - 0.03EM -0.00003Alt
	ADG	132	0.47	22.17	ADG= 99.88 + 25.64TMC - 30.09$BW^{0.75}$ - 0.72EM -0.002Alt
	EM	132	0.81	4.22	EM= 112.74 + 1.61TMC - 23.21$BW^{0.75}$ - 0.10ADG -0.003Alt

[1] Nutrient: E= energy (kcal/b/d); CP= crude protein (g/d); TL= total lysine (g/d); TM= total methionine (g/d), TMC= total methionine+ cystine (g/d). [2] Nutrient partitioning: $BW^{0.75}$= metabolic body weight (kg); ADG= average daily gain (g/d); EM= egg mass (g/d/b). [3] Number of Observations. [4]In each equation: Alt= altitude (m); Lgn= longitude digit (degree).

Conclusion Prediction equations of current meta-analysis have more accuracy if they use for floor-reared Ross 308 broiler breeder hens during 32-to-52 wk of age than 24-to-31 and 53-to-64 wk of age. These findings would be used to determined relevant environmental factors for inclusion in further research and modelling efforts with regard to performance of broiler female parent stock. In present study all available data were used for model development therefore evaluation of the models against an independent database is suggested.

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Effects of feed form on dietary *Aspilia africana* leaf utilization by broilers

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Implications Dietary inclusion of 5% *Aspilia africana* leaf in crumble-pellet form improves the growth performance and carcass yield of broilers.

Introduction The physical form of feed (mash, pellet) is a crucial factor in meat yield of broiler. Whole grains due to its bulkiness are less palatable thus resulting to poorer broiler performance. Improved performance in pellet feeding is attributed to a decrease in feed wastage, reduced selective feeding, destruction of pathogenic organism and improved palatability. However, pellets are about 10% more expensive than that of mash feeds. Strategies are therefore sort to improve feed manufacturing cost while maintaining broiler performance. One of such strategies is to provide diets in mash form using roller mill ground corn to control flow problems (Dozier III *et al*., 2010) or in crumble pellets by the crushing the pelleted feeds to a consistency coarser than mash (Mirghelenj and Golian, 2009). The ban on the use of antibiotic growth promoters in animal feeds led to the search for alternative feed management strategies that will improve the performance and feed efficiency of broiler chickens. Leaf meal supplementation (phyobiotics) is one such strategy considered as the ideal alternative that will promote broiler growth and health (Oko *et al*., 2012). *Aspilia africana* leaf (AaL) is currently being explored as a potential phytobiotic due to its vast antimicrobial activities (Oko and Agiang, 2011). This experiment was conducted to assess the effects of dietary *Aspilia africana* leaf provided in mash and crumble pellet forms on broiler performance.

Material and methods *Aspilia africana* leaf was harvested from blooming stands within the vicinity of the teaching and research farm of the University of Calabar, Nigeria. The leaves were processed by shredding, air drying (24 hr) and milled. The corn-soybean (antibiotic–free) basal diets were formulated according to the Hubbard Recommendation for Anak 2000 broilers. To assess the phytobiotic effects of *Aspilia africana* leaf, two forms (mash using roller mill ground corn and crumble pellet) of AaL diets at four levels were studied. Eight treatments comprising of either mashed or crumbled pellet of the basal diets (0% AaL – negative control) 2 and 5% AaL/kg basal diets and 0.2% terramycin/kg basal diets (positive control) were investigated. 320 commercial broilers (Anak 2000) were used in a randomized complete block design with 8 treatments and 4 replications (pen) of 10 birds each. Broilers were managed under the deep litter system in a 2-phase feeding program during a 42-d production period. Diets were identical in nutrient specifications during the same age period. Birds were offered feed and water *ad libitum*. Birds and feed were weighed on a pen basis at 1, 7, 14, 28, 35 and 42 d of age for the determination of growth rate, feed intake, and feed conversion. Mortality was recorded daily. On d 43, 4 birds per pen were randomly selected, weighed and processed for carcass evaluation. Data were statistically evaluated using the ANOVA procedure of SAS 2004. Means were separated by Tukey's honestly significant differences procedure when the overall *F*-test was $P \leq 0.05$. Preplanned orthogonal contrasts were used to divide treatment effects with 4 df into 1) crumble pellets vs. mash, 2) Negative control vs. AaL diets, 3) 2% AaL vs. 5% AaL, and 4) AaL vs. Positive control. Statistical significance was established at $P \leq 0.05$.

Results There were significant differences ($p<0.05$) in body weight (2007.50 - 2275.33g) weight gain (41.08- 45 64g/d) and feed consumption (121.15- 125.22g/d) between birds fed AaL diets compared to those on the control diets. It was also observed that at the starter and finisher phases, birds fed crumble-pellet diets had higher growth rate and feed consumption than birds fed mash diet. Inclusion of 5% AaL in all diets tends to increase ($p<0.05$) broiler performance throughout the 42-d study. Feed conversion was marginally higher ($p>0.05$) in birds fed AaL crumble-pellet diet than in birds fed other dietary treatments. Growth rate of birds on 0.2% terramycin were comparable those on 2% AaL but lower than those on 5% AaL diets. Carcass yield (73.43 – 82.65%) and relative breast meat yield (23.41- 25.55%) were higher ($p<0.05$) in broiler fed AaL crumble-pellet diet than other treatments.

Conclusion Inclusion of 5% AaL in crumble- pellet diet improved the growth and carcass performance of broilers.

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A comparison of production parameters of two strains of layer hens producing brown and white hens

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Implications There has been worldwide interest in providing consumers with high quality table eggs. Brown eggs are more preferable by consumers as their appearance is closer to the local eggs which are in turn closer to the nature. The thick shell of brown eggs makes it in favour of commercial companies to reduce breakage loss. Overall productive performance of brown hens is better than that of the white one. Also, brown eggs are better in nutritional value than the white eggs. All these reasons make brown eggs more preferable by consumers than the white ones.

Introduction Improving production efficiency is essential for producers to reduce their costs and be able to improve profitability and compete with the imported products in terms of price and quality. This can be achieved by utilizing strains which produce brown eggs with high quality. Interestingly, The nutritional values of brown eggs are known to be better than that of the white eggs. It was reported by Bell and Weaver (2002) that brown eggs have less lipids (8.49% vs. 10.04%) and more vitamin A (317 IU vs. 260 IU) and vitamin B2 (0.254 mg vs. 0.180) than white eggs. The objective of the current study was to investigate the differences between the production performance between brown and white strains.

Material and methods Two strains of layer hens were used: Lohmann LSL-Classic strain which was white egg producer and Lohmann Brown-Classic strain which was brown egg producer. One day old pullets, 600 from each strain. One pullet house was used for birds grown from one day until 19 weeks of age and one layer house was used for birds raised from 19 weeks until 70 weeks of age. For each strain, the pullets were distributed between three batteries providing space of 333-364 cm^2 per pullet. At 19 wks of age, pullets were transferred to the layer house until 70 wks of age. Each cage contained six hens providing 500 cm^2 per hen. Pullets and hens were fed *ad libitum*. Mortality, temperature and humidity were recorded daily. The pullets were fed grower ration from day one until 8 wk of age (18.5% protein, 2750 kcal kg), developer ration from 8 wk-16 wk of age (14.5 % protein, 2750 kcal kg), and pre-lay from 16 wk until 24 wk of age (17.5% protein, 2750 kcal kg), and laying ration from 24 wk of age to the end of the laying period (18.0% protein, 2900 kcal kg). The pullet production data collected included body weight. Total feed consumption and overall feed efficiency was calculated. Overall percent egg production, overall egg weight, overall egg mass and total feed consumption, and overall feed efficiency were calculated till 70 weeks of age. In addition, components of brown and white eggs, shell thickness, and HU were measured. Finally, economic impact study on the return on investment from raising brown or white hens was conducted. The data were analyzed using ANOVA utilizing the S-Plus statistical program (Crawley, 2002). Means were separated using Tukey's test, and the significance was set at $P< 0.05$.

Results Overall body weight gain and feed efficiency for the brown pullets were significantly ($P<0.05$) better than the white pullets. Mass of eggs and feed efficiency in case of brown hens were significantly ($P<0.05$) higher than that of the white hens for the last period (58-69 wk of age). Overall average percent egg production for the 48- wk period reported here for the brown hens (90.0%), was slightly higher than that for the white hens (89.6%), but the difference was not significant ($P>0.05$). The results showed no significant difference ($P>0.05$) between the overall weight of the brown and the white eggs Overall feed consumption for brown and white hens was similar with no significant difference ($P>0.05$); however, feed consumption for the brown hens for the last period measured (58-69 wk of age) was significantly ($P<0.05$) less than that of the white hens. Results of yolk, albumen and Haugh Unit for both brown and white eggs showed no significant difference. Shell thickness of the brown eggs was significantly higher than that of the white hens. In general, the production performance of the brown strain was better than that of the white strain. This could enhance diversity of poultry products in the country and provide consumers with high quality eggs. The economic impact study results showed that the brown strain is more economic than the white ones because they can be kept for a longer period for egg production as they have better feed efficiency at a later age (58-69 wk of age). This means that they eat less as the hens get older. Also, the higher shell thickness of the brown eggs than the white one makes them more resistant to breakage during handling and transport. This will definitely have economic impact in terms of saving money by commercial companies.

Conclusion In general, the production performance in terms of overall body weight gain, feed efficiency, feed consumption (for the period 58-69 wk of age) and egg mass of the brown strain was better than that of the white strain. This could enhance diversity of poultry products and provide consumers with high quality eggs which they will prefer. The brown hens were more economic than the white hens.

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Effect of parental live weight on the reproductive performance of Japanese quail *(Cortunix cortunix japonica)*

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Implications Results obtained from this study would assist quail breeders in the production of a breeder stock with optimum productivity and survival rate.

Introduction As in other poultry productions, one of the main prerequisite for efficient and profitable quail breeding is to produce fertile eggs and to obtain highest hatchability in these eggs. Production of fertile eggs is affected by many factors related to both parents and environment. A high level of genetic correlation exists between the parental live weight and egg weight of female breeders (Coban *et. al.,* 2008; Haq *et. al., 2011*).

Material and methods The data used for this study were obtained from an experiment conducted at the Quail Unit centre Songhai, Port-Novo, Benin Republic, consisted of 3,195 records obtained from male and female Japanese quail. The records were further categorized into four mating groups based on the parental live weight as follows: Light male ≤170g (LM) × Light female ≤190g (LF); Heavy male >170g (HM) × Heavy female >190g (HF); Light male ≤170g (LM) × Heavy female >190g (HF); Heavy male >170g (HM) × Light female ≤190g (LF). The data was analyzed with General Linear Model of SAS (2003) for One- way analysis of Variance. Means were separated using Duncan New Multiple Range Test.

Results Means ± SEM of external egg quality traits, hatching and post hatching body weights, Percent fertility, hatchability, hatch and mortality from the four crosses are shown on the Table below:

Parameters	LM x LF	HM x HF	LM x HF	HM x LF
Egg weight(g)	11.18±0.03^{b}	10.93±0.06^{c}	11.50±0.03^{a}	10.87±0.10^{c}
length Egg (cm)	3.13±0.005^{c}	3.17±0.008^{b}	3.21±0.004^{a}	3.15±0.01^{b}
Egg circumference (cm)	7.83±0.01^{b}	7.81±0.01^{b}	7.89±0.009^{a}	7.77±0.02^{b}
BWT (g) at hatch	6.64±0.07^{a}	4.78±0.19^{b}	6.46±0.07^{a}	4.11±0.29^{c}
BWT (g) week 1	17.62±0.20^{a}	10.83±0.46^{b}	16.75±0.20^{a}	9.08±0.72^{c}
BWT (g) week 2	35.84±0.40^{a}	22.53±0.97^{c}	33.55±0.40^{b}	19.21±1.52^{d}
BWT (g) week 3	68.32±0.75^{a}	41.18±1.73^{c}	62.70±0.73^{b}	34.99±2.72^{d}
BWT (g) week 4	91.83±1.01^{a}	59.11±2.46^{c}	84.91±0.98^{b}	49.03±3.79^{d}
BWT (g) week 5	120.33±1.31^{a}	80.46±3.32^{c}	111.16±1.27^{b}	67.42±5.20^{d}
BWT (g) week 6	150.83±1.64^{a}	100.32±4.15^{c}	138.68±1.58^{b}	83.60±6.52^{d}
BWT (g) week 7	183.08±1.20^{a}	123.64±5.10^{c}	168.29±1.92^{b}	101.26±7.99^{d}
Fertility (%)	96.40^{a}	89.72^{b}	93.58^{a}	88.64^{b}
Hatchability (%)	98.34^{a}	78.44^{b}	90.66^{a}	70.62^{c}
Mortality (%)	2.54^{b}	10.19^{a}	4.48^{b}	12.7^{a}

^{abc} Means with different superscript along rows are significantly different ($p<0.05$); BWT = Body weight

Significant differences ($p<0.05$) were obtained in egg weight, egg length and egg circumference. At hatch and 1 week old, body weight from LM x LF and LM x HF were not significantly different ($p<0.05$) but were different from HM x HF and HM x LF. Significant differences ($p<0.05$) exist in all the crosses for post hatching weight at week 2 to week 7 with LM x LF having the highest value. There was no significant differences ($p<0.05$) in the percent fertility of HM x HF and HM x LF crosses but they were different from LM x LF and LM x HF with LM x LF having the highest fertility. Percent hatchability in LM x LF and LM X HF crosses were not significantly different. Mortality was highest in HM x HF and HM x LF crosses indicating that most of the chicks obtained from these crosses are probably not strong as indicated in their body weights thus the lower survival.

Conclusion Light male and light female crosses gave chicks with better post hatching performance and lowest mortality rate than all other crosses. The use of light males is therefore recommended to quail breeders for optimum productivity.

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Evaluating the interval from calving to first service, and calving to conception in high yielding lame and non-lame Holstein-Friesian dairy cattle

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Implications The number of days from calving to first service, and calving to conception are increased in lame dairy cows, therefore reducing lameness and its impact through additional supervision is key to improving fertility.

Introduction Resumption of normal ovarian cyclicity following parturition is essential for successful productivity in dairy herds. Typically dairy cows have been reported to resume ovarian activity and ovulation within 15-45 days postpartum, with regular cycles approximately every 18-24 days. Early postpartum ovulation is associated with improved reproductive fertility (Galvão *et al.,* 2010). It is well documented that lameness is a painful and debilitating condition that can have a detrimental effect on reproductive performance, oestrus behaviour and intensity (Sprecher *et al.,* 1997; Hernandez *et al.,* 2001; Walker *et al.,* 2008). Several studies have reported that when compared to non-lame cows, lame cows had delayed cyclicity; increased calving to first service interval; increased calving to conception interval, and an increased number of services to conception (e.g. Sprecher *et al.,* 1997). The aim of this study was to evaluate the number of days from calving to first service, calving to conception, and average number of inseminations to conception in lame and non-lame dairy cattle.

Material and methods Approximately 100 high yielding Holstein-Friesians (10975 kg) with access to pasture from February to October were studied. Following calving cows were assessed for lameness weekly (Flower and Weary 2006). Cows were locomotion scored post milking, on a flat concrete alley. Each cow was given a locomotion score from normal to severely lame (locomotion scores 1 to 5, respectively). Calving day, parity, days to first service, days to conception, and number of inseminations to conception were recorded. Number of days from calving to first service was recorded from n=94 cows (non-lame n=69, lame n=25). Number of days from calving to conception was recorded from n=71 cows (non-lame n=49, lame n=22), n=5 cows were culled before pregnancy could be established, and n=18 cows were not diagnosed pregnant at the time of data analysis. The cows studied had a mean parity of 2.38. (±SE 0.14). Non-lame cows had a mean parity and locomotion score of 1.45 (± 0.06) and 1.86 (± 0.14) respectively, and lame cows had a mean parity and locomotion score of 3.84 (± 0.27) 3.08 (± 0.06) respectively. Data were normally distributed, and student t-test was done (GenStat 16th edition).

Results Lame cows had a longer interval from calving to first service and calving to conception ($p<0.05$). However, there was no significant difference in the average number of inseminations to conception for lame and non-lame cows. The effect of lameness on fertility parameters is shown in Table 1.

Table 1 Effect of lameness on fertility parameters

	Lame	Non-lame	s.e.d	P
Days from calving to first service	63.8	53.6	3.875	0.009
Days from calving to conception	113.5	84.2	11.26	0.011
Number of inseminations to conception	2.5	2.1	0.3391	n.s

Conclusion The increased number of days from calving to first service, and from calving to conception in lame dairy cows indicates that lameness affects reproductive efficiency, and therefore productivity.

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Effect of preimplantation factor on the secretion of prostaglandin $F_{2\alpha}$ and E_2 from bovine endometrial tissue following a lipopolysaccharide challenge *in vitro*

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Implications New treatments are needed for bovine endometritis. Preimplantation factor (PIF) has the potential to be a candidate; however, the present study shows that PIF did not alter the prostaglandin response in an endometritis model.

Introduction Endometritis is a common inflammatory uterine disease in dairy cattle caused by entry of bacteria into the uterus. Secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2) from endometrial tissue is increased during the pro-inflammatory immune response caused by bacteria. Current treatments are antibiotics and hormones, however, with growing resistance to antibiotics (Santos *et al*., 2010) and the ineffectiveness of hormone treatment (Haimerl *et al*., 2012), new treatments are needed. Preimplantation factor (PIF) is a pregnancy specific peptide secreted by the embryo that has immune modulatory roles during pregnancy and has been shown to exert its effects in models of inflammatory based autoimmune diseases (Weiss *et al*., 2012). The aim was to assess if synthetic PIF (sPIF) has immune modulatory roles in a lipopolysaccharide (LPS; a component of the cell wall of *Escherichia coli)* model of endometritis.

Material and methods Bovine endometrial tissue from cows (n=12) with a stage I corpus luteum present (CL cows) was weighed and cultured separately in media alone or stimulated with LPS (1µg/ml), sPIF (50, 100, 500nM) or LPS (1µg/ml) and sPIF (50, 100, 500nM). Media were sampled at 24, 48 and 72h and analysed by radioimmunoassay (RIA) for $PGF_{2\alpha}$ and PGE_2 concentrations. Serum was obtained for all cows from blood samples taken immediately after slaughter and analysed by ELISA for progesterone concentration. Cows were grouped into high (>3ng/ml) and low progesterone (<3ng/ml) concentrations. The experiment was then repeated using endometrial tissue from follicular cows. Cow type was recorded so that both dairy (n=3) and beef cows (n=4) were used. Media was analysed by RIA for $PGF_{2\alpha}$ concentrations. Data was expressed as production of prostaglandin per mg of tissue and analysed in GenStat using a repeated measures ANOVA, with the following factors: CL data: sPIF, LPS and progesterone group; follicular data: sPIF, LPS and cow type.

Results *CL cows:* CL cows were split into two groups based on progesterone concentration at slaughter: low progesterone (1.93±0.34ng/ml; n=8) and high progesterone (10.39±2.43ng/ml; n=4). There was no effect of progesterone on $PGF_{2\alpha}$ or PGE_2 secretion ($P>0.05$). There was a significant increase in $PGF_{2\alpha}$ and PGE_2 secretion over time in culture ($P<0.001$). LPS treatment induced a significant increase in $PGF_{2\alpha}$ and PGE_2 secretion from explants ($P<0.001$). There was a significant interaction between LPS treatment and progesterone group with explants within the high progesterone group secreting less $PGF_{2\alpha}$ than those in the low progesterone group when not challenged with LPS ($P<0.05$). There was also an interaction between LPS and time, with explants stimulated with LPS secreting more PGE_2 between each time point compared to those not stimulated with LPS (<0.001). sPIF had no effect on $PGF_{2\alpha}$ or PGE_2 secretion at any time point with or without LPS treatment ($P>0.05$). Variation between cows in response to sPIF was large, therefore cow type was recorded for follicular animals and samples were split into groups based on cow type. *Follicular cows:* There was no overall effect of cow type (beef or dairy) on $PGF_{2\alpha}$ secretion ($P>0.05$). There was a significant increase in $PGF_{2\alpha}$ secretion over time ($P<0.001$). LPS treatment induced a significant increase in $PGF_{2\alpha}$ secretion from explants ($P<0.001$). There was a significant interaction between LPS treatment and cow type; explants from beef cows secreted more $PGF_{2\alpha}$ in response to LPS treatment than dairy cow explants ($P<0.01$). Furthermore there was a significant interaction between time point and cow type; explants from beef cows secreted greater concentrations of $PGF_{2\alpha}$ between each time point than dairy cows ($P<0.001$). There was no effect of sPIF and high variability was seen in both dairy and beef cow explants in response to sPIF treatment ($P<0.05$).

Conclusion LPS significantly increased $PGF_{2\alpha}$ and PGE_2 production, demonstrating a clear pro-inflammatory immune response from both luteal and follicular tissue. However, the effect of sPIF was highly variable and had no overall effect on the pro-inflammatory response to LPS challenge in bovine endometrial tissue. It was hypothesised that this variability was due to cow type (beef or dairy, not recorded for the CL cows), however when cow type was recorded in follicular cows, breed did not affect results. The effect of sPIF on other pathways integral to the innate immune response of the bovine endometrium to LPS remains to be investigated.

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Body weight change in the dry period and milk yield deviation from the expected in early lactation as indicators of transition diseases in dairy cattle

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Implications Knowledge gained from this analysis will help in determining the value of including body weight and milk yield change in prediction models for metabolic disease during the transition period.

Introduction Timely identification of disease is becoming increasingly important in modern dairy herds, the average size of which continues to increase. Interest has been fuelled in the use of automated systems of disease detection however, uncertainty still exists as to which cow-level indicators best reflect health status and allow for the prediction of disease. A growing body of research on transition biology demonstrates the importance of this period. The physiologically challenging nature of this period means that it is characterised by a high disease incidence. It is estimated that 30-50% of cows are affected by some form of metabolic or infectious disease around calving (LeBlanc, 2010). The objective of the current analysis was to determine the effect of specific diseases on body weight change and milk yield variation, as an initial step in evaluating their value as indicators.

Material and methods Data were obtained from the research herd of Holstein Friesian cows on a long-term 2x2 factorial genotype x feeding project at the SRUC Dairy Research Centre, Scotland. Prior to the period applied in this analysis, cow feeding management had been changed from the low forage and high forage systems to the more extreme home-grown feed (HG) and purchased by-products (BP) feeding systems. In each feeding system there were cows of moderate UK-average (Control) and high (Select) genetic merit, giving rise to four systems. Approximately 4 weeks before calving, cows were fed a transition diet which comprised 30% of the average daily dry matter intake of milking cows of the HG or BP complete diet. Data from 26 cows with clinical vet diagnoses in the first 30 days of lactation were used in this analysis. Diseases included were milk fever, left displaced abomasum, retained placenta and acute sickness. 4 cows had no body weight data and were included only in the milk yield analysis. Day of diagnosis was assigned the value of 'Day 0', with days preceding diagnosis having negative values. In order to eliminate noise from day-to-day variation in milk yield and live weight data, an exponential smoother was applied. For milk yield, a fitted lactation curve for each cow based on the dairy production system, days in milk and slope of lactation (Friggens *et al*., 1999) was used as the target trajectory for each cow. The difference between expected and actual milk yield, was calculated for the first 30 DIM, which represented deviation from target yield on a daily basis. Body weight change over the dry period was calculated as a percentage of weight at dry-off, thus body weight change equals calving weight minus drying weight, divided by drying weight, multiplied by 100. A generalised linear model (GLM) that included system, lactation and days to disease as covariates was used to analyse milk yield deviation and body weight change data and produced LS Means in SAS (v9.3)

Results Cows diagnosed with milk fever, left displaced abomasum and retained placenta had lower than expected yields which were significantly different from one another, -5.36, -1.27 and -11.80 litres respectively. However, cows diagnosed with acute sickness had above expected yields (2.32 litres). Cows diagnosed with left displaced abomasum lost the greatest amount of body weight (2.43%) in the dry period while those with milk fever had not lost any body weight.

Table 1 LS Means for daily milk yield deviation from expected yield (litres) and body change weight in cows with different diseases in the transition period (different superscripts within group indicate significant difference ($p<0.001$))

Variable	Milk yield deviation from expected (litres)[2]		Body weight change %[3]	
Disease	Mean	Standard error	Mean	Standard error
Milk fever	-5.36[a]	1.18	3.2[a]	1.02
Left displaced abomasum	-1.27[b]	1.04	-2.43[b]	0.77
Acute sickness[1]	2.32[c]	0.84	-0.79[bc]	0.61
Retained placenta	-11.80[d]	1.12	0.25[c]	0.84

[1]Acutely sick cows were defined as those which had any of these conditions, off-feedness, hospitalisation, metritis, dull post calving, and ketosis;
[2]n = 26; [3]n = 22

Conclusion Body weight change and milk yield deviations have potential as indicators of diseases during the transition period. Body weight changes in the dry period show potential as an indicator of left displaced abomasum and acute sickness. Deviations from expected milk yield in early lactation could be used to indicate the presence of milk fever, retained placenta and left displaced abomasum.

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Increased beta-hydroxybutyrate production interrupts splenic immunity in dairy cows with *postpartum* negative energy balance

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Implications Dairy cows with *postpartum* negative energy balance (NEB) have raised circulating concentrations of beta-hydroxybutyrate (BHB) and we show here that this is associated with altered splenic gene expression, implying effects on immune function.

Introduction Increased energy demands to support lactation and decreased feed intake capability in *postpartum* cows lead to NEB which impairs body immunity, including a decrease in both the number and functional capacity of immune cells (Hoeben *et al*. 1997; Wathes *et al*. 2009). The catabolism of stored lipid results in excessive BHB production. Both *in vivo* and *in vitro* studies have shown that BHB interrupts various aspects of immunity (Hoeben *et al*. 1997; Wathes *et al*. 2009). The spleen plays important roles in both cell-mediated and humoral immunities. However the mechanisms by which circulating BHB levels may affect splenic immunity are not well understood. The aims of this study were to investigate the association between increased circulating BHB concentrations and splenic gene expression in dairy cows with *postpartum* NEB at both genomic and pathway levels using microarray and bioinformatics techniques.

Materials and methods *Postpartum* NEB model was developed in 12 cows using different milking and feeding protocols, producing 6 cows in mild NEB and 6 in severe NEB (Morris *et al*. 2009). The cows were slaughtered at 14 ± 0.4 days *postpartum*. Circulating BHB concentrations before slaughter were quantified using a commercial kit. After slaughter, spleen samples were collected for RNA extraction and used for hybridisation to Affymetrix 24K GeneChip Bovine Genome Arrays. The probe expression was normalised using a Robust Multichip Average. The association between BHB concentrations and normalized splenic gene expression values were established with a Pearson correlation and tested using a 2-tailed t-test with Benjamini and Hochberg correction to control False Discovery Rate. Genes for further analysis were selected based on the correlation strength (R^2) and significance levels (P) and subjected to pathway analysis using an Ingenuity Pathway Analysis (IPA) server.

Results The BHB concentrations at slaughter ranged from 0.3 to 4.5 mmol/L, in which its higher concentrations were associated with the severe NEB cows and its lower concentrations with the mild NEB cows ($P<0.001$). Numerous genes/probes (3893) were significantly correlated with the BHB concentrations at $P<0.05$, of which 2253 showed negative correlation and 1640 positive correlation. Among them 618 genes/probes had $R^2>0.6$ and $P<0.01$ and were selected for further investigation. Of these, 459 genes were annotated with biological functions, with 127 involved in various immune and inflammatory processes and 50 in metabolism. Most of the immune genes (89/127, 70%) were negatively correlated with BHB levels whereas most of the metabolic genes (34/50, 68%) were positively correlated. Many genes important in immunity and inflammation, such as *IL2RG, INFG, CCL4, CCL6* and the collagen system (including *SPARC* and over 10 *COL* subunits) were negatively correlated with BHB. IPA analysis showed that various aspects of functions and pathways associated with immunity/inflammation and metabolism were significantly altered, including NF-kB activation, T and B cell signalling, superoxide radical degradation, *etc*. Many genes involving in other biological functional categories, such as reproduction (24 genes), cellular development (127 genes), *etc*., were also significantly correlated with the BHB levels.

Conclusion The present study demonstrated that plasma BHB levels were significantly associated with the changes in splenic gene expression, especially genes involved in various aspects of immunity. As the predominant correlations between plasma BHB and the splenic immune genes were negative, increased BHB production in the cows with *postpartum* NEB may down-regulate the immune system and predispose the animal to infections.

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Enteric methane emissions from non-lactating pregnant dairy cows while grazing

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Implications This study has delivered methane (CH_4) emission values for non-lactating pregnant dairy cows when grazing. These can be used in the development of an improved greenhouse gas (GHG) inventory for the United Kingdom (UK).

Introduction Within the UK dairy cattle represent an important source of CH_4 emissions from the livestock sector. While CH_4 emissions from confined dairy cattle have been extensively researched, and are now well understood, there is much less information available on CH_4 emissions from grazing dairy cattle. This is particularly the case with non-lactating ('dry') dairy cattle while grazing. However, having accurate CH_4 emission factors for different livestock categories, and for livestock within different production states, is essential to enable the development of robust greenhouse gas inventories for the agricultural sector. The current study was undertaken to provide information on CH_4 emissions from non-lactating dairy cows while grazing.

Material and methods Enteric CH_4 production was measured from a total of 68 pregnant non-lactating Holstein-Friesian dairy cattle (23 primiparous and 45 multiparous) during three successive years (23, 22 and 23 cows in Years 1, 2 and 3, respectively). Cows used in these studies ranged from 19 – 69 days prior to their actual calving date. Each year CH_4 emissions were measured using the sulphur hexafluoride (SF_6) technique over six consecutive 24-hour periods, with measurements commencing 28 August, 16 September and 15 September during Years 1 – 3, respectively. The mean release rate of SF_6 from the permeation tubes within this experiment was 5.01 mg/day. During each of Years 1 – 3, cows were given access to a fresh grazing area on the first and fourth day of the measurement period, with fresh herbage offered to provide a nominal daily herbage allowance of 14 kg DM/cow/day (mean pre and post grazing sward heights, 12.6 and 5.2 cm, respectively). While cows were not offered a concentrate supplement during this time, they were given access to a 'dry cow' mineral/vitamin supplement. Mean daily herbage intake during each six-day CH_4 measurement period was estimated for each cow by 'back calculation'. This was calculated as the sum of the metabolisable energy (ME) requirement for maintenance plus pregnancy (using Feed Into Milk equations: Agnew *et al*., 2004), divided by the ME content of the herbage grazed (derived using Near Infrared Spectroscopy). No account taken of live weight change.

Results Primiparous and multiparous cows had a mean live weight of 592 and 679 kg, respectively, while calculated ME intakes were 92 and 101 MJ/day, respectively. Total CH_4 emissions were 206 and 221 g/cow/day for primiparous and multiparous cows, respectively, while the corresponding CH_4/DMI was calculated as 25.6 and 25.1 g/kg, respectively.

Table 1 Description of cows on the study, intake and methane production

	Primiparous				Multiparous			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Mean live weight (kg)	592	55.1	496	696	679	77.7	497	871
Condition score at drying off	2.5	0.21	2.3	3.0	2.6	0.21	2.3	3.0
Parity					3.1	1.41	2	7
Day of pregnancy	243	10.3	226	261	243	11.3	216	266
Milk yield during previous lactation (kg; 305d)	6816	889.1	5514	8429	9402	1427.6	6637	12062
ME intake (MJ/d)	92	7.7	80	106	101	10.5	79	123
Herbage DM intake (kg/d)	8.1	0.76	6.8	9.7	8.8	0.97	6.8	11.2
CH_4 production (g/d)	206	32.5	146	276	221	43.6	145	327
CH_4/DMI (g/kg)	25.6	4.01	17.5	33.3	25.1	4.33	17.5	34.7

Conclusion Enteric CH_4 production for non-lactating pregnant dairy cattle while grazing was 206 and 221 g/day for primiparous and multiparous cows, respectively.

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Lameness and other risk factors associated with hair loss on the hock, in a longitudinal study

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Implications The cause and effect of the relationships between hock lesions and lameness remains unknown, lameness may precede the development of hock lesions, or vice versa.

Introduction Hock lesions have been reported as positively associated with lameness; both are welfare concerns in dairy cattle (Kielland, *et al*. 2009). The aim of this study was to investigate the association between lameness with the presence of hair loss on the hock and other related factors.

Material and methods Holstein-Friesian heifers calved between July 2008 and July 2009 were recruited from three herds which housed adult cattle in cubicles with mattresses bedded with sawdust. The body condition score(BCS), mobility score and cleanliness score of all recruited animals were measured monthly from September 2008 until March 2010. The severity of hair loss on both hocks were measured using a scoring system (score 0-3) described previously (Huxley & Whay 2006). Milk records were provided by farmers. A logistic regression model with two levels was set up and outcome variable was no hair loss (hock score 0) and any hair loss (hock score 1-3). A lameness predictor with five categories was included in the model: lameness for three continuous visits (previous (t-1), current (t) and next visit (t+1)) was assessed. Variables with $p \leq 0.05$ were retained in the final model.

Results A total of 1475 records (739 records for left hocks and 736 records for right hocks) from 70 animals were used in the analysis (Herd 1, 23; Herd 2, 30; Herd 3, 17). The provisional logistic regression model is presented in Table 1. Cows which were lame at the previous visit and recovered (OR: 8.65; 95%CI: 2.07-36.26) or continue to be lame (OR: 7.01; 95%CI: 2.15-22.89) at the current visit had higher odds of having hair loss on the hocks compared with the cows not lame at three continuous visits respectively. Farm 3 (OR: 7.46; 95%CI: 1.27-43.77) had higher odds of having hair loss on the hocks compared with Farm 1. Milk yield and cleanliness score were both associated with having hair loss on the hock.

Table 1 The list of predictors in the logistic regression model

Variable	Categories	Freq.	Coeff.	O.R.	P value	95% CI	
Intercept		1475	0.61				
Left/right	Left	739	Ref.				
	Right	736	-0.25	0.78	0.37	0.45	1.34
Milk yield (kg)	3.0-21.7	326	Ref.				
	21.8-27.2	327	1.17	3.22	*<0.001	1.43	7.24
	27.3-32.1	320	1.47	4.35	*<0.001	1.60	11.82
	32.2-48.0	321	1.88	6.56	*<0.001	2.00	21.56
Cleanliness score	0-5	513	Ref.				
	6-7	739	0.62	1.85	0.07	0.95	3.61
	8-11	211	1.12	3.06	*0.04	1.08	8.66
Farm	1	593	Ref.				
	2	508	1.27	3.57	0.10	0.79	16.14
	3	374	2.01	7.46	*0.03	1.27	43.77
Lameness (t-1,t,t+1)	Not lame at three visits	474	Ref.				
	Not lame at previous and current visits, being lame at next visit	64	0.15	1.16	0.79	0.39	3.46
	Lame at previous visit and recovered at current visit	82	2.16	8.65	*<0.001	2.07	36.26
	Not lame at previous visit and being lame at current	102	0.50	1.66	0.34	0.59	4.62
	Lame at previous and current visits	211	1.95	7.01	*<0.001	2.15	22.89

*P≤0.05

Conclusion Our provisional results suggest that cows which are detected as lame in the previous month (whether they are lame or recover in the current month) had a higher risk of having hair loss on the hock in the current month compared with animals which were not lame in the previous and current month. Our provisional results imply that lameness precedes hair loss on the hock i.e. lame cows develop hair loss rather than hock hair loss makes animals lame in the future.

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Locomotion score as an early diagnosis tool for lameness in Holstein cows

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Implications Results from this study cast insights into the importance of early diagnosis of lameness through close monitoring of dairy cows using locomotion score. Early diagnosis would lead to early treatment of lameness.

Introduction Lameness is the third most common reason for involuntary culling after infertility and mastitis (Whitaker *et al*. 2004). It results in direct and indirect economic losses; the former are due to treatment and the involuntary culling of cows and the latter are due to milk yield reduction and fertility deterioration (Green *et al*., 2002). Total cost per case has been estimated to range from 48 to 886 € (Noordhuizen, 2012). Early treatment means more chances for cows to recover. This requires an early diagnosis which includes frequent observation and a clear definition of lameness. The objective of this study was to assess the hoof condition of cows considered mildly lame e.g. with a locomotion score (LS) 2, in order to conclude whether they should be trimmed and treated.

Material and methods The study was carried out in a large commercial farm located in Northern Greece and included 237 first lactation and 66 second lactation Holstein cows that calved between 2008 and 2010. Cows were locomotion scored weekly on a five-point scale, starting six weeks before calving and throughout lactation. A trained veterinarian, with proficient skills in detecting lameness assessed all cows. Those with a locomotion score ≥ 2 were considered lame and were hoof trimmed within a week of observation. All lesions were recorded in a designated sheet and scored on a three-point scale. Lesions were categorized into three types: contagious diseases (CD), claw horn disorders (CHD) and both CD and CHD; the latter were also split depending on which type of lesion was predominant. One-way analysis of variance was used to detect differences in lesion severity between cows scored LS=2 and LS>2.

Results In total 248 cows were diagnosed as lame (81.8%). Most cases occurred in the first 3 weeks after calving (Figure 1). Percentage of each LS at first lameness incident is shown in Table 1. Frequency of CD was 20.9%, of CHD was 16.5% and 62.5% of cases had both type of lesions; CD was predominant in 41.9% of these cases, CHD in 39.4% and in 18.7% of the cases CD and CHD were equally present. Table 2 shows that lesions were present and similarly distributed between cases scored 2 and those scored 3 or 4. In addition, severity of lesions did not differ between them (P>0.05). This implies that cows with LS=2 should be considered lame and included in the treatment hoof trimming schedule of the farm, if early diagnosis and treatment of lameness is our concern.

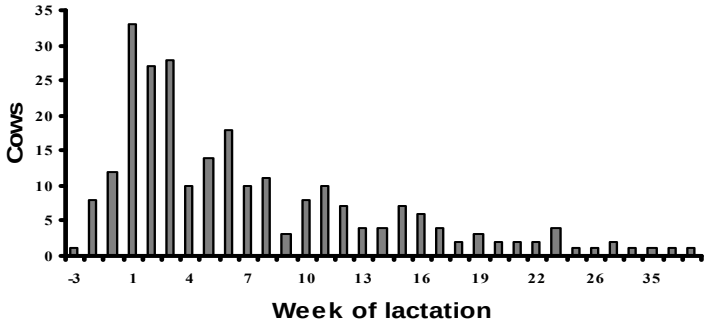

Figure 1 First lameness incidents before and after calving

Table 1 Locomotion score at first lameness incident

	%
2	75.4
3	23.4
4	1.2
5	0.0

Table 2 Frequency of lesions (%) per locomotion score

Lesion	LS = 2	LS > 2
CD	21.9	18.0
CHD	16.0	18.0
CD+CHD	62.0	63.9

Conclusion Performing locomotion scoring on a weekly basis, at least for the early lactation cows and considering cows with a locomotion score 2 as lame will help farmers to detect lameness early and proceed with timely treatment of lesions.

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Force plate analysis as an alternative to visual lameness scoring in detection of lameness in Holstein Friesian dairy cattle

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Implications This research aims to benefit dairy cow welfare; reduce culls for lameness and help ensure the prolonged soundness of cows in adequate housing conditions by comparing the reliability and feasibility of existing methods of lameness detection.

Introduction Early and consistent detection of lameness is paramount in the UK dairy industry; with recent studies showing 36.8% prevalence (Barker *et al* 2010) it is one of the primary reasons for decreased milk yield and culling of animals. The number of producers is ever decreasing, with no let-up in demand for milk, hence it is important to determine the most efficient and cost effective means of lameness detection; ensuring viability in a farm setting and minimising disruption to the daily routine. A way to better understand this time consuming and economically important problem is to utilise both subjective (i.e. visual) lameness scores with objective information garnered from repeated measurements on a force plate over which cows walk at least twice daily. This may help to deduce which method detects lameness more quickly and accurately.

Material and methods In order to compare the usefulness of visual lameness scoring (VLS) to scoring through objective electronic means, 38 lactating Holstein-Friesian cows, identified with electronic ear tags at a single farm, were walked over five AMTI three-axis, twelve channel hall effect force plates twice daily over 10 months. Focus was on the change in the centre of pressure during the impact phase of the stride (CCOP), with a working hypothesis that CCOP would increase in unsound animals. Data recorded were compared to regular Manson and Leaver (1988) visual mobility scores as well as key performance indicators recorded on Interherd. Data pertaining to CCOP were highly positively skewed and therefore transformed using log10 for the purpose of analysis using mixed model analysis in ASReml.

Results CCOP was related to VLS; the higher the VLS, the higher the CCOP ($P < 0.05$). CCOP values were consistently higher in the right limbs and in the forelimbs (Table 1). For parities 1-4, CCOP increased with parity. As lactation progressed, CCOP values increased. 24 animals in the test group were reported lame according to VLS either during or after the experiment. Out of these 24 animals, 16 had an above average 305d yield. Overall CCOP decreased with increased 305d yield.

Table 1 Mixed model analysis summary for CCOP and visual lameness core (VLS). (*** = $P < 0.001$).

Measurement	Mean	S D	Cow	Month	Parity	305d yield	DIM	Foot
CCOP	158.7	340.0	***	***	***	***	***	***
VLS	1.65	0.33	***	***	***	***	***	N/A

Conclusion In agreement with previous studies, animals with above average 305D yields are more likely to become visually lame, however this association is not reflected in CCOP. The fact that CCOP was higher in right limbs could be due to the direction of turns into and out of the parlour on this farm. Increases in the CCOP of visually lame animals reflect findings from some equine studies using pressure mats (Oosterlinck *et al* 2012), suggesting that CCOP may be a viable method of indicating lameness. These findings warrant investigation of further measurements garnered from force plates to aid in consistent identification of lameness, potentially offsetting the problem of reduced human observation of large herds and the subjectivity of visual scoring. Research into further methods of lameness detection and prevention, such as exploiting genomic indicators of future lameness susceptibility is ongoing.

Acknowledgements The authors gratefully acknowledge funding from The Royal Veterinary College.

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Influence of lameness on the lying behaviour of straw housed, zero-grazed lactating Jersey cattle

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Implications Housing cattle in straw yards may be beneficial for lame cows as it does not alter their behaviour compared with non-lame cows.

Introduction Studies have shown that in conventionally-managed dairy herds, lame cows spend more time lying down than non-lame cows (e.g. Singh *et al*, 1993) and a similar pattern has been seen in zero grazed Holstein cows (Blackie *et al*, 2011). However, there is limited evidence of the impact of lameness on the lying behaviour on straw bedded systems and very limited research into the impact of lameness on lying behaviour of Jersey cattle specifically. The general behaviour of non-lactating Jersey and Holstein cattle has been compared on deep bedding and those on free stalls where it was found that Jerseys spend less time lying down prior to calving in both housing types (Campler *et al*, 2012). The aim of the present study is to investigate the effect of lameness on lying behaviour of Jersey cattle in a straw based system.

Material and methods The study was conducted on a 525 cow, straw housed dairy herd in the south east of the UK from June 3rd 2013 to June 28th 2013 using 35 Jersey cows (29 primiparous and 6 multiparous). The cows were housed all year round on a straw based system and received fresh straw daily. Feed passageways were grooved concrete and scraped 2-3 times a day by a tractor with a scraper attached. Feed was pushed up once daily at approximately 06:00 and cows received a TMR feed once daily. Cattle were milked twice daily throughout the study. Lameness was assessed using the locomotion scoring of Flower and Weary (2006) method (locomotion score 1-5; score 1 normal gait; score 5 severe lameness). No cows scoring 4 or 5 were used in the study as there were too few animals of this score. For analysis cows were grouped according to locomotion score (1, 2, 3). Groups were balanced for stage of lactation and parity LS1 (n=12) parity 1.3 ± 0.22 and 222.1 ±33.11 days in milk, LS2 (n=12) parity 1.4 ± 0.29 and 189.4 ± 38.55 days in milk and LS3 (n=11) parity 1.4 ± 0.24 and 228.3 ± 32.41 days in milk. Immediately after locomotion scoring, activity monitors (IceTag™, Ice Robotics Ltd, Roslin, UK) were attached to the back right leg above the fetlock for 4 days to measure standing and lying behaviour. Data from the IceTags were used to determine the percentage of time that the animal spent standing, lying or active; the data were converted to hours/day. Frequency of lying was determined as the mean number of times a cow lay down in a period of 24 hours. Daily milk yield was measured over the week of study. All data were normally distributed (using Pearson's skewness test) and differences between groups were assessed by one-way Analysis of Variance using Genstat (16th Edition).

Results There were no significant effects of locomotion score on the time spent active, lying and standing (Table 1) within this study. The cows had similar numbers of lying bouts across the three locomotion score groups and had similar milk yield.

Table 1 Influence of locomotion score on behaviour and production of lactating Jersey cattle

	Locomotion Score 1 (n=12)	Locomotion Score 2 (n=12)	Locomotion Score 3 (n=11)	S.E.D	P- Value
Active (hours per day)	1.16	1.22	1.19	0.10	0.844
Lying (hours per day)	9.50	9.65	9.42	0.63	0.933
Standing (hours per day)	13.34	13.13	13.40	0.61	0.900
Steps per day	3301	3496	3336	295.4	0.779
Lying bout count per day	13.31	15.58	16.00	1.39	0.131
Milk Yield (litres per day)	20.67	23.00	23.18	2.75	0.595

Conclusion The results found indicate that housing cattle on straw yards may positively influence the behaviour of lame Jersey cows. Further research is needed into the activity budgets of lame and non-lame Jersey cattle specifically over a range of housing conditions.

Acknowledgements The authors gratefully acknowledge help from the farm manager and staff throughout the study.

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The effect of housing system on oxidative stress of Holstein dairy cows in early lactation

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Implications Differences in housing systems are a considerable source of variation on oxidative stress in dairy cows. Hence, appropriate housing for cow comfort should be the main component in stall designs and management programs when the aim is to maximize profitability and maintaining health cows.

Introduction Housing systems and management practices are directly associated with cow comfort and welfare. The notion is that improper housing of dairy cows plays a key role in initiating a variety of stressors which may result in health disorders, especially in critical periods of the productive life of cows when they are more prone to oxidative stress. The aim of this study was to assess the effect of the housing system on oxidative stress indicators in Holstein dairy cows during early lactation.

Material and methods The study was carried out using 42 clinically healthy Holstein cows in early lactation that were kept in 6 commercial farms in Northern Greece. In three farms the cows were housed in a straw yard (SH) and in the remaining farms in cubicle housing (CH). All farms had earthen paddocks. The initial selection of cows was based on their days in milk (DIM≤35). Thereafter, the cows of each farm were allocated according to values of somatic cell counts (SCC) in their milk following two samplings with a monthly interval. Hence, a low group (LSCC, n=3) and a high group (HSCC, n=4) were created. Blood samples were obtained by tail venipuncture (one with EDTA and one without anticoagulant). Milk samples were also collected at evening milking twice to assess chemical composition and microbial load. Body Condition Score, Rumen Fill Score and Locomotion Score were evaluated using the methodologies described by Ferguson (1994), Atkinson (2009) and Sprecher (1997), respectively. RBC, WBC and PLT counts as well as HGB, HCT, MCV, MCH and MCHC values were measured by an automated hematology analyzer (Advia® 120, Siemens, Germany). Urea, uric acid and albumin concentrations were determined with a biochemical analyzer (Clinical Chemistry Analyzer, Flexor E., Vital Scientific N.V., Netherlands). Oxidative stress (O.S.) was estimated by measuring serum reactive oxygen metabolites (ROMs) using a photometer (Free Carpe Diem Analyzer, Diacron, Italy). Categorical variables were analysed using chi-square test or Fisher's exact test when appropriate. Comparison consisted of 3 or more groups were conducted with one-way ANOVA. Student's t-Test was used to compare mean differences in the binary independent variables. All statistical analyses were done with SPSS® version 20.

Results Overall level of oxidative stress was significantly different between SH and CH cows (P<0.05). Significant differences were also observed between sampling months, regardless of housing system and somatic cell count (P<0.05). During the first month of lactation SH-HSCC animals had significantly higher oxidative stress (P<0.05) in comparison to the second month, however the SH-LSCC cows did not seem to have such dissimilarity (Table 1). Total HGB, HCT, MCV, MCH and protein levels were also significantly different between SH and CH cows (P<0.05). Moreover, a significant linear correlation with negative direction (Rho:-0.324, P<0.05) between O.S. and urea levels was found in both samplings.

Table 1 Oxidative parameters in cows housed in different systems

	SH		CH			SH-HSCC 1st month		SH-HSCC 2nd month		
	Mean	±SE	Mean	±SE	P	Mean	±SE	Mean	±SE	P
O.S.	65.1	2.71	73.1	2.48	0.05	75.6	2.65	63.6	2.22	0.012
	1st month		2nd month			SH-LSCC 1st month		SH-LSCC 2nd month		
	Mean	±SE	Mean	±SE	P	Mean	±SE	Mean	±SE	P
O.S.	74.6	2.79	63.3	2.11	0.00	73.2	3.11	62.9	2.08	0.081

Conclusion The significant differences in oxidative stress between SH and CH cows throughout the experiment are indicative of the impact of housing system a fact that merits further investigation.

Acknowledgements
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A survey of bovine colostrum management practices on Irish dairy farms

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Implications The findings of the present study indicate that colostrum management on farms can be improved to maximise immunity in neonatal dairy calves.

Introduction To ensure adequate immunity, calves must get 3 litres of good quality colostrum within 2 hours (average 40kg calf; Chigerwe *et al*, 2008). If not, it may result in failure of passive transfer (FPT). In 2012, ~70% of Irish calves tested in regional veterinary laboratories had FPT thus indicating a problem (DAFM and AFBI, 2012). The hypothesis of the present study was that colostrum management practices on Irish dairy farms may not be maximising immunity in neonatal calves.

Material and methods The study population was selected from a dairy farm database that was part of the Irish Cattle Breeding Federation (ICBF) HerdPlus group (n=320). Selection was random, based on herd size and geographical location, aimed to represent the Irish national average. The questionnaire consisted of four sections: 1) cow management, 2) calving management, 3) calf management and 4) colostrum management (investigated in the current abstract). A final total of 15 colostrum related questions were selected. Questions included details on collection and storage as well as feeding practices such as volume and timing of the calf's first feed. Surveys were distributed via post to selected farmers between 11 July and 15 August 2013. Responses received were entered onto the online survey software package SurveyMonkey (www.surveymonkey.com). On final collection, coded responses to each questionnaire were downloaded to one file and Microsoft Excel (MS office, 2010) was used to collate the data. A chi squared analysis (significance level $P<0.05$) was performed (PROC FREQ in SAS; SAS Institute 9.3) using two independent variables: (i) 'quota' (Qcat), and (ii) 'enterprise'. Quota category 1 (Qcat 1) was suppliers with a quota ≤380,000L; Qcat 2 was >380,000L and <600,000L; and Qcat 3 was ≥600,000L. Enterprise was divided into specialist dairy farms (SD) & dairy farms with another enterprise (DO).

Results The final survey response rate was 85% (n=271). In terms of collecting colostrum, Qcat 3 respondents were more likely to collect colostrum at the next herd milking post-calving than Qcat 1 and 2 (majority had collected colostrum within 2 hours post-partum; $P<0.05$). Additionally, SD farms most commonly waited to collect colostrum until next herd milking (43%) compared to DO farms (26%; $P<0.01$). More Qcat 1 respondents fed calves colostrum from their own dam (79%); however this category also more commonly allowed their calves to suckle the dam (48%). A larger number of Qcat 3 used a pooled colostrum supply for the calf's first feed (32%) compared to both Qcat 1 and 2 (*Fig.1*). Bottle and teat was the most common method used to feed colostrum among all respondents and more Qcat 3 farms used this method than either other category. Of those that knew the intake volume at first feed, the majority fed 2-3 litres (43%); 51% of Qcat 3 fed this volume while 37% of Qcat 1 offered 2-3 litres (*Fig.2*). As an alternative source to the calf's own dam, 55% of farms said they would use colostrum from any cow. Alternatively, transition milk (15%) and colostrum from neighbouring farms (5%) were used for the first feed. First feed was earlier in Qcat 1 (45% in 1 hour) compared to Qcat 3 (35% in 1 hour; $P<0.05$).

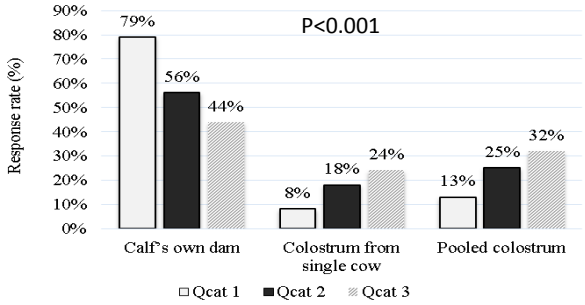

Figure 1 Effect of Qcat on primary colostrum source

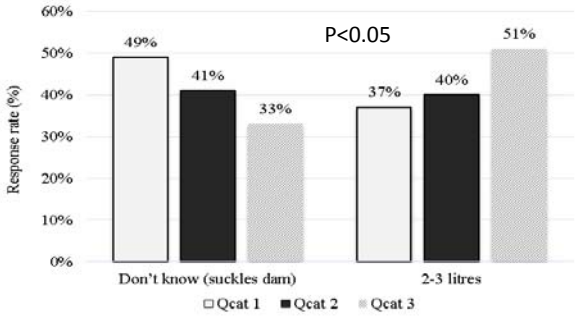

Figure 2 Effect of Qcat on the volume of the calf's first feed

Conclusion On most farms surveyed in this study, the volume of the calf's first feed is immeasurable or inadequate. Many farms feed within an adequate time period; however primary colostrum source used is not recommended. In light of these findings, colostrum management on Irish dairy farms needs to be reviewed to ensure adequate immunity in neonatal calves.

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Effects of feeding dairy heifer calves once daily from 28 days of age

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Implications Once daily feeding of dairy heifer calves from 28 days can reduce labour requirements, but correlates with lower pre-weaning growth rates, previously associated with increased mortality and lower first lactation production.

Introduction DEFRA has recently issued clarification that all calves must be fed milk twice daily until 28 days, this study aims to see how once daily feeding from this age affects growth rates, body shape, disease and Insulin-like Growth Factor 1 (IGF-1). Previous work has shown that low IGF-1 at one month is associated with death before six months of age[1].Higher growth rates pre-weaning are associated with lower age at first calving and increased first lactation yields[2]. Conversely, once daily feeding reduces labour requirements on-farm[3]. This study aims to assess the impact of once daily management on mixed breed dairy heifer calves in a spring block calving grass based dairy system. A skim milk based milk replacer was fed to the once a day group and a whey based powder to the twice daily group reflecting the typical choice made by farmers.

Material and methods On a commercial farm in Dorset, 194 heifers were recruited at birth and assigned to a once or twice daily feeding group. Data was collected for calving information and all calves were housed in the same barn with blocks of pens assigned to each treatment group. All calves were fed colostrum by oesophageal tube within six hours, and again within 12 hours, of birth. Calves were fed waste colostrum for the first week. All calves were fed milk replacer until 8 weeks old. The ONCE group were fed 600g/day skim based milk powder mixed at 20% split into two feeds for calves under 4 weeks old and given as a single feed after this age. The TWICE group were fed 900g/day of whey based milk powder mixed at 15% twice until weaning. Weekly checks were carried out by the first two authors who were blind to treatment group and all calves were measured and checked for clinical disease in weeks 1,2,4,6 and 8. Ponderal index was calculated as weight (kg) / (withers height (m) + trunk length (m))^3 to provide an index of leanness. A blood sample was taken in the first week to assess IgG and IGF-1 and a second sample was taken between four and seven weeks to measure IGF-1. Data were analysed in R, *t*-tests were used to test for differences between groups and linear models used to assess the factors affecting growth rates, ponderal index and IGF-1.

Results Calves in the TWICE group grew significantly faster than the ONCE group with larger differences in growth rates seen in the first month of life when all calves were fed milk twice daily (Table 1). Differences in skeletal growth and ponderal index persisted into the second month of life and ONCE calves did not catch up in the second month of life and the TWICE calves were on average 7.4kg heavier ($p<0.01$) and 4.4cm taller at the withers ($p<0.001$) at eight weeks.

Table 1 Differences between calves fed skim based milk replacer once daily or whey based milk replacer twice daily. Continuous variables tested using t tests and proportions tested using chi-squared tests. ADG: Average daily gain

Variable	TWICE Group		ONCE Group		P value
	Mean	95% CI	Mean	95% CI	
Total Protein (mg/ml)	61.3	42.0-80.7	60.4	42.6-78.1	0.49
ADG (1-4 weeks) kg/day	0.582	0.031-1.131	0.442	-0.165-1.049	0.002
ADG (4-8 weeks) kg/day	0.570	0.096-1.045	0.527	-0.002-1.056	0.30
ADG (1-8 weeks) kg/day	0.583	0.276-0.900	0.0502	0.190-0.815	0.008
Ponderal Index (8 wks)	18.9	13.62-24.1	19.9	14.62-25.2	0.02
Total BRD Score	7.6	0-15.6	8.1	1.5-14.6	0.37
Percent with Pneumonia	25.2		27.7		0.83
Total Faecal Score	2.8	0-5.8	3.4	0.3-6.4	0.01
Percent with Diarrhoea	61.2		68.7		0.3

Conclusion A management system using twice daily feeding and a whey based powder was associated with higher pre-weaning growth though in this early spring calving group neither treatment group grew at the recommended 0.7-0.8kg/day1. Greater differences were found in skeletal growth, ponderal index and IGF-1 than in ADG between management groups.

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A survey of current practice among dairy farmers out-wintering replacement heifers in Great Britain

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Implications Dairy farmers out-winter heifers mainly to reduce the cost of rearing replacements and to improve animal health and welfare. Farmers out-wintering heifers consider that acceptable animal performance is achieved in these systems.

Introduction Out-wintering replacement heifers is a low capital cost option for grass based dairy systems or where winter housing is a limiting factor to dairy herd expansion. Dairy heifers adapt well to cold outdoor conditions if provided with shelter and a dry lying (Redbo *et al.,* 2001), and out-wintering mature cattle on forage crops can achieve similar levels of animal performance to housing (Keogh *et al.,* 2009). Out-wintering systems in Great Britain are farmer-led innovations (Barnes *et al.,* 2013), therefore a survey of these farmers was conducted to assess current practice with a view to informing dairy farmers who are considering employing this system of heifer rearing.

Material and methods In April 2012, 120 questionnaires were sent to dairy farmers known to out-winter replacement heifers with names supplied by local grazing societies, pasture based discussion groups, businesses servicing pasture based farmers, an on-line out-wintering discussion group and farm consultants. The questionnaire comprised 66 questions in four main categories: farm characteristics, reasons for out-wintering, management of out-wintering system, performance and success of out-wintering. Seventy questionnaires (58%) were returned, ranging from South West Scotland, Wales and Cornwall. Data were collated into SPSS v. 19 and analysed for mean, standard deviation (s.d.), max, min, median and mode.

Results Experience of out-wintering ranged from one to 40 years (mean 9.7) per farm. The majority of respondents had seasonal calving herds; 69% spring calving, 2% autumn calving, and 14% spring/autumn calving. Production characteristics reflected this with a mean milk production of 5360 litres per cow (s.d. 1498), from cows with a mature live weight of 527 kg (s.d. 62.4). Mean herd size was 368 cows (s.d. 206.3) and almost two thirds (63%) of respondents indicated they were expanding herd numbers. The top reason given for out-wintering was to reduce the cost of heifer rearing, followed by improvements in animal health and welfare, irrespective of whether the herd was expanding or not (Table 1).

Table 1 Top four reasons[1] for out-wintering heifers on dairy farms that were either expanding or not

	Herds expanding		Herds not expanding	
	Mean	s.d.	Mean	s.d.
Reduce the cost of heifer rearing	4.56	0.813	4.52	0.790
Improve animal health and welfare	4.02	0.976	4.04	0.878
Alleviate pressure on buildings	3.72	1.297	3.68	1.644
Reduce labour input	3.59	1.106	3.96	1.224

[1]as rated on a five point Likert scale (1 not important, to 5 extremely important)

The most popular forage used for heifers older than one year was pasture (55%) followed by kale (42%), fodder beet (32%), hybrids (25%) and stubble turnips (23%). This pattern was similar for heifers less than one year old although fodder beet was less popular than both hybrids and stubble turnips. The most common supplementary feed was grass silage bales (87% and 83% respectively for heifers >1 year old and heifers <1 year old). Supplementary feed composed 35% and 44% of the total diet offered to heifers >1 year and heifers <1 year old respectively. Severe weather was most commonly dealt with by offering additional supplementary feed (41%) and/or offering additional crop (43%). The most common methods for providing dry lying areas were selecting free draining fields (57%) and having a grass runback area and wide field headlands (47%). Housing was the most common strategy for underperforming animals (54%) although only 17% of farms were monitoring live weight change. Despite this, average live weight gain was reported as 0.56 kg/d. Farmers perceived body condition and milk production of out-wintered heifers in their first lactation to be similar to housed animals.

Conclusion Farmers out-wintering dairy heifers have larger than average herds and are more likely to be expanding, although the most important reasons cited were to reduce costs and improve animal health and welfare. Monitoring heifer performance is not widely undertaken during the out-wintering period, although farmers out-wintering generally consider performance is equal to or exceeds their experience of housed heifers.

Acknowledgements The farmers who responded to the questionnaire and DairyCo for funding are gratefully acknowledged

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Relationship between enteric methane emissions, gross energy and live weight of Holstein Friesian Heifers

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Implications Strong relationships have been presented between methane (CH_4) emissions and the variables DM intake, GE intake and live weight which can be used in the refinement of the UK National Greenhouse gas inventory.

Introduction Agriculture accounts for 9.6% of the total UK Greenhouse gas (GHG) emissions based on 2011 estimates with enteric fermentation being one of the largest contributors (NAEI, 2011). Little information is available on the effects of dietary, animal and management factors on CH_4 emissions from young cattle therefore an evaluation of CH_4 emissions from dairy young stock during the grazing period was undertaken. Current CH_4 emissions are estimated using Tier 2 methodology of Intergovernmental Panel on Climate Change (IPCC) with the assumption that 6.5% of GEI is lost as CH_4 in dairy and beef cattle (3.0% for feedlot cattle). Using the sulphur hexafluoride (SF_6) technique the objective of the present study was to quantify CH_4 emissions of grazing dairy herd replacements over a range of developmental ages.

Material and methods In total 72 Holstein Friesian heifers sourced from the Hillsborough Research Institute dairy herd were allocated based on age into three developmental age groups of 12 animals. Calves were between 6-9 months, yearlings between 12-15 months and in-calf heifers between 18-21 months of age. Pasture was predominantly perennial ryegrass and no supplementary feeds were offered. Methane emissions were estimated using a modified SF_6 technique as described by Johnson *et al*. (1994). Linear regression analysis using REML was performed between CH_4 emissions and LW, DMI and GEI testing for differences between age groups with season fitted as a random effect.

Results A significant linear relationship was found between DMI and CH_4 emissions (g/d) ($P<0.001$, Figure 1) when including age as a factor ($P<0.001$, Pseudo $R^2=0.8617$) regression slopes for individual age ranges were as follows: calf Y= 13.60 X + 29.010, yearling Y = 12.94 X + 70. 480 and in-calf heifer Y= 3.47 X + 138.620. A significant linear relationship was also found when regressing CH_4-E with GEI ($P<0.001$, Figure 2). When age was included as a factor this resulted in a significant relationship ($P<0.001$, Pseudo $R^2=0.862$). Regression slopes for individual age ranges were: calf Y= 0.040 X +1.5920, yearling Y=0.039 X + 3.924 and in-calf heifer Y= 0.012 X + 7.6520. The relationship between CH_4 emissions and LW was highly significant ($P<0.001$). When including age as a factor this was also highly significant ($P<0.001$, Pseudo $R^2=0.812$) with individual age range regression slopes as follows: calf Y= 0.379 X +14.050, yearling Y=0.239 X + 74.770 and in-calf heifer Y= 0.097 X + 120.070.

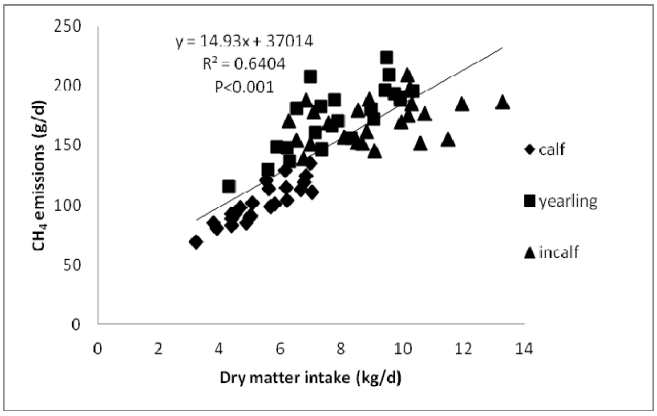

Figure1 Relationship between CH_4 emissions and dry matter intake

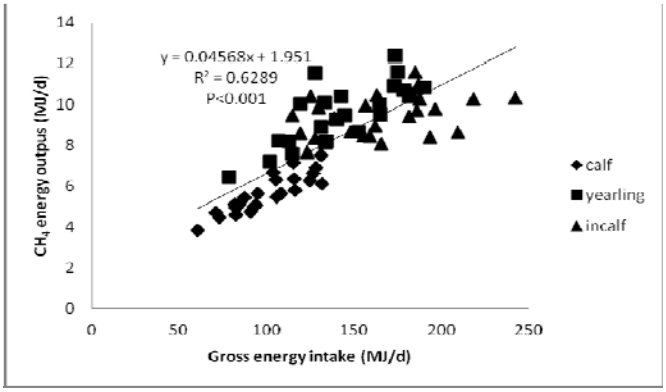

Figure 2 Relationship between CH_4 emissions and gross energy intake

Conclusion This study clearly shows that DMI and GEI are drivers of CH_4 emissions (g/d and MJ/d) with larger live weights significant of increased CH_4 emissions (g/d). Development of this data set is prudent in reducing the gap in knowledge of CH_4 emissions from different age ranges of Holstein Friesian heifers.

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A survey of Jersey heifer calf rearing practices among UK dairy farmers

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Implications A wide range of systems are being used by farmers rearing Jersey heifer calves on UK dairy farms. The findings of the survey indicate that mortality rates to weaning are within acceptable limits. However, there may be opportunities to improve current rearing practices by knowledge transfer.

Introduction Dairy farmers in the UK are looking for alternative breeds of cow for milk production as evidenced by the increase in coloured and cross-breed heifer calves being registered with BCMS. Those selecting the Jersey breed have limited UK information available to them on how best to rear their replacement heifer calves (Jaster, 2005), with most research and industry recommendations based on knowledge of the Holstein breed. Therefore a survey was conducted among farmers rearing Jersey heifer calves to determine current practice with a view to informing knowledge transfer and future research.

Material and methods In December 2012 questionnaires were posted to 122 milk producers that had registered Jersey heifer calves with UK Jerseys during the previous 12 months. The questionnaire included 7 main sections: 1) farm details, 2) calving cow & new-born calf, 3) colostrum management, 4) milk feeding, 5) pre-weaning concentrate & forage, 6) weaning strategy, 7) post weaning management. Each section included a mix of open and closed questions with multiple choices. Farmers were asked to answer questions using data for the previous 12 month period. Mortality rates were calculated from the number of heifer calves reported on the farm. The data were analysed for statistical associations using chi-square tests or analysis of variance using Genstat version 15. There were no significant associations.

Results Responses were received from 38 of those sent a questionnaire (31.1% response rate). Jersey cows had been kept on the farms for an average of 42.5 years (median; range 2 to 100 years). The median herd size was 162 cows (range 35 to 300) and the mean 305 day lactation yield was 5910 litres (range 4457 to 7830 litres). Typically, 60 (median; range 10 to 180) heifer calves had been born on the farm during the 12 month reporting period, a total of 2375 heifer calves were born on the respondent's farms during this time. The calves were left with their dam for 16.5 hours after birth and 32.4% of respondents reported that calves received their colostrum by suckling from their dam. Other respondents fed colostrum using stomach tube (13.5%), teated bottle (18.9%) or combined suckling from the dam with artificial feeding (35.1%). Colostrum quality was tested 'always' (2.7% of respondents), 'sometimes' (24.3%) but most often 'never' (73.0%). A milk replacer (149.5 g powder/l; 3.9±0.22 litres per day; mean ±sem) was offered to calves by 50% of farms, most often twice per day (78.9%) and from a teated feeder (55.3%). Other milk feed options were colostrum (15.8%), transition milk (5.3%) waste milk (7.9%), whole milk (2.6%) or combinations of these. During the milk feeding stage the calves were offered a concentrate from 5 (range 4 to 7) days of age, this was most often offered *ad libitum* and was either pelleted (55%), coarse mix (26%) or other. Forage were offered *ad libitum* from 7 days (range 4 to 10), this was typically either barley straw (39%) or hay/haylage (27%). Water was made available from 5 days of age (3 to 7 days) and 92% of calves were allowed continuous access. The mean age at weaning was 8.8±0.40 weeks. The calves were generally selected for weaning according to age (32.4% of farms), with concentrate intake (13.5%) and weight (5.4%) being the other means of selection. Gradual weaning was employed by 78.4% of farms, with 21.6% of farms choosing to abruptly wean calves. The gradual weaning process involved switching to once a day milk feed (40.7%), reducing daily milk volume (22.2%), replacing milk with water (3.7%), or combinations of these (33.3%). The mortality rates among calves on the respondent's farms are shown in Table 1.

Table 1 Mortality rates at key stages to weaning

	median	interquartile range
stillborn	5.0%	2.3 to 7.1%
died within 24h of birth	0.0%	0.0 to 1.0%
died between 24h and weaning	2.6%	2.0 to 7.7%
died before weaning	11.1%	6.3 to 15.0%

Conclusion The results indicate that wide ranges of calf rearing practices are used among farmers rearing Jersey heifer calves as replacements for the dairy herd. Mortality rates within the surveyed farms generally fall within acceptable levels. However, there are aspects of colostrum and milk feeding management that do not meet with recognised best practice.

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Evaluation of feeding high (750g/d) or standard (500g/d) levels of milk replacer on the performance of artificially reared dairy-bred beef calves to 12 weeks

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Implications With restricted milk fed dairy-bred beef calves it is common practice to feed 500g/d calf milk replacer (CMR) split into two feeds per day to weaning. The results presented here show that increasing the CMR to 750g/d increases growth rate and feed costs and that the calves fed 500g/d CMR did not exhibit compensatory growth. This increase in feed cost could be negated by earlier slaughter of the bulls.

Introduction Utilising the correct CMR feeding regime to achieve optimum growth rates whilst maintaining health and development and allow a smooth transition from milk to solid feed is vital for the success of any effective artificial calf rearing programme. Extensive work has been undertaken to look at the effects of CMR intakes on the growth of calves although these differ in methods and conclusions (Davies & Drackley, 1998). However commercially artificially reared calves are commonly fed 500g of CMR per day usually split into two feeds per day. The objective of this experiment was to evaluate the effect of feeding a standard (500g) or high (750g) level of CMR on calf performance to 12 weeks on a twice-a-day bucket rearing system.

Material and methods Forty Holstein and Beef cross Holstein bull calves with a mean age of 19.6 days were artificially reared and assigned in a randomised block designed experiment to one of the following treatments: High, calves received 750g(air-dry weight)/day CMR ('Enerlac', Volac International Ltd) in warm (37^{o}C) water (187.5g CMR/L) fed via buckets at 4 litres per day in two equal feeds*;* Standard, calves fed 500g(air-dry weight)/day CMR in warm (37^{o}C) water (125g CMR/L) at 4 litres per day in two equal feeds. The calves were individually penned and offered *ad lib* straw, water and concentrates (Start n' Wean, Wynnstay Group Plc) and weaned at 42 days. The calves were moved into group pens at weaning. The CMR and concentrates were analysed to contain 198 and 46g/kg oil and 213 and 205g/kg CP respectively. The data were analysed by ANOVA with calves blocked according to weight and breed.

Results The calves fed 750g/day of CMR recorded significantly higher (P<0.05) DLWGs from start to 3 weeks, higher 3 week weights and gained an extra 4.8kg in weight from start to 12 weeks.

Table 1 Effect of CMR feed rate on liveweight (kg)

	Standard	High	s.e.d	Sig
Start weight	49.6	50.0	0.85	NS
3 week weight	60.6	64.4	1.74	*
Weaning weight	76.5	79.0	2.41	NS
12 week weight	129.4	134.6	5.26	NS

Table 2 Effect of CMR feed rate on DLWG (g)

	Standard	High	s.e.d	Sig
Start - 3 weeks	518	692	42.6	*
Start - weaning	635	695	38.7	NS
Weaning - 12 weeks	1259	1324	37.1	NS

Table 3 Feed intakes (kg/head) and feed conversion ratio (FCR) start to weaning

	Standard	High	s.e.d	Sig
Conc intake (start - weaning)	36.4	29.4	4.01	NS
Conc intake (wean - 12 weeks)	139.2	146.0		
Milk replacer	19.1	27.9		
FCR start - wean (kg feed: kg gain)	2.44	2.41	0.319	NS

Concentrate intake from start to weaning tended to be higher (P =0.124) for the calves fed the Standard CMR feed rate however there were no significant differences in FCR. Feed costs per calf were increased by £14.18 and by 10p/kg live weight gain with the 750g/day CMR based on the feed costs prevailing at the time of the study (October 2102).

Conclusion The calves reared on both 500g and 750g/day of CMR exceeded the recognised growth targets for purchased bucket reared calves at 12 weeks of 119-122kg. The calves fed 750g/day of CMR recorded significantly higher DLWGs from start to 3 weeks and 3 week weights and by 12 weeks the High CMR fed calves had gained an extra 4.8kg in weight. The calves fed 500g/day of CMR having recorded lower DLWGs to weaning did not exhibit compensatory growth.

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Teat *versus* bucket feeding systems for calves

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Implications The results of this study show that feeding milk to artificially reared calves via a bucket with a teat at head height compared to a bucket on the floor without a teat resulted in improved calf health with a reduced incidence of bloat.

Introduction In a recent survey on rearing systems for artificially reared calves some 83% of farms feed milk via a bucket which is assumed to involve a restricted quantity of milk fed to individually housed calves. Of the 83% of farms feeding milk via a bucket 29% feed milk from buckets with a teat; therefore 71% of these farms feed milk from buckets without teats. The objective of this experiment was to investigate the effect of rearing dairy-bred beef calves on calf milk replacer (CMR) from either a bucket with a teat at head height or from a bucket without a teat placed on the floor on performance to 12 weeks.

Material and methods Forty eight Holstein and Beef cross Holstein bull calves with a mean age of 15.6 days were artificially reared and assigned in a randomised block designed experiment to one of the following treatments: Teat, calves fed warm (37^{o}C) milk replacer ('Enerlac', Volac International Ltd) mixed at 187.5g plus 812.5ml of water to give 1 litre of mixed milk twice per day at 4 litres per day to weaning at 42 days to supply 750g/day CMR from buckets with teats (Twin Bucket Feeder, Wydale Plastics) placed at head height. Bucket, calves fed the same type and quantity of CMR via a bucket without a teat placed on the floor. The calves were gradually weaned, individually penned and offered *ad lib* straw, water and concentrates. The CMR and concentrates were analysed to contain 198 and 46g/kg oil and 213 and 205g/kg CP respectively. The data were analysed by ANOVA with calves blocked according to weight and breed.

Results The calves fed milk via a teat gained an extra 1.4kg in weight after 12 weeks. However there was no significant difference (P>0.05) in method of milk feeding on calf growth rate.

Table 1 Effect of method of feeding on liveweight (kg)

	Teat	Bucket	s.e.d	Sig
Start weight	46.8	46.7	2.37	NS
3 week weight	56.5	57.4	3.11	NS
Weaning weight	71.1	70.8	4.45	NS
12 week weight	114.7	113.2	7.11	NS
DLWG Start - 12 weeks	0.808	0.792	0.065	NS

Calf health was determined using recognised scoring systems with lower scores indicting normal (better) health status. The following health scores were recorded; hydration score (Ely & Guthrie, 2000); cough score, nasal discharge and eye discharge score (Linderoth, 2007); ear score and faecal scores (McGuirk, 2009).

Table 2 Effect of method of feeding on calf health scores

	Teat	Bucket	s.e.d	Sig
Start	1.62	1.67	0.334	NS
Week 6	1.78	2.31	0.549	NS
Week 12	1.45	1.98	0.225	NS

Table 3 Incidence of disorders and disease (number of calves treated)

	Teat	Bucket
Scour	14	13
Respiratory	9	12
Bloat	2	4

There was a gradual improvement in health status of the calves on the teat system from week 1 which by 12 weeks approached statistical significance (P=0.052). It can be seen from table 3 that there was an increase in the incidence of bloat on the bucket system. Fortunately this was quickly identified and treated otherwise this could have had a significant effect on calf performance with potential mortality.

Conclusion Feeding method had no significant effect on weight gain to 12 weeks but the calves fed CMR via a teat at head height tended to have improved health scores with a reduced incidence of bloat. Therefore it would be recommended that artificially reared calves are fed milk via a teat at head height.

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Dynamics of detectable immunity: a study of bovine herpesvirus 1 specific antibodies in the bovine neonate

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Implications Bovine herpesvirus 1 (BHV1) seroprevalence in Irish cattle is estimated at 70%. Clinically, BHV1 exerts fertility & respiratory effects, and can cause the exclusion of infected bulls from AI studs. Perinatal dam to calf transmission is a real risk, & the very early life of the calf is a critical control point in reducing BHV1 infection. Reliable categorisation of calves based on BHV1 antibody (no exposure, maternally derived passive immunity, suspected field infection or vaccination) would allow risk-based control to be developed. This study aims to provide interpretative direction on BHV1 test results over the perinatal period. The work also informs risk based decision-making for the pre-selection of BHV1-free bulls for entry into AI studs.

Introduction ELISA serological testing allows the detection of antibodies against specific viral antigens: glycoproteins gB & gE & as all Irish BHV1 vaccines are gE-deleted, the animals BHV1 status may be inferred. Both gB & gE antibodies are transferred in bovine colostrum. Pairs of dam/calf serum samples are collected nationally as part of the Irish BVD eradication programme. These provide a unique large scale opportunity to explore the relationship between dam & calf BHV1 antibody levels in the first weeks of life.

Material and methods 1794 pairs of dam/calf serum were tested. Overall the age of the calves ranged between 4 & 116 days, with a mean (±SD) of 41 (±13.8) days. Dam & calf sera were tested under same conditions using commercial ELISA (IDEXX IBR gB & gE antibody ELISA kits) according to manufacturer's instructions. Results reported as % blocking & Sample/Negative ratio (S/N) as instructions. Pearson correlation coefficients (r) were calculated using Excel (Microsoft, Seattle, WA, USA)

Results In 982 (54.7%) dam/calf pairs, both calf & dam samples were negative to both gB & gE ELISA – indicative of no BHV1 infection. In 236 (13.2%) pairs, both calf & dam were positive to both gB & gE ELISA, consistent with field BHV1 infection while in 201 pairs (11.2%) both calf & dam were positive to gB and negative to gE ELISA, typical of BHV1 gE-deleted vaccination. In 374 (21%) the dam & calf BHV1 gB and gE serology results were inconsistent with the above 3 scenarios.
Initial analysis concentrated on the 236 dam/calf results suggestive of field BHV1 infection, this group had a mean calf age (±SD) of 41 (±14.0) days. There was a strong correlation (r=0.74) between dams & calves gB antibody levels but much weaker relationships between the calves gE antibody levels and either the dams gB or gE level, r=-0.04 and r=0.24 respectively. In this group, calf age had little effect on gB or gE antibody level, r=-0.27 and r=0.15 respectively.
In the 201 dam/calf results, suggestive of BHV1 vaccination, the mean calf age (±SD) was 39 (±12.2) days. Again the strongest correlation was between dam gB antibody and calf gB antibody levels with r=0.75, while that with calf age was much lower (r=-0.06).

Conclusion
A deeper understanding of the predictive value of current BHV1 serology results should maximise the information yield from any single early-life sampling event, benefiting viral control programmes and AI bull selection. Partitioning the various factors which determine specific BHV1 antibody levels in the calf is an essential step. The fact that over a fifth of sample pairs tested had BHV1 results atypical of no exposure, maternal antibody, field infection or vaccination must be considered in the future design of control programmes for this virus.

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