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Comparing the assessment of animal health and welfare in farm assurance scheme, organic standards and legislation

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Introduction Farm assessment has been developed in the UK to ensure the safety, quality and traceability of food and welfare of livestock to consumers. The standards of assessment may focus on resource, such as housing conditions and husbandry practices and the outcome of those resource on animals, such as the physical condition (Burkholder, 2000; Roche *et al*., 2009) or behaviour of animals. An approach of assessing outcomes is, however, thought to better measure animal welfare and help scheme to identify potential husbandry problems (Main, Whay, Lee, & Webster, 2007). The aim of this study was to examine the use of outcome-based observations within the assessment reports.

Material and methods This study focused on the Assured Dairy Farm (ADF) standards set by the Assured Food Standard (AFS) organization, known as Red Tractor and compared with Soil Association (SA) organic standards and DEFRA Cross Compliance Scheme (CC) animal welfare reports. The data of this study came from ADF reports, collected from three major certification bodies which carry out inspections, Soil Association (SA) inspection reports, which were conducted by the SA assessors against the Organic standards and reports from the DEFRA Cross Compliance legal requirements. The evidence that the assessors provided in comment boxes in reports which supporting their decisions were defined as outcome-based comments, for example: physical condition of cows, or resource-based comments, for example: housing condition, by key words.

Results Amongst the 449 ADF reports, there were 49718 comment boxes in total, and 30240 (60.82%) comments were made. Of the 30240 comments, 29189 (96.52%) were resource-based comments, 850 (2.81%) were outcome-based and 202 (0.67%) were not very clear to be labelled either as outcome-based or resource-based comments.
Within the 37 SA inspection reports, a total of 1228 comments were made. 883 (71.91%) comments were resource-based comments, 334 (27.2%) were outcome-based comments and 11 (0.9%) were unclear comments.
There were a total 364 comment boxes in the 26 CC reports, only 60 comments were made (16.5%) to support the answers and the scores. 41 (68.3%) of all the comments were resource-based comments, 18 (30%) were outcome-based comments, and in just one case (1.7%) was unclear.
Out of the 850, 334, 18 outcome-based comments from the ADF, SA, CC reports respectively; a total of 15, 9 and 9 comments, respectively, were relevant to non-compliance and the rest were related to compliance of the standards.

Table 1 Summary of three organisations with the numbers of comments.

	Total questions per report	Mean of Comment made per report	Mean of resource comment per report	Mean of outcome comment per report
ADF	111	67.3	65.0	1.9
SA	33	33.2	23.9	9.1
CC	14	2.3	1.6	0.7

Conclusion To summarise, a major finding is that the number of comments made and outcome comments per reports varied between organisations. This might reflect the difference in the assessors' training and the instruction of the assessment protocols. It is believed that a balance of outcome-based and resource-based evidence could be used to identify welfare concerns (Webster, 2005). After the review of the reports from the Red Tractor scheme, and comparison with the SA inspection and CC legal requirements, the results indicated that more outcome-based comments could be used to support non-compliance decisions. The use of outcome measurements in different farm assessment remains intriguing in need of further study.

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002

Survey of UK stakeholder opinion on suitability of measures and use of outcomes for dairy cattle welfare assessment schemes

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Introduction On-farm animal welfare assessment protocols are becoming more widely used, as consumers demand more information about the quality and safety of animal produce. Understanding how all stakeholders in the industry from farmers to consumers view the whole process of welfare assessment is critical to having welfare assessment schemes accepted by the industry and the public. For any particular welfare assessment scheme to be accepted for use by all stakeholders in the dairy industry, it must fulfil a number of criteria. It must measure important aspects of welfare, and measure those aspects adequately and reliably. The level of involvement of the farmer during assessments and the way in which the results are presented to the farmer, and are used by the scheme owner is also important. Therefore, the aim of this study was to collate stakeholder views on how dairy cow welfare assessment schemes can be delivered to maximise acceptance.

Material and methods A review of previous dairy cow welfare assessment protocols was made to determine what aspects of welfare have been measured, and what assessment methods have been used. Discussions with welfare assessment scheme managers were held to determine what options were available for delivering the schemes on farm. From this, an on-line questionnaire was constructed with 49 questions. The first 4 questions established the training and experience of the respondent. The second part of the questionnaire investigated respondents' opinion of how the assessment should be conducted, the third part asked respondents to rate a range of welfare criteria (e.g. mobility, living of a natural life, disease status) from extremely unimportant to extremely important to measure, and asked about the reliability and practicality of the current measures for these aspects of welfare and the final section asked for opinion on how the results should be used and presented. The major groups of stakeholders invited to take part were representatives from the dairy industry/trade organisations, dairy research scientists, dairy advisors, dairy veterinarians, dairy farmer representatives, consumer and animal welfare charity organisations, milk buyers and retailers. SNAP Professional (v10) webhost software was used to carry out the survey and collate the data. Results are presented as respondents in each answer category as percentage of total number of respondents to that question.

Results Of the 175 invitations sent, there were 108 responses representing all stakeholder groups (Table 1). Most respondents (97%) favoured the idea that welfare assessment protocols could be used as an advisory/management tool for farmers. Most also thought these protocols could be used by external assessors for certification purposes (89%). There was a broad range of answers on who should bear the costs of running the schemes with milk retailers, milk purchasers and consumers being the most favoured (67%, 62% and 51% respectively). In terms of what aspects of welfare should be measured, mobility (lameness), presence of disease and presence of injuries were thought to be most critical aspects of welfare to assess (rated as extremely important by 86%, 79% and 66% of respondents respectively). Assessing pain caused by management, adequate resting, and thermal comfort were also thought to be important (53%, 50% and 41% respectively), with cleanliness and living of a natural life least important (23% and 12% respectively). There was broad agreement across the stakeholder groups, with mobility and disease included in the 'top five' welfare criteria for all groups. Some aspects of welfare have methods available that are thought to be valid and reliable. These include mobility, feeding and drinking, and the use of farmer data on some of the diseases and injuries. However, there were thought to be few reliable measures for pain management, social interactions or positive emotional state. In regards to the outcome of the farm assurance assessment, when a non-compliance against a scheme standards is found, most respondents favoured actions that allowed the farmer to discuss the non-compliance with the assessor (73%), or the farmer being given time to perform any required action (65%). Alternatively the assessor should further investigate the cause of the non-compliance (66%). There was less support for penalising farmers (35%) and very little support for the farmer using the information as they see fit without any further action for external sources (2%).

Table 1 Percentage of questionnaire respondents classified by occupation or employing organisation

	Academic/ Researcher	Working for a farmer organisation	Working for an assurance scheme	Consultant	Milk buyer/ processor	Welfare charity	Practising Vet	Dairy Producer	Retailer	Other
%	27	11	11	8	8	8	5	5	5	10

Conclusion There was a high response rate to the questionnaire indicating interest across the dairy industry. There is broad agreement on what aspects of welfare to measure across all stakeholder groups. Some of these measures, such as mobility, have practical and reliable measurement methods available, but measures for the other important welfare aspects need more research. It is clear that the stakeholders view welfare assessment protocols as tools for use by the farmer on his/her own farm as well as a tool for certification bodies or other assessors. Further consultation is required to determine what body should be responsible for the costs of the scheme. This information is valuable for those developing new welfare assessment packages by providing data on user expectations and needs.

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Seeking the most characteristic quantitative movement changes in lame pigs – potential for automatic herd-lameness tracking on farms

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Introduction Lameness is a common cause of lost productivity for the pig industry worldwide and a significant threat to animal welfare. Lameness may arise from poor conformation, lesions in the hoof or integument and other disorders in the musculoskeletal and nervous systems. Common diagnostic methods require assessors with accurate diagnostic abilities and time for individual assessment, raising questions about the reliability of visual gait assessment. The objective of this study was to investigate movement changes in juvenile female pigs with advanced lameness compared to those with clinically sound locomotion based on a biomechanical method, to develop an additional tool in the selection of superior breeding animals.

Material and methods A total of 84 female pigs were enrolled in a longitudinal gait study in a period from January to July 2012. The experimental population grew gradually, increasing by typically five animals every three weeks, reflecting the three-week batch rotation system used for gilt/sow management on the Newcastle University pig unit. The youngest gilts were 39kg (SD 3.8) and the oldest 146kg (SD 13) at point of entry to the study; the oldest animals underwent just one data collection before they entered the commercial breeding herd. A multiple camera-based motion capture was applied at regular intervals to the pre-breeding gilts, during which 3D coordinate data of reflective skin markers attached to head, neck, trunk and leg anatomical landmarks were collected. 15% of the gilts developed spontaneous lameness at some point during the enrolment period and before they entered the breeding herd at 220±10days. Animal movement was captured on scheduled motion capture dates when there was willingness to follow a human along a walkway with an apple reward. Lameness was clinically diagnosed on the day of capture using a subjective scale from 0 to 3 (where 0=normal, 1=stiffness, 2=lameness detected, 3=minimal weight bearing on affected limb (adapted from Main *et al*., 2000). Head, neck and trunk marker trajectories, elbow (front leg) and knee (hind leg) joint angles, hoof lift and the step-to-stride length ratio of 5 lame pigs (lameness score 3, 114kg BW SD 20) were compared against the same movement parameters of 5 normal pigs (lameness score 0, 124kg BW SD 2). Depending on whether the data were normally distributed or not, Mann-Whitney tests and t-tests were applied (Minitab v.16, USA) to compare key intervals (differences between local maxima and minima (amplitudes)) on displacement curves over time for normal pigs and pigs with front and hind limb lameness.

Results The frequency of irregular steps was increased (P<0.001front lame, P=0.0015 hind lame) in both front and hind legs for lame pigs. However, lameness generally induced more irregularity in the front legs (P<0.001). Front lame pigs had more front leg step irregularity (P<0.001) compared to pigs with lameness in hind legs, whereas pigs with hind lameness had increased hind step irregularity (P=0.038) compared to pigs with front lameness. The amplitudes of the vertical head, neck and spine displacement over time were different in normal pigs compared to pigs with front or hind lameness. Pigs with front limb lameness had greater head (P=0.003), neck (P=0.003)) and spine (P=0.024) displacement (Table 1), but decreased frequency of vertical head (P=0.008) and neck (P=0.019) movement within a gait cycle (stride). Hind hoof clearance (lift) during the swing phase was less (P=0.008) in pigs with hind leg lameness compared to normal pigs. Total range of motion at the elbow joint was decreased for pigs lame in a hind leg (P=0.043), while the total range of motion for the knee was unaffected by lameness in either hind or front legs.

Table 1 Gait parameters in gilts with different locomotive status

	Normal pigs (N=5)		Front lameness (N=2)****		Hind lameness (N=3)	
Gait parameter	mm*	% gait cycle (time)**	mm*	% gait cycle (time)**	mm*	% gait cycle(time)**
Head vertical displacement	30[a]	27[a]	83[b]	50[b]	33[a]	28[a]
Neck vertical displacement	18[a]	27[a]	76[b]	49[(b)]	25[a]	23[a(b)]
Spine vertical displacement	15[a]	25[a]	35[b]	49[a]	32[b]	37[a]
Hoof lift front (mm)	43		48		40	
Hoof lift hind (mm)	57[a]		65[a]		41[b]	
Step-to-stride ratio (0.5 represents perfect symmetry)	0.45-0.55 in 90-93% of measures		73% of measures <0.45 or >0.55 in front legs		34% of measures <0.45 or >0.55 in hind legs	
Knee range of motion (º)	44		37		42	
Elbow range of motion (º)	34[a]		35[a]		30[c]	

*Distance perambulated by the marker from lowest to highest position. ** % gait cycle for movement from lowest to highest position of the marker. ***Abc superscripts denote significance; brackets indicate tendency for significance (P<0.08). ****N=2 only, but difference was substantial and within-pig SD small for named parameters.

Conclusions Although the number of lame pigs was relatively low, lameness in front legs caused pronounced changes in the vertical movement patterns of the frontal trunk and head of pigs. Hind-limb lameness affected leg movement characteristics, such as hoof clearance, the step-to-stride ratio and the range of motion at the elbow joint. The Step-to-stride ratio appears to be a useful monitor of the stepping symmetry of animals and, like vertical movement of the spine, detects lameness in both front and hind legs.

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004

Comparison of video recordings and rumination collars for measuring rumination activity in cubicle housed commercial dairy cows

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Introduction Amongst other factors, ruminal pH is affected by the amount of time a cow spends ruminating. Measuring rumination activity can be costly, time consuming and labour intensive. The development of automated equipment to record rumination is necessary. Among these devices is the HR Tag (Lely, SCR Engineers), a rumination monitoring system. The HR Tags have been validated under controlled circumstances (observation pen) (Schirmann *et al*., 2009). Therefore the aim of the present study was to validate the system in less controlled circumstances, using cubicle housed dairy cows in a commercial farm setting, by comparing the measures obtained with the HR Tags with data obtained from video recordings.

Material and methods Fourteen multiparous cows (mean ± SEM body weight 689.3 ± 62 kg, parity 4.43 calvings ± 1.22 and days in milk 103.93 days ± 12.3) were allocated to two groups of seven cows each. Cows were given two weeks to adapt to the facilities and diets, and all measurements were recorded in the third week. Cows were offered a total mixed ration diet (TMR = 47% grass silage, 19% wheat, 14% concentrate, 9% water, 7% crimped maize and 4% molasses) with concentrate fed to yield in the milking parlour. Water was supplied *ad libitum*, and the cows were milked twice daily. A HR Tag to record rumination activity was fitted to each cow. Cow behaviour was recorded using sixteen video cameras positioned in the shed so that all cows were easily viewed. The animals were video recorded for 24 hours each day during the measurement week (except for milking when the cows left the shed). Behaviours were recorded by one observer, using The Observer® software. During the measurement week, 12 two hour blocks per cow were analysed to record rumination time (min/2hrs), with these periods exactly matching the periods reported by the HR Tags. To determine observer reliability, the observer scored rumination time twice on 20% of the total observed 2hr blocks and the correlation coefficient was calculated (Minitab 16). The relationship between the time recorded by video analysis and the time reported by the HR Tags were assessed using correlation coefficient and linear regression. All procedures related to animals were approved by the Animal Ethical Review Committee (VERC 2011-88) of the Royal (Dick) School of Veterinary Studies, the University of Edinburgh.

Results The two observer recorded rumination times were highly correlated: r = 0.99 (P<0.001). A high correlation between the time recorded by video analysis and the time reported by the HR Tags was obtained r= 0.87 P = 0.001 and R^2 = 76.4 (P<0.001) (Figure 1).

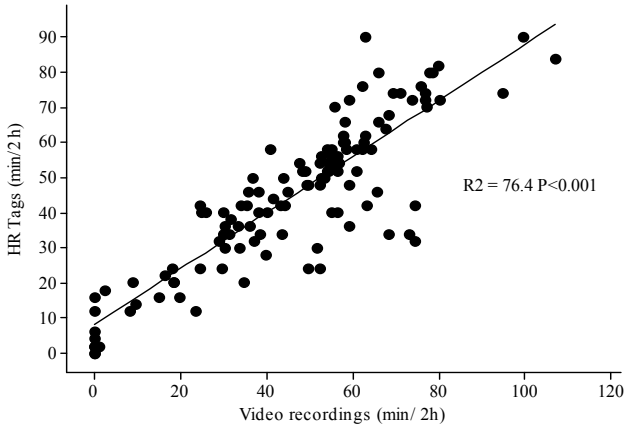

Figure 1 Relationship between rumination time (min/2h) obtained by analysis of video recordings and HR Tags.

Conclusion Measurements of rumination time obtained with the HR Tags proved to be acceptable for the conditions of this study, although they were lower than previous reports of the automated system (Schirman 2009). The results obtained suggest that the use of the HR Tags in commercial farms can be advised for the determination of rumination activity. The identification of illness through changes in rumination behaviour may be appropriate, although further research is now needed to improve the applications of such automated systems of monitoring rumination activity.

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005

Exploratory behaviour and performance of suckling piglets fed novel creep diets in two housing systems

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Introduction The intake of solid creep feed by suckling piglets can promote both behavioural and physiological adaptation to the abrupt change in diet which occurs at weaning. Creep feed intakes have been reported to be very low during lactation (Kuller *et al*., 2010), hence the need to study ways of stimulating the exploratory behaviour and acceptance of creep feeds by suckling piglets. It is known that exploratory behaviour can be stimulated by novelty. Therefore, the aim of this study was to investigate the effect of increasing creep feed variety by use of different sequential flavours on solid food intake of piglets before weaning in two housing systems with different degrees of complexity and freedom for the sow, and the consequences for intake and pig health and performance after weaning.

Material and methods Thirty six sows (Large White and Landrace crossbred) were used in a 2x2 factorial design. At 5 days before farrowing, the sows were randomly allocated to either a farrowing crate or a loose farrowing pen (PigSAFE). On day 10 of lactation, litters in each system were further divided into two groups, matched for litter size and mean piglet weight, and allocated to a flavour variety regime or consistent flavour regime of creep feeding. In the flavour variety regime, the control creep feed was treated by addition of 5 different flavourings (toffee, apricot, red fruit, butterscotch, apple) which were given sequentially on a daily basis. The flavours were added at the rate of 500g/t, according to manufacturer's advice, by mixing the appropriate amount (0.5g) to treat 1kg of feed with 30g of water and spraying this onto the creep feed, after which it was air dried before feeding. Control creep feed was sprayed with water alone to correct for any effects of wetting and drying. Creep feed intake was monitored daily and piglet weight monitored weekly during lactation. Piglets were weaned in the fourth week (approx 28d of age) and moved as litter groups to weaner accommodation with fully-slatted flooring in all-in all-out, controlled environment rooms. A sequence of standard commercial creep feeds was offered *ad libitum* according to the normal farm routine and the feed intake and growth of each litter monitored for the first 2 weeks after weaning. The health of the litter was also monitored. The data collected were, after checking for normality, subjected to a two-way analysis of variance using the general linear model (GLM) procedure in MINITAB v 16.0.

Results There was no effect of the lactation housing system on piglet feed intake and performance before or after weaning, and no interaction between housing and creep feeding regime. There was a significant increase ($P<0.05$) in the amount of creep feed consumed by the piglets fed flavoured feeds during lactation. Weight gain in the two weeks after weaning was also significantly higher ($P<0.05$) in piglets previously fed flavoured creep (Table 1).

Table 1 Main effect of flavour diversity from day 10 of lactation to weaning on feed intake and weight gain of piglets.

	Control	Flavour	SEM	P value
Number of piglets/litter	10.74	10.65	0.18	0.72
Weaning age (days)	28.09	28.11	0.33	0.96
Total feed intake/pig (g)				
15-22 days	8.46	30.94	22.77	**0.01**
22 days-weaning	38.55	80.28	42.52	**0.03**
10 days-weaning	54.04	118.28	17.37	**0.01**
Weight/pig (kg)				
Initial weight (10 days)	2.94	2.92	0.16	0.93
Weaning weight (~28 days)	7.70	7.74	0.39	0.87
Post weaning performance (kg/pig)				
Feed intake - weaning to 2weeks post weaning	3.93	3.96	0.23	0.92
Weight gain in the 2weeks post weaning	4.07	5.11	0.33	**0.03**

Conclusion Sequential feeding of different flavoured creep feeds to piglets during lactation increased their solid food intake prior to weaning and increased weight gain during the first two weeks after weaning.

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006

Do farmed juvenile mink (*Mustela vison*) prefer multiple tube platforms of wire or plastic construction?

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Introduction Although farming animals for their fur is illegal in the UK, it is a financially significant industry in countries such as Denmark, China, Finland, USA and Russia with the International Fur Trade Federation reporting a $15 billion turnover for 2011. Breeding mink have been selected against fear behaviour resulting in animals that are more curious of humans. However, other abnormal responses are performed, such as stereotypies and fur-chewing that are associated with the barren wire cage housing used in intensive farming (Hansen *et al*. 2007). Studies of environmental enrichment have shown that provision of a shelf in the form of a tube can reduce stereotypic behaviour (Axelsson *et al*., 2009), but this is in conflict with other findings (Hansen *et al*., 2011). In Norway, new legislation from 2015 will extend the existing requirement for enrichment with every cage needing a single shelf hanging from the cage roof. Evidence is currently lacking as to what is the best material for these tubes and whether two shelves are necessary for animals that are invariably reared in male/female pairs. The aim of this study was to record the behaviour of mink provided with one or two hanging tubes of plastic or wire.

Material and methods Juvenile mink (n=80) were housed in 40 adjacent cages in male/female pairs inside a commercial unit and fed a commercial diet (a moist mash based on fish byproducts). Cages were industry standard measuring 0.90x0.30x0.45 m (length, width and height) with a separate nestbox and a loose plastic tube on the floor (statutory enrichment). The study used a 2 x 2 factorial design with hanging tubes of two materials, plastic and galvanised wire, presented either singly or as a pair. Four cages (one of each treatment combination) were videoed at a time for 12 hours of daylight (06.30 to 18.30). Behaviour was recorded using instantaneous sampling every minute without individual identification of the animals. Residual plots confirmed by a Shapiro-Wilk normality test, showed the data to be parametric and were analysed using a two-way ANOVA (no. tubes vs. materials) except climbing, which was analysed using a Kruskal-Wallis test. For the double tube treatments only, a t-test analysed what behaviour was performed by the second mink, when the first was in a hanging tube.

Results There was no significant difference in behaviour associated with material, but the number of tubes provided did affect time spent in their use. Mink provided with two hanging tubes, spent significantly longer (P<0.01) occupying this enrichment compared with when just one tube was provided (Table 1). However, when just comparing when two tubes were provided when one animal was in a tube (4.5 % of observations), the other would be much more likely to be active or in the nestbox, occupying the other tube only for approximately ten per cent of these observations only (10.0 vs. 8.4 ± 3.34 %, mean plastic vs. wire ± SED, respectively; Figure 1). Actual stereotypic behaviour was not observed in any of the cages used in this study.

Table 1 Behaviour of mink provided with 1 or 2 hanging tubes of plastic or wire

	Plastic		Wire				
Behaviour (% observations)	1 tube	2 tubes	1 tube	2 tubes	Tube SED	Number SED	T x N SED
In nesting house	55.5	53.0	54.3	51.7	3.04	3.04	4.30
Active in cage	16.8	15.5	14.2	15.0	1.22	1.22	1.72
Asleep in cage	21.9	24.4	24.6	23.9	3.83	3.83	5.42
Using tube	3.6	4.9	3.7	5.9	**0.55****	0.55	0.78
Eating/drinking	1.9	2.1	2.7	2.4	0.31	0.31	0.44

n.b. ** indicates significance at P<0.01

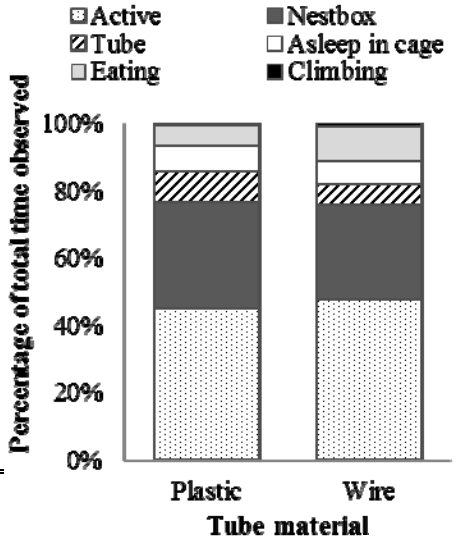

Figure 1 What the second mink was doing, when the first was in a hanging tube

Conclusion The results seem to support new legislation that only one hanging tube is required, as the mink pair rarely used both tubes at the same time and that no stereotypic behaviour was observed in this study. However, use of the tubes did significantly increase with the provision of a second tube. It is possible that the mink may have preferred their own tube, but this could not have been ascertained without individual identification and was not possible in this study. Until it is determined how important that short period might be to the animals' welfare we are still lacking conclusive evidence as to the best solution.

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Lamb mortality in a prolific flock managed in an intensive grassland system: effect of lamb factors

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Introduction Ewe prolificacy is a key determinant of flock productivity and financial performance under lowland conditions in Ireland; but a major issue associated with higher litter size is increased lamb mortality. Survey data for Irish farms indicate that lamb mortality is 8.5% for lowland flocks while mean litter size is ~1.5 (Hanrahan 2010). A reduction of 3 percentage points in lamb mortality would be worth ~€10 million to the lowland sheep sector in Ireland. The aim of the current study was to quantify the sources of variation in lamb mortality using data from a flock managed under an intensive grassland system, and to evaluate the relationship between mortality and birth weight. Information on the latter was used to estimate optimum birth weight.

Material and methods The data consisted of 2905 lambs (332 singles, 1638 twins, 810 triplets and 120 quads) born between 2006 and 2011, inclusive, to crossbred ewes in a flock at Athenry Research Centre that was used for studies on nutritional and grazing management factors in the context of intensive (ewe stocking rate ~14/ha) grassland-based sheep production. The ewes were born to S Blackface dams and were sired by either Belclare or Chamoise rams. Ewes entered the flock at 1.5 years of age and were usually culled at 5.5 years. Lambs were by Suffolk rams (except for a small proportion by Texels in 2 years). Lambing was indoors and live lambs were tagged within 24 h of birth. Average litter size was 2.12 and 1.63 for Belclare-X and Chamoise-X ewes, respectively. Two mortality traits were defined: perinatal (i.e., born dead or dead at tagging) and total mortality (= perinatal deaths plus subsequent deaths to weaning at ~14 weeks of age). Mortality was analyzed as a binomial variable using Proc GENMOD (SAS, 2003); the model had effects for year, dam breed, birth type, sex, damage and dam; birth weight was included as a covariate when examining the impact of this variable on mortality. All 2-factor interactions were examined but only significant (P<0.05) interactions were included in the final model. Birth weight data were analysed using mixed model procedures with fixed effects as for mortality and dam as a random term.

Table 1 Effects on lamb mortality and birth weight (± s.e.)

Effect	Mortality		Birth
	Born dead	Total	weight (kg)
Birth type	***	***	***
Single	0.06 (0.040-0.088)†	0.06 (0.040-0.095)	5.58±0.046
Twin	0.04 (0.033-0.055)	0.07 (0.059-0.087)	4.53±0.032
Triplet	0.12 (0.092-0.158)	0.21 (0.168-0.249)	3.64±0.041
Quad	0.16 (0.089-0.270)	0.28 (0.191-0.384)	3.15±0.092
Sex‡	**	***	***
Male	0.054 (0.040-0.074)	0.090 (0.071-0.115)	4.60±0.042
Female	0.033 (0.023-0.049)	0.057 (0.043-0.077)	4.45±0.042
Dam breed‡	***	***	**
Belclare-X	0.029 (0.021-0.040)	0.054 (0.045-0.071)	4.62±0.033
Chamoise-X	0.061 (0.041-0.090)	0.095 (0.088-0.167)	4.43±0.058

†95% Confidence interval. ‡Means expressed on a twin-lamb basis

Results Overall litter size and total lamb mortality were 2.00 and 10.1%. Estimates of effects on mortality traits and birth weight are summarised in Table 1. Year was a significant factor for both mortality traits; least squares estimates of total mortality of twins varied from 5.9 to 8.0% among years. Mortality was significantly higher for triplets and quads compared with singles or twins. The difference between the dam breeds was 4 percentage points for total mortality and was not accounted for by the difference in birth weight. Effects of birth type on total mortality were no longer significant when adjusted for birth weight. The only significant interaction was sex-by-birth type for both perinatal and total mortality (males > females if singles or twins), and was independent of birth weight. The relationship between mortality and birth weight was quadratic with an intermediate optimum (Figure 1) and varied with birth type (P<0.001). Optimum birth weight was 6.0, 5.6 and 4.7 kg for singles, twins and triplets, respectively. The actual means (Table 1) are below the optima likely due to the range of late-pregnancy nutritional treatments being evaluated (Keady and Hanrahan 2009, 2012). While birth weight is a function breed and system the optima can be used to evaluate system adequacy by comparing weight of twins and triplets to that of singles since the latter are less likely to be affected by management system. Predicted total mortality for populations of single, twin and triplet lambs with mean birth weight at optimum is 6.8, 4.2 and 10.7%, respectively.

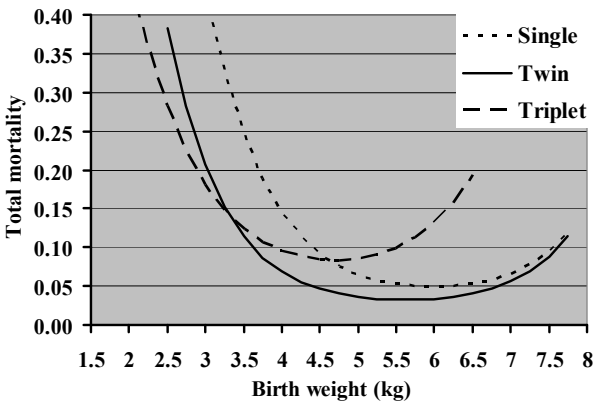

Figure 1 Relation of total mortality to birth weight (kg)

Conclusions The optimum birth weight for singles and triplets is 0.93 and 0.78 times that of singles; these values can be used to assess the likely impact of nutritional or other interventions on the risk of mortality for lambs born as multiples. The results imply that mortality can be reduced by ~3 percentage points nationally. The interaction of birth type and birth weight has implications for the implementation of genetic evaluation for lamb survival traits.

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008

What is the relationship between level of infection and 'sickness behaviour' in cattle?

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Introduction It is well recognized that health and welfare challenges can lead to changes in animal behaviour. However, the question whether the onset and extent of the behavioural changes are related to the size of a health challenge, e.g. the infectious dose of a pathogen, remains largely unanswered. Knowledge of the relationship between behavioural changes and size of the challenge has both theoretical and diagnostic value, as it would allow targeted interventions, thereby enhancing animal health and welfare. The objective of this study was to investigate the relationship between the infective dose of a common macro-parasite, *Ostertagia ostertagi,* and a number of behaviours in growing cattle. It was hypothesised that below a certain health challenge threshold changes in behaviour are of the same magnitude and that only at high pathogen doses, above the threshold, changes in behaviour are linearly related.

Material and methods Twenty-four Holstein-Friesian cross beef bulls between 5-6 months of age were randomly allocated to one of four groups consisting of 6 animals each. *O. ostertagi* L3 larvae were given to the first three treatments diluted in water and by gavage in equal doses on Days 0, 7 and 14 making a total of 300,000 for the High (H), 150,000 for the Medium (M) and 75,000 L3 for the Low (L) treatment. The fourth group functioned as the controls and received a water gavage on the same days. Bulls were weighed twice a week and faecal egg counts (FEC) were taken on the same days starting from Day 0. Blood samples were taken once a week to measure pepsinogen concentration. An activity sensor (Icetag) was fitted to the front leg of each bull to record activity and posture. The experiment was concluded on Day 55 post first infection. Lying and standing bouts were identified according to Tolkamp *et al.* (2010). Feeding behaviour was monitored with the use of video recordings. The results were analysed using a repeated measures ANOVA, after transformation of the raw data if these were not normally distributed.

Results The FEC were positive from Day 20 onwards for all parasitized bulls. Parasitized animals had increased pepsinogen levels by Day 20 which remained elevated throughout and showed a linear relationship with dose ($P < 0.001$). Average daily weight gain differed ($P < 0.001$) between treatments from Day 27 onwards, with the H treatment showing the lowest daily gain (0.429 kg/day), followed by M (0.929 kg/day) when compared to the L (1.31 kg/day) and Control (1.27 kg/day). The M treatment however showed compensatory growth and returned to control levels by Day 37 (Figure 1). Regarding behaviour, posture was affected from Day 29, with the frequency of lying and standing episodes being lower ($P = 0.038$) for the H treatment (Figure 2) and average lying episode duration being longer ($P = 0.011$) for H when compared to L. The total number of steps was lower ($P = 0.038$) for the H treatment between Days 36-46 compared to the other treatments. There was no effect on feeding behaviour for any of the levels of infection.

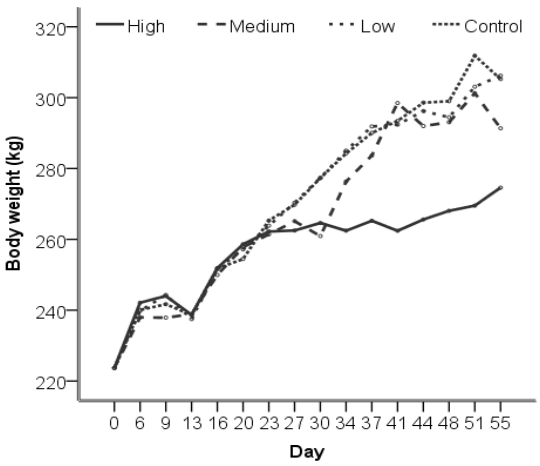

Figure 1 Body weight (in kg) for the High (300,000), Medium (150,000), Low (75,000) and an uninfected control.

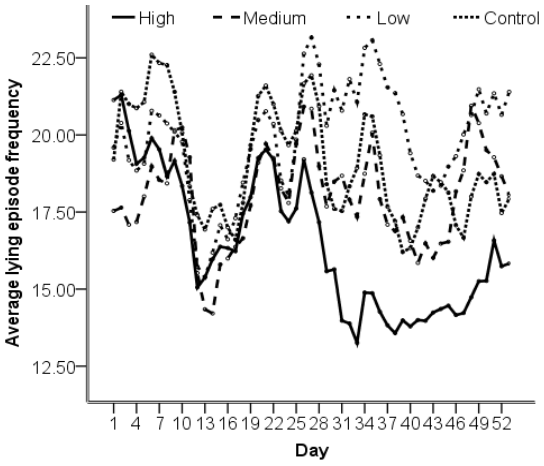

Figure 2 Lying episode frequency (3 day rolling mean) for the High (300,000), Medium, (150,000), Low (75,000) and an uninfected control.

Conclusion It is concluded that the relationship between Medium (150,000), Low (75,000) and an uninfected pathogen dose and behavioural change has a threshold below which no behavioural changes from the norm are detected. If the effect is similar across subclinical infections has yet to be investigated. Performance can be affected before a detectable change in behaviour occurs; but recovery through compensatory growth took place quickly without intervention.

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009

Behaviour of New Zealand pasture based cows offered access to differing free stall bed types, compared with pasture

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Introduction New Zealand (NZ) dairy cows have typically been grazed all year round, however higher milk production per farm unit (Luo *et al*., 2008) and nitrogen losses has led to the need to reduce grazing time during wet conditions. This has motivated more farmers to use cow housing during inclement weather (Arnold *et al*., 2009), but the majority of dairy farmers and cows have never experienced housing. As a consequence, the aim was to assess the uptake and use of differing free stall bed types by adult dairy cows, with no previous experience of cow housing, and to comapre this with cows at pasture.

Material and methods This work was completed in accordance with the ethical procedures of Massey University, Palmerston North, between November 2011 and May 2012, using 36 adult dairy cattle (5 to 13 years of age), which were selected at random and allocated according to age and live weight to one of three groups (1, 2 or 3) of 12 cows, such that the groups were equalized for these factors. During the bed comparison periods cows were grazed for a restricted period of 4 h/d (11.00 to 15.00 h), stood on a concrete for two 2 h simulated milking periods (9.00 to 11.00 & 15.00 to 17.00 h) and for 16 h/d (17.00 to 9.00 h). Group 1 and Group 2 were housed alternately in pen A and B, both of which were fitted with 13 of the same free stall beds, while Group 3 were kept at pasture. Cows readily used freestall beds and following this period cows were rested on pasture for 5 d, while the stalls in Pen A were fitted with 13 dual chamber water beds and Pen B with 13 deep litter sand beds. Pens or pasture were offered to the cows in an incomplete Latin square design, between 17.00 and 9.00 h, in which groups 1 and 2 cows were first acclimatized to one bed type, while group 3 were on pasture for a 5 day period, during which lying of individual cows was monitored at 3 h intervals, followed by which the maintenance behavior of all individual cows (lying, walking, feeding, standing, drinking, standing & lying bouts, bed No's used) were recorded manually by scan sampling (Daylight: 5 minute intervals; Darkness: 15 minute intervals) over 3 consecutive 24 h periods (72 h). The cows had a 5 day rest period at pasture, followed by which housed cows were offered the alternative bed type and the procedure was repeated, such that all the housed cows were assessed on both of the bed types. Activity meters (Ice Tags TM) were fitted to 7 cows in each group and used to validate manual observations. Much of the behavior data was found to be not normally distributed and was analyzed using the non-parametric Kruskal Wallis procedure in Minitab (16.0) with bed (water, sand or pasture) type included in the model as a fixed effect, while differences between medians were assessed, using individual standard deviations and a confidence interval of 95%.

Results Cows were offered access to water beds had significantly (P<0.05) lower total lying and greater standing time than cows offered access to sand beds or pasture. Pasture and sand beds resulted in similar total lying time and number of lying bouts.

Table 1 Lying, standing, walking and feeding behaviour of cows offered access to water or sand beds or pasture

		Night bed type			
		Pasture	Sand	Water	P value
Housing/pasture (16 h)	Lying (h)	10.4 (2.64) [a]	10.4 (2.17) [a]	7.35 (3.51) [b]	<0.0001
	Standing (h)	1.5 (2.46) [b]	1.7 (1.44) [b]	2.9 (2.6) [a]	<0.0001
	Feeding (h) ‡	4.0 (0.92) [a]	3.1 (1.19) [b]	3.1 (1.00) [b]	<0.0001
	Walking (h)	0.04 (0.044)	0.00 (0.043)	0.00 (0.056)	0.3000
	Lying bouts (No.)	1.0 (0.73) [b]	1.0 (1.03) [a, b]	2.0 (1.19) [a]	<0.0001
Restricted grazing (4 h)	Lying (h)	0.75 (0.569) [c]	1.17 (0.632) [b]	1.67 (0.620) [a]	<0.0001
	Standing (h)	0.33 (0.367) [a]	0.08 (0.314) [b]	0.08 (0.296) [b]	<0.0001
	Grazing (h)	2.67 (0.565) [a]	2.50 (0.647) [a]	2.00 (0.648) [b]	<0.0001
	Walking (h)	0.12 (0.092)	0.12 (0.082)	0.12 (0.135)	0.8430
	Lying bouts (No.)	6.0 (2.49) [b]	9.0 (3.05) [a]	5.0 (3.15) [b]	<0.0001
Milking pad (4 h)	Lying (h)	0.00 (0.180) [c]	0.08 (0.431) [b]	0.25 (0.486) [a]	<0.0001
	Standing (h)	3.75 (0.488) [b]	3.58 (0.525) [b]	3.33 (0.460) [a]	<0.0001
	Walking (h)	0.25 (0.136) [b]	0.25 (0.130) [b]	0.33 (0.176) [a]	0.0370
	Lying bouts (No.)	0.0 (0.52) [c]	1.0 (1.30) [b]	1.5 (1.58) [a]	<0.0001

[a, b] Medians in the same row followed by differing superscript letters differ significantly P<0.05

Conclusions Cows found pasture and sand beds equality acceptable, more so than dual chamber water beds. Housing reduced nightly feeding time.

Acknowledgements Authors would like to acknowledge funding from DairyNZ, Fonterra, Beef and Lamb NZ.

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010

Development and validation of a standardised system for the assessment of lameness in captive elephants

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Introduction It has been documented that as many as half of all captive elephants suffer from foot problems at some stage in their life (Mikota, 1994). Foot pathology is the greatest cause of morbidity and mortality in captive elephants (Csuti, 2001), with a change in locomotion often being the only clinical sign. Evaluating locomotion can have a positive effect on elephant welfare by not only assessing current lameness, but enabling an assessment of the efficacy of analgesia and any subtle changes in gait, which may indicate improvement or deterioration. The aim of this study was to design a practical and repeatable scoring system to quantify lameness that could be used by handlers and veterinarians on a daily basis regardless of husbandry system.

Material and methods A total of 72 elephants out of a possible 73 in the United Kingdom and Ireland were filmed from behind, in front and from both sides. Using a questionnaire and a select specialised panel of elephant specialists, a zoo veterinarian and a locomotion expert a Numerical Rating Scoring (NRS) system was proposed. Lameness was scored on a four point scale (0-4) with numerical values that are ascribed to the presence of specific criteria: 0=clinically sound; 1=stiffness; 2=abnormal tracking; 4=reluctance to weight bear. The intra- and inter-observer repeatability of five veterinary surgeons using this system was determined, and compared to a Visual Analogue Scale (VAS). Lameness scores for front and hind limbs were compared using a Pearson Chi Squared test. Agreement between and among observers were investigated using Cohen's kappa (κ). Intra- and inter-observer reliability of the VAS was assessed by calculating the 95% limits of agreement and width of agreement within and between observers. NRS and VAS were compared using a Kendall tau-b test.

Results Overall intra-observer reliability was moderate (κ =0.676), and inter-observer reliability was fair (κ =0.37) for the presence of lameness altogether. Inter-observer agreement improved from the first score to second scoring from slight agreement to fair agreement for stiffness and reluctance to bear weight. Abnormal tracking had moderate intra-observer agreement for both scoring sessions. There were wide widths of agreement for the VAS inter-observer (67mm); however they were narrower intra-observer (33mm).

Conclusions The proposed NRS can be used to evaluate elephant locomotion using simple criteria for an elephant specific NRS. It can be used on freely moving elephants with good intra observer repeatability, and moderate inter-observer repeatability.

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011

Impact of an omega-3 ration on bone formation, resorption, and quality in laying hens

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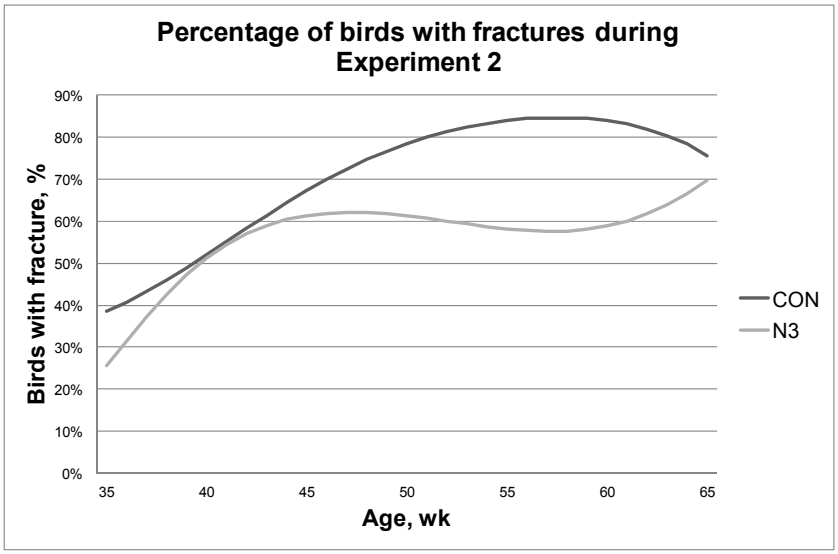
Introduction Keel fracture in the laying hen is the most critical animal welfare issue facing the egg production industry and will likely worsen as extensive systems are employed in response to the 2012 EU directive banning traditional battery cages. Given the gravity of the situation, an effective solution to reduce the frequency and severity of keel fractures is urgently needed. The current effort sought to develop a solution utilizing dietary omega-3 polyunsaturated fatty acids (PUFA) as a means to improve bone health and reduce mineral loss from bones of laying hens.

Material and methods Our study utilized a standard commercial unit within a single shed comprised of two barns located side by side. Each barn was divided into two sections by a middle barrier that contained nest boxes for that section and then further divided by wooden barriers resulting in each barn containing eight individual pens (N=24,000; n=1,500 hens/pen; n=8 pens/barn). Birds were provided ranging area through barn specific popholes that led to outside areas separated by electrical wire. Pens of one shed received a ration enriched with omega-3 (N3) and the other a standard control (CON). Data was collected over two experiments where the N3 flocks received rations differing in the short:long chain omega-3 content (E1 - 0.67; E2 -1.5) from 10 birds/pen at five time points (25, 35, 45, 55, and 65 weeks). Birds were euthanized at the farm site, keels assessed for damage, and the keel, tibia, and humeri removed and frozen until analysis. Quantification was made of the occurrence of keel fractures, serum osteocalcin, and concentrations in the humerus of: $CaPO_4$, alkaline phosphatase (ALP), matrix metalloprotease, Tartrate-resistant acid phosphatase (TRAP), immature and mature cross links, and multiple histomophological measures (e.g., osteoclast size, trabecular dimensions). Additional data was collected including behaviour, production endpoints, and bone density and biomechanics, are reported elsewhere. Statistical analysis was performed to model each response's relationship with age, mass (if appropriate), and treatment using MlwiN. To develop the final model, a full model was produced from which backwards stepwise regression was performed until all model terms were considered effective predictors ($p < 0.05$).

Results Contrary to our expectations, diet proved little benefit to prevalence of fractures or their severity during Experiment 1, though during Experiment 2, fractures during were reduced in N3 pens reaching a maximum predicted difference of 27% at 57 weeks. During both experiments, ALP and TRAP were greater in CON birds indicating a heightened rate of bone remodelling in CON flocks. Additional measures of bone formation (including osteocalcin, $CaPO_4$,) were also increased strengthening the position that greater bone formation was occurring in birds receiving the standard ration, although the ratio of immature to mature cross links was greater in N3 flocks bone. Our findings conflict with previous efforts from our lab using an entirely short chain ration which found that birds receiving the N3 ration had increased measures of formation and resorption, although the cross link measures appeared to follow a similar pattern.

Despite the unexpected pattern of decreased bone remodeling in pens receiving the N3 ration, the diet appeared to meet our objectives with decreased appearance of fractures. Related work from the experiment reported elsewhere suggests differences in biomechanical responses, particularly measures of compliance in agreement with the cross-link results from the current abstract suggesting mechanisms independent of bone remodeling.



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012

Herd health plans for farm assurance: dairy farmers' opinions

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Introduction Farm assurance schemes were developed to re-establish consumer confidence in UK produce (Duffy and Fearne, 2009) after high profile breaches of food safety (Duffy *et al*., 2005) and concerns regarding animal welfare (Main *et al*., 2001). Herd health plans (HHPs) are compulsory for farm assurance and are intended to be used as a tool to assist with proactive herd health planning (DEFRA, 2010). However, Bell *et al* (2006) found that some farmers viewed HHPs as a tick box exercise mainly of benefit to external organisations. The current study explores farmer use and opinions on HHPs and methods to improve engagement with the written document.

Material and methods In November 2011, a postal questionnaire was sent to 1403 dairy farms within the West Midland counties of England. The questionnaire covered three main sections; demographical information; HHPs for farm assurance and possible improvements; herd health management (HHM) and diseases of most concern. The definition of HHP was the written document to comply with farm assurance schemes whereas the term HHM was defined as additional disease recording and monitoring of herd performance. The questionnaire contained 34 multiple choice questions; 15 of which were attitudinal statements using a Likert scale, nine short answer questions and a table for farmers to fill in five disease concerns in their milking herd and three disease concerns amongst calves. A reminder was posted two weeks later when responses started to decline. The study was carried out in accordance with the Harper Adams University College research ethics policy. Responses were entered into a SPSS v.19 database which was used to analyse different groups of respondents using chi-squared tests and two-step cluster analysis.

Results The number of useable questionnaires returned was 293 (20.8 per cent). The most frequent provider of the HHP was the farmer's vet (48.6 per cent; n=141) with the farmer themselves being the providers for 26.6 per cent (n=77). Annual updates were made to the majority of the HHPs (63.8 per cent; n=185). The most negative response to the general statements relating to HHPs was *'I have noticed the benefits of having a HHP for my farm profits,'* with 59.3 per cent (n=172) of respondents strongly disagreeing or disagreeing. Almost the same number of respondents (59.0 per cent; n=171) strongly agreed or agreed with the statement, *'I value the input of my vet in the HHP.'* However, the most positivity was demonstrated by the response to farm assurance accreditation being an advantage with 82.4 per cent (n=239) strongly agreeing or agreeing. Regarding the possible disadvantages, the statement which received the most negativity was, *'HHPs increase the amount of paperwork.'* Nearly three quarters of respondents strongly agreed or agreed with this statement. Associations were found with respondents who described the contact with their vet to include active HHM in six of the 15 attitudinal statements. The respondents with active HHM were more inclined to give positive responses to the general statements and potential advantages of HHPs and less negative about the possible disadvantages (P ranged from 0.001 to 0.021). Generally the farmers with routine fertility visits were more positive about seven of the 15 statements than those farmers who had less frequent or no routine fertility visits (P ranged from 0.001 to 0.026). There was an association (χ^2 (4) = 21.908, P<0.001) between the respondents grouped according to the provider of their HHP and responses to one statement, *'I value the input of my vet in the HHP;'* the majority of the respondents who indicated that their vet or the farmer and vet together provided the HHP were more likely to strongly agree or agree. Similarly, two step cluster analysis identified the same trend with respondents having more positive views with respect to the HHP and cattle health when they had more frequent fertility visits and perceived they were receiving active HHM from their vet. Regarding the potential improvements to HHPs; the most popular suggestions were: '*goals set from your herd health records*,' '*regular feedback from your vet*,' '*summary action page at the front of the document*' and '*national standardised HHP format.*' Respondents were more divided over the suggestions which included the use of technology.

Conclusions This study had demonstrated that the respondents largely considered the HHP to be an inactive document, although respondents with more positive opinions towards HHPs had more frequent routine veterinary fertility visits and were more likely to describe their contact with their vet to include active HHM. Possible practical methods to increase farmer engagement with HHPs have been identified such as goal setting and veterinary feedback.

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013

Methods used to collect farmers' attitudes, motivators and barriers toward cattle production: a rapid review

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Introduction On-farm disease control is extremely important as the consequences of a disease breakdown can affect not only animals on a particular farm, but other farms and industries within the farming sector. Current work at the University of Nottingham is investigating the motivators and barriers to implementing disease control via biosecurity and vaccination on UK dairy farms. Farmers' motivators and barriers are important to understand as part of the decision making behind behaviour (DEFRA, 2008). There have been a number of methods used to collect and analyse motivators and barriers to implementing disease control and little, if any, published research has attempted to summarise the work in this area. The aim of this study was to review and appraise previous research investigating opinions, motivators and barriers of farmers relating to cattle farming. A second aim was to identify and appraise the study methods that have been used previously to elicit this type of information to inform future research in this area.

Material and methods A rapid review framework was used to assess the published literature in this area. The database CAB Abstracts (Ovid) was searched independently by two authors in July 2012 for relevant papers using specific keyword search terms relating to farmers, beliefs and opinions. No exclusion criteria were applied to the search strategy. Papers identified by two researchers were compared, and relevant publications were then subject to specific inclusion criteria independently. Those that were not readily accessible electronically or in paper format from The University of Nottingham library were requested through inter-library loans and subject to the same inclusion criteria. Information regarding areas such as topic of interest, population of interest and methods of data collection and analysis were identified. Critical appraisal was performed on all selected papers independently by two researchers and compared. The data were then analysed descriptively and summarised.

Results A total of 57 papers met the inclusion criteria and were therefore included in the review. The earliest paper identified was published in 1989 with the total numbers of papers published annually generally increasing to the present day. Data collection was carried out in countries worldwide, although nearly three quarters of the papers (42/57) focused on cattle production in developed countries. Over half of the papers (31/57) focused on dairy production, with the remaining papers looking at beef (10/57), veal (1/57), both beef and dairy (2/57), or an unspecified cattle production type (13/57). Papers included covered a broad range of subjects, with topics such as management, technology adoption and prevention and control of diseases commonly occurring. A variety of methods were used by researchers although inconsistencies in terminologies used to describe methods were identified. Most researchers used tools such as questionnaires and interviews to collect data. The selected papers were assessed to have varying levels of quality in relation to defined critical appraisal points.

Conclusions The results of this review highlight that investigation of farmer opinions in relation to cattle production is not a new phenomenon although it is still relatively novel within veterinary research. This type of research has been shown to be applicable to a variety of topics covering many aspects of production. There appears to be a lack of information in many of these studies in relation to the methods used to collect and analyse data, and any theories that may underpin these methods. This is a barrier to other investigators building on previously conducted research to avoid unnecessary duplication of work. Future studies aiming to research motivators and barriers need to include more methodological details in their published work.

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014

A study to evaluate the diagnostic potential of adult ewe carcases at fallen stock collection centres

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Introduction Annual ewe mortality rates in the UK are estimated at 5-7% (Scott 2007), of a current breeding ewe population of around 15 million, equating to around 750,000 dead ewes annually. Many of the diseases resulting in death will also cause losses through involuntary culling (*e.g.* due to poor condition). The profile of diseases which contribute to the mortality and involuntary culling of adult ewes in the UK is at present probably best estimated using VIDA (Veterinary Investigation Diagnosis Analysis) data generated by the AHVLA scanning surveillance for sheep programme, funded by Defra. This programme examines 5-700 ewes annually. This system may have inherent biases (Nevel & Stark 2009), there may under-representation of important endemic diseases, and the sample size is small. By law, all carcases must be collected by a licensed fallen stock collector, for appropriate disposal, so this material is already transported to a central location. If of diagnostic quality, this material could represent an opportunity to (a) provide useful information on causes of ewe mortality on individual flocks and nationally and (b) attribute mortality rates to specific diseases, and thus their costs to the industry and individual farms. Detailed studies on the usefulness of fallen stock for livestock disease surveillance are lacking but good examples of active surveillance in this area include the National Fallen Stock Survey for scrapie (Del Rio Vilas *et al.* 2005) or encephalitic listeriosis (Overmann *et al* 2008).

Material and methods Between February and November 2012, an average of nine adult ewes were necropsied per session on eleven separate occasions at a fallen stock collection centre. The ewes were collected from a wide catchment area across North East England. All carcases were examined anonymously, with no clinical history and no knowledge of the farm of origin. Where a diagnosis considered sufficient to account for death could not be made based on gross *post mortem* findings alone, limited further testing was undertaken. All necropsies were performed by the authors, and diagnoses made according to agreed minimum diagnostic criteria. Contemporaneous notes were made at the time of necropsy, detailing ewe breed, estimated age, body condition, degree of autolysis, and gross *post mortem* findings by body system. Further laboratory testing was performed at or through the AHVLA regional laboratory at Thirsk. Carcases which were obviously severely autolysed or decomposed were avoided.

Results A total of ninety-nine ewe carcases were examined in eleven sessions. Autolysis precluded a diagnosis in nine cases (9%), and a diagnosis could not be made in a further 17 cases, with or without further laboratory testing, despite carcases being of diagnostic quality. In 66% of cases, a diagnosis considered sufficient to account for death was made. In a further 6%, the diagnosis may or may not have accounted for death but may, with further clinical history, have been useful for flock health planning (*e.g.* high worm egg counts). Of the carcases in which a diagnosis was made, mastitis (11%) was most commonly diagnosed, with most diagnoses being between May and July. Acute fluke, of which the first diagnosis was made in late October, was next commonest at 7% of all carcases. Johnes disease (diagnosed using a faeces PCR with gross pathology characteristic of multi- or paucibacilliary disease), Ovine Pulmonary Adenocarcinoma (OPA) (confirmed histologically), bacterial bronchopneumonia (gross pathology +/- bacterial confirmation), chronic suppurative pneumonia (multifocal pulmonary abscessation) (gross appearance) and neoplasia (gross appearance +/- histopathology) were all diagnosed with an incidence of 6% each. *Corynebacterium pseudotuberculosis* was cultured from a caseous abscess in the tracheobronchial lymph node of one ewe. Dosing gun injuries, ruminal acidosis, focal peritonitis, vegetative endocarditis and metritis were diagnosed in a minority of ewes. Enteritis associated with *Salmonella* 61:K, 1,5,7 was seen but was not considered an adequate cause of death. Bacterial cultures were hampered by *Proteus* overgrowth in some cases, despite attempts to sear surfaces and the use of charcoal transport swabs.

Conclusions Necropsy of carcases of fallen (or euthanased) ewes collected at licensed fallen stock collection centres can yield useful diagnostic information which can be used to inform flock health planning, and surveys of disease prevalence. Thus attribution of mortality rates to specific diseases can be derived, perhaps to inform industry priorities. In this study, clinical history may have improved the diagnostic rate in some carcases by informing appropriate further laboratory testing. It is also possible that if sheep farmers knew that a diagnostic service was available, they may request prompt collection of carcases thus minimising the effects of autolysis on carcase quality. If these additions could be implemented, we consider that fallen stock necropsy could have an important role to play in surveillance for endemic diseases in the sheep industry, and can inform on-farm interventions to improve productivity.

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015

Classification of dairy goat farming systems in Greece by principal component analysis and cluster analysis

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Introduction Greece has the largest national herd of dairy goats among European countries but the lowest milk yield per animal. It seems that the dairy goat sector has been neglected from the mainstream research topics in agriculture. Contrary to the increased implementation of intensive management systems in most European countries, in Greece, the majority of herds are reared in low input systems and there is a remarkable scarcity of information regarding management practices. Our objective here was to describe the existing systems using principal component analysis (PCA) and cluster analysis (CA).

Material and methods A total of 85 dairy goat herds (30,644 goats) from 20 prefectures were surveyed from September 2011 to August 2012. Data were collected during pre-scheduled on-farm visits, using a case-specific questionnaire. The latter comprised questions about labour, livestock, facilities and equipment and general management practices (land use, grazing and feeding management, reproduction etc.), properly selected in order to facilitate the classification of the herds. A multivariate statistical approach was used on the data to obtain the most appropriate classification. From the 34 initially considered variables, 22 were not used due to high correlation coefficients with the 12 variables which finally retained and forced into PCA. The 12 retained variables were, facilities and equipment score, number of female adult goats, goats' replacement rate, annual milk yield per milking goat, non-irrigated land per livestock unit, irrigated land per livestock unit, duration of grazing during winter, distance covered during grazing in winter, goats to bucks ratio, kids' weaning age, farmers' years of experience and total working units. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and the Barlett's test of sphericity were calculated to test sampling adequacy and whether the correlation matrix was an identity matrix, respectively. The rotation of the component matrix was performed using the Direct oblimin rotation. The uncorrelated variables (principal components, PC), produced by PCA were used for the consecutive cluster analysis. Cluster analysis was performed in two stages. Initially, the number of clusters was decided using hierarchical cluster analysis (HCA) based on Ward's method. Then, the farms were allocated in the predefined by HCA number of clusters using k-means cluster analysis.

Results The KMO measure of sampling adequacy was 0.688 and the Barlett's test of sphericity was significant ($P<0.001$), indicating that PCA was appropriate and revealing a high relationship among variables, respectively. From the produced PC, only three had eigenvalues greater than 1 explaining about 52% of the total variance and were retained for the successive CA. The description of the PC was based on the eigenvectors of weights of the initial variables for each one of the PC; The three PC are described below: **PC1:** High investments on facilities and on the cultivation of both irrigated and non-irrigated land for self-production of feedstuff together with high annual milk yield; reduced duration of grazing and low distance covered during grazing in winter; low experience of the farmers who implement early weaning of kids and a low replacement rate scheme. **PC2:** Large herds with sufficient labour, investing on cultivation of non-irrigated land for grazing but not on irrigated land for feedstuff harvesting. **PC3:** High replacement rate for goats and late weaning for kids; low goats to bucks ratio. Based on the three PC and using an HCA it was decided to allocate the herds in four clusters. K-means CA allocated herds into four clusters, which counted 11, 29, 40 and 5 herds, respectively. Table 1 shows the final cluster centers for the three PC; the four clusters are described below.

Table 1 Final cluster centers for the three principal components

	Cluster			
Principal Components (PC)	1 (11herds)	2 (29 herds)	3 (40 herds)	4 (5 herds)
PC1	0.63	0.32	-0.62	1.70
PC2	1.93	0.36	-0.12	-1.23
PC3	-0.14	-0.90	0.50	1.53

Cluster 1 (11 herds, 12.9%): Milk production oriented, large herds, owned by young and rather inexperienced farmers investing on facilities, labour and land. The labour force is sufficient with the personnel being occupied, also, on the cultivation of land for the self-production of feedstuff and for grazing. Early weaning is the dominant practice which leads to high annual milk production per goat. **Cluster 2** (29 herds, 34.1%): Herds with low replacement rate of goats, high goats to bucks ratio and early weaning of kids. A less intensive genetic selection program and reproductive management scheme may be implied. **Cluster 3** (40 herds, 47.1%): Herds owned by experienced farmers, with low investments on facilities, labour and land. Feeding management is based mainly on grazing even in winter months; goats are covering long distances at pasture. High replacement rate of goats possibly due to high losses and late weaning is the norm in this cluster. **Cluster 4** (5 herds, 5.9%): Small herds investing on facilities and land used for feedstuff harvesting but not for grazing, which is limited. The farmers have low experience and are self-occupied in the farm, minimizing the use of employees. Low goats to bucks ratio is observed in this cluster, whereas the replacement rate was high and the weaning of kids, late.

Conclusions The proposed classification using data reduction and clustering methods can be a useful tool for an *a posteriori* assessment of the dairy goat sector in Greece. Thus, it can provide the means for strategic and innovative planning in existing herds.

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Effects of clove supplementation to reduce the ammonia nitrogen loss in goat

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Introduction The environment at large is greatly predisposed by the ammonia emissions from livestock production. Ammonia nitrogen loss not only affects the environment but also causes a loss to the farmers as among the livestock feed the protein part is most costly. Out of the total intakes of protein, amino acids or other nitrogen consumption of ruminants, 71 to 85% are excreted in faeces and urine. So reduction of ammonia nitrogen loss would also be helpful to the farmers. By strongly binding to dietary protein condensed tannins can reduce proteolysis and nitrogen loss during mastication and rumination (Min *et al*., 2003). If the nutrient wastage in ruminants can be lowered, it will increase the overall production of ruminants as well as helpful for the environment. Clove has tannin and some essential oils like eugenol, β-caryophyllene, Acetyl- eugenol which may change some microbial activity in rumen that may decrease the ammonia nitrogen loss in ruminants (Khan and Chaudhry, 2012). Therefore, the present study was undertaken to examine the effect of clove supplements on rumen fermentation with the specific objective of reducing ammonia nitrogen loss.

Material and methods Eight young bucks (Black Bengal Goat) usually 1 to 1.5 years with average body weight 7.65 kg were used to assess the *in vivo* digestibility of mixed feed. They were housed on a woody floor covered with wood shavings at the Govt. Goat Development Farm, Tilaghor, Sylhet, Bangladesh. Eight bucks were weighed by weighing balance and divided into two groups containing four bucks in each group. One for control and the other for clove supplement. The animals of both groups were transferred into the two metal pens during the periods of experiment. Animals were kept at a fixed level of feeding with (500 g/day/animal) napier grass and (250 g/day/animal) concentrate diet to fulfil their maintenance requirement. For clove supplemented group (Clo) 5 g/day/animal clove was added with the concentrate. Animals received their daily feed allowance in two equal portions at 09 and 16 h. First seven days were adjustment period and later ten days were collection period. Daily urine and faeces were collected from the metal pens for chemical analysis. A curved polythene sheet was placed underneath the wooden floor of metal pens. Total volume of urine was collected from the polythene sheet containing 1 (N) HCl. Finally the representative part of urine was kept in a sample tube for determining the NH_3-N concentration. Total collections of faeces were made from the net which was placed on the floor of metal pens. The representative part of the faeces was kept in refrigerator for further chemical analysis. The representative parts of supplied feed (forage and concentrate mix) and faeces were analyzed for determining the dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF) and ether extract (EE). Acidified urine was analyzed for determining the NH_3-N concentration. The *in vivo* data were analyzed by using ANOVA following the principles of CRD using computer package (Lawes Agricultural Tust, 1997) and SEM and SED differentiated treatment means. Body weight gain was measured for 10 days in the experimental period.

Table 1 Chemical composition (g/100g DM) of feed supplied to the goat and faeces of goat

		DM	OM	CP	CF	EE	NFE
Feed items	Napier	19.3	89.2	8.7	29.2	2.1	49.2
	Con. mix	87.6	92.7	15.6	11.9	9.3	56.4
Faeces	Control	69.2	89.6	8.2	17.6	1.9	61.9
	Clove Supp.	50.5	91.0	8.7	17.1	1.9	63.4

Table 2 Digestibility**,** body weight gain (BWG) and urine NH_3-N concentration of goats providing two types of feed

	Digestibility (g/kg)				BWG	NH_3-N con.
	DM	CP	CF	OM	(g/d)	(mg/L)
Control	802.1	879.3	795.3	806.5	18	232
Clove supp.	856.1	906.3	856.7	857.2	24	170
SED	3.10	12.53	13.10	1.89	2.54	10.71
P<	0.001	0.06	0.001	0.001	0.01	0.001

Result The proximate composition of napier grass, concentrate mixture used during the experimental period and faeces for control and clove supplemented group were shown in Table 1. The DM and CF were lower in faeces of the Clove supplement than the control group. On the other hand, CP, OM and NFE were higher in the Clove supplement than the Control. Digestibility of DM, CF and OM were significantly higher ($p>0.001$) in the Clove supplement than the Control (Table 2). The increase in mean CP digestibility by clove supplementation approached statistical significance ($P=0.06$). Daily body weight gain was significantly higher ($P<0.005$) in Clove supplement than Control. Clove reduced NH_3-N concentration in urine significantly ($P<0.001$) and it might be partly due to the higher level of tannin and essential oils present in clove. Though the CP (g/100g) was higher in control but due to higher DM (g/100g) in the Control, total CP (g) loss was higher in the Control than the Clove supplement. The result indicated that clove can reduce NH_3-N wastage from ruminants. In this way clove can increase nitrogen retention in ruminant and can increase the growth. In addition, fall of ammonia emission through urine and faeces can also reduce environmental pollution.

Conclusions It could be concluded that the efficiency of N utilization can be improved with clove supplementation, by this clove can enhance growth of ruminants and increase the production and finally can raise profit to the farmers. Further research could be carried out using some other types of tannin as novel supplement to reduce ammonia nitrogen loss in ruminants and can save the environment as well as farmers.

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017

Comparing *Labeo rohita* and *Channa marulius* for heavy metal profile from the Chashma Lake

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Introduction Increased anthropogenic activities can enhance the amount of heavy metals in the environment, especially in aquatic ecosystems. Pollution of heavy metals in aquatic ecosystem is growing at an alarming rate and has become a worldwide problem (Malik *et al*., 2010). Heavy metals in aquatic environment are a major concern because of their toxicity and threat to plant and animal life, disturbing the natural ecological balance. Due to the increasing awareness about the nutritional importance of fish, its demand has increased all over the world. Fish meat contains the large proportion of protein, vitamin, minerals and healthy fats which make it a healthy diet as it is easily digested by the human body. Conversely the presence of heavy metals in fish can be a potential health risk for the fish meat consumers. Therefore, this study assessed the heavy metal profile of *Labeo rohita* and *Channa marulius*, which are among the most commercially important and preferred fish species in Pakistan.

Material and methods The study was carried out on fish from Chashma lake, which is the 3rd largest water reservoir of Pakistan, located at 32° 25′ N, 71° 22′ E; southwest of Mianwali to Dera Ismail Khan at River Indus. The study followed a 2 x 3 factorial arrangement by involving 2 fish species, each with 3 dead weights as described later. We selected *Labeo rohita (Lr)* because of its taste and *Channa marulius (Cm)* for its high nutritive value and its perceived wound healing attributes. Fishing was performed with the help of local fishermen. Sixty samples of each fish species were selected from three weight categories (W1≤1.0, W2≤1.5 and W3≤2.0 Kg) on the basis of their routine weights at which they are caught and sold in the study area. The fish were transported on ice to a laboratory where these were washed with de-ionized water before fish muscles were collected and stored at -20 ºC until analyzed. Selected heavy metals (Cr, Mn, Ni, Zn, Hg and Pb) in fish muscles were analyzed by a Varian Vista-MPX CCD simultaneous ICP-AES (Varian Inc, Australia) machine located at Newcastle University. Metal concentrations were calculated as mg kg^{-1} wet weight. Minitab 16 software was used to test the main effects of fish species (Sp), weights (W) and their interaction for each metal for significance at $P<0.05$. The mean metal contents in fish meat were also compared with their permissible levels of European Commission (EC, 2006) and World Health Organization (WHO, 1986).

Results Table 1 shows the mean concentration (mg/kg wet weight, ww) of heavy metals alongside their standard error of means (SEM) and significance for the effects of Sp, W and Sp x W at $P<0.05$ or else . Most mean metal concentrations were well below the permissible levels of EC or WHO for food fish, except Mn in *Lr*. Generally metal concentrations increased with increased weight of fish. More metals concentration was found in meat of all weight categories of *Lr* than *Cm*. The order of metal concentration in *Lr* was Zn>Ni>Hg>Pb>Mn>Cr and in *Cm* was Zn>Pb>Ni>Hg>Cr>Mn. Highly significant differences were observed for Sp, W and Sp x W interactions at $P<0.001$ for Cr, Mn, Ni and Zn.

Table 1 Mean concentration (mg/kg ww) of heavy metals in *Labeo rohita* (*Lr*)and *Channa marulius* (*Cm)* of Chashma lake

	W1≤1000g		W2≤1500g		W3≤2000g		Permissible Levels (mg/kg ww)		SEM and significance		
Metals	*L r*	*C m*	*L r*	*Cm*	*L r*	*Cm*	EC	WHO	Sp	W	Sp x W
Chromium	BDL	BDL	0.011	0.011	0.023	0.012		0.05	0.004^{***}	0.005^{***}	0.007^{***}
Mercury	0.014	0.011	0.019	0.014	0.035	0.024	0.5		0.001^{***}	0.002^{***}	0.001^{NS}
Manganese	0.012	0.003	0.02	0.007	0.033	0.009	-	0.01	0.0002^{***}	0.0002^{***}	0.0003^{***}
Nickle	0.027	0.007	0.035	0.016	0.036	0.050	-	0.7	0.0005^{**}	0.0006^{***}	0.0008^{***}
Lead	0	0.015	0.024	0.017	0.041	0.041	0.2	2	0.004^{NS}	0.005^{*}	0.005^{*}
Zinc	0.445	0.261	0.716	0.459	0.814	0.580		50	0.01^{***}	0.02^{***}	0.02^{***}

*, ** and *** were significant effects at $P<0.05$, $P<0.01$ and $P<0.001$ respectively whereas NS is non-significance if $P>0.05$

Conclusions Smaller fishes appeared to have less metal contents, so these should be preferred by the consumers. Relatively lower metal contents in meat from *Cm* than *Lr* suggest that it may be preferred for human consumption, though in none of these species were the levels above WHO recommendations except Mn in *Lr*. The lower metal contents in *Cm* could be attributed to its preference for deep and clear sandy or rocky bottoms of water containing low metal contents.

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018

Evaluation of oestrus detection methods in different housing systems and determination of reproductive performance of lame and non-lame dairy cattle

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Introduction Poor reproductive performance and increasing rates of lameness in dairy cows are major factors in reduced profitability among dairy farmers (Maatje *et al*., 1997; Shearer and Amstel, 2000). A key contributor to low reproductive performance lies with poor oestrus detection (Maatje *et al*., 1997). Particularly, the modern Holstein Friesian does not display oestrus as overtly as her predecessors; this is even more evident in the high yielding cow (Lopez *et al*., 2004). This poses a crucial problem within the farming community, as the accurate interpretation of signs of oestrus behaviour is fundamental to overall conception rates and productivity. It is documented that lameness has a detrimental effect on oestrus behaviour (Walker *et al*., 2008). Additionally Walker *et al.* (2008) determined that lame cows had reduced oestrus intensity, lower progesterone concentrations during the 6 days prior to oestrus, and shortened periods whereby herd-mates mounted the lame cows. That is, the lame cow in oestrus cannot display in the conventional manner, and therefore the lame cow remains undetected. The aim(s) of this study were to: (1) provide insight to the oestrus detection methods used depending on the housing method employed; (2) to determine if the same oestrus detection methods are used for both lame and non-lame cows; (3) to determine reproductive performance for both lame and non-lame cows; and (4) to identify if farmers notice altered oestrus behaviour in lame cows.

Material and methods A survey was developed using an online website designed to host student surveys' at no cost. The survey was comprised of multiple choice (n=10) and open (n=15) questions. The survey consisted of questions related to: 1) general herd information (n=11); 2) reproductive management strategies for lame and non-lame cows (n=7); 3) lameness and fertility (n=7). A link to the survey was distributed from Sept 2012-Dec 2012 to dairy farmers internationally (e.g. Great Britain, Europe, Canada, The United States of America, Australia, New Zealand, Japan, African countries etc.), through electronic messaging systems, public forums, and other social networking websites. All responses remained anonymous. Statistical analysis was carried out using the Chi square test in Excel, to determine if there was a significant association in the number of inseminations required for lame and non-lame cattle. Abbreviations for the oestrus detection methods in (Figure 2.) are as follows; visual observation (Vis), tail paint (TP), chalk (CH), mount detector (Mount), pedometer (Ped.), radio telemetry (Radio.), and teaser animals (teaser).

Results The response rate was 11% (192 responses out of 1780). The countries that had the most respondents were: U.S.A., Australia, Great Britain and Canada. The most common housing method reported was free stall housing (n=109), followed by fully pastured (n=42), and tie stall (n=41). 89 respondents answered the question regarding the number of inseminations required for lame and non-lame cows. It was determined that lame cows required significantly more inseminations to conception than non-lame cows ($p<0.001$) (Figure 1). It was also determined that differing oestrus detection methods were employed depending on what housing method was used (Figure 2.). 142 respondents answered the question 'Are the same oestrus detection methods used for lame cows and non-lame cows?' Of these respondents, 132 of them use the same oestrus detection methods for both lame and non-lame cattle, and 112 of those respondents noticed altered behavioural changes associated with lameness (reduced oestrus expression). Therefore 10 of those respondents that noticed altered behaviour use differing oestrus detection methods for lame cows.

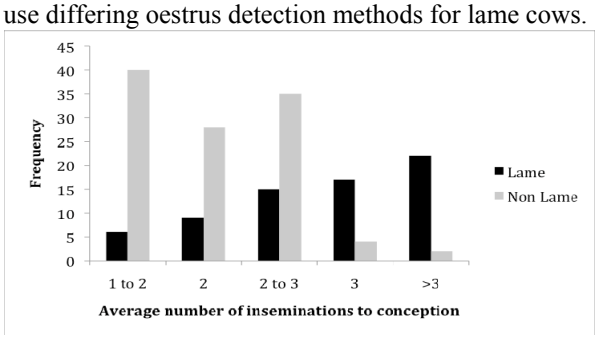

Figure 1 Frequency of number of inseminations to conception for lame and non-lame dairy cows

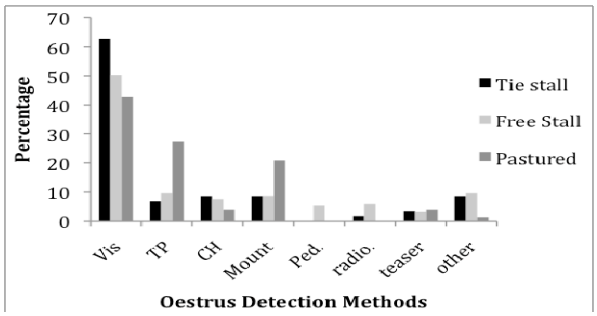

Figure 2 Different oestrus detection methods for three housing methods

Conclusions These results indicate that oestrus detection techniques are influenced by housing methods, and that lame cows require significantly more inseminations to conception. It was also determined that many farmers do notice altered behaviour in lame cows, however the same oestrus detection techniques are used for both lame and non-lame cows. As lame cows may alter their behaviour when in oestrus, it may be beneficial to design an oestrus detection protocol specifically for lame cows.

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Effect of anthropogenic activities on heavy metals, total oxidants, antioxidants and histology of liver of *Labeo rohita* from the River Indus

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Introduction Agricultural, industrial and domestic effluents containing various pollutants, such as heavy metals, pesticides and fertilizers are, invariably, discharged into small rivers and streams without their proper treatments. Such contaminants like heavy metals are very toxic for aquatic fauna. Therefore this study investigated the effects of heavy metals in water as pollutants on the metal profiles, total oxidants, antioxidants and histology of livers of *Labeo (L.) rohita*.

Material and methods This completely randomised study was carried out in Mianwali District by involving four sites around the River Indus which were exposed to domestic and municipal sewerage and agriculture runoffs. Site 1 was intense, Site 2 was medium and Site 3 was less polluted while Site 4 was used as the control as it was comparatively a clean site around the River Indus. Eighty samples of *L. rohita,* representing 20 samples per each site, of about 1.5 kg weight per fish were collected with the help of professional fishermen. The fish were humanely killed, washed, dissected to obtain livers for the estimation of their heavy metal, total oxidant and antioxidant status and histology. About 1-2 mm slices of fish liver were fixed in 10% formalin for 24 hours before their embedding in paraffin and sectioning with microtome (Microm HM310) into 5-6µm sections which were stained with haematoxylin and eosin. The stained sections were examined under a microscope and photographed by Moticam 1000 (Motic® China). One gram sample of liver was used for the estimation of total oxidants (TOS) of livers by using a novel automated measurement method (Erel, 2005) and the total antioxidants (TAS) were assessed by the automated colorimetric method. Remaining liver samples were freeze dried, ground, digested in nitric acid and analysed for heavy metals by ICP-OES. The numerical data were statistically analysed by using Minitab software 16 to observe the main effects of the sampling sites on the metals, total oxidants and antioxidants of fish liver at $P<0.05$. Tukey's test was used to compare the means for significant differences at $P<0.05$. The mean metal profiles were also compared with the permissible levels by the World Health Organisation (WHO, 1986). The histological micrographs were compared to illustrate apparent changes in the stained liver samples.

Results Table 1 shows significant differences in heavy metal profiles, total oxidants and total antioxidants of fish livers sampled from selected sites ($P<0.01$). The concentration of heavy metals at polluted sampling sites (S1, S2 and S3) exceeded the fish from control site (S4) and the WHO standards of food fish. The order of heavy metal concentration in fish liver was Zn>Pb>Cr>Ni>Cd. While fish liver sampled from the cleaner site showed normal histology (Fig. 1a), numerous degenerative changes were observed for livers of fish sampled from the polluted sites of the River Indus (Fig. 1b, c, d). There was no apparent difference in the histology of fish liver between the polluted sites (S1, S2, and S3).

Table 1 Mean (± SE) metal (mg/kg), total oxidant and antioxidant (µmol/L) contents and WHO standards (mg/kg) for fish

	Site 1	Site 2	Site 3	Site 4 (Control)	Maximum permissible levels (WHO, 1986)
Cd	0.18±0.01 ^{c}	0.28±0.01 ^{a}	0.26±0.02 ^{b}	0.02±0.005 ^{d}	0.05
Cr	6.18±0.21 ^{a}	4.77±0.20 ^{b}	3.69±0.12 ^{c}	0.04±0.01 ^{d}	0.05-0.15
Pb	11.96±1.21 ^{a}	7.93±0.24 ^{b}	5.84±0.12 ^{c}	0.67±0.02 ^{d}	2.0
Ni	0.57±0.34 ^{a}	0.90±0.23 ^{b}	0.39±0.12 ^{c}	0.30±0.005 ^{d}	0.5-1
Zn	59.63±2.5 ^{a}	44.85±1.67 ^{b}	40.35±1.50 ^{c}	8.72±0.22 ^{d}	40
Oxidants	3.29±0.44 ^{a}	2.44±0.27 ^{c}	2.74±0.05 ^{b}	0.13±0.04 ^{d}	-
Antioxidants	2.15±0.12 ^{a}	1.58±0.13 ^{c}	1.88±0.23 ^{b}	1.54±0.26^{c}	-

(Means with different superscripts in the same row differed significantly; $P<0.05$)

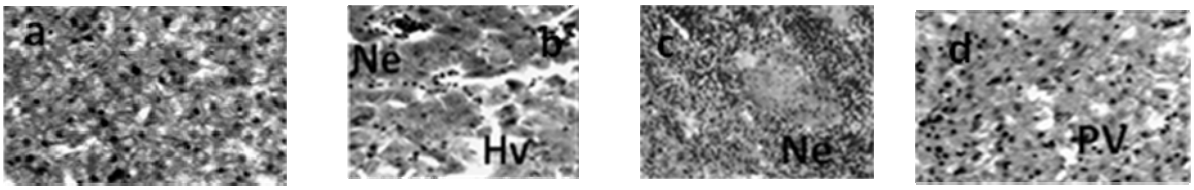

Figure 1 Light micrographs of fish liver inhabiting clear (a) and polluted water (b,c,d,) of the River Indus showing normal structure (a); necrosis (Ne) and highly vacuolated (Hv) hepatocytes (b); large area of necrosis (c) and heterogeneous parenchyma with different spectrum of poorly vacuolated hepatocytes (Pv) and pyknotic nuclei (d).

Conclusions High metal concentration in fish tissues of this study may cause metal related damage in these fish and their consumers. Pollutants were able to change the histology, oxidant and antioxidant status of fish liver even at low levels.

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020

Identification of tuna species from fresh and processed tissue using direct sequencing

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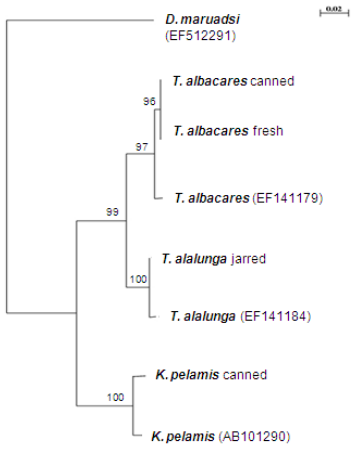
Introduction Tuna is of significant commercial importance in both the human and companion animal food industries. It is a key ingredient in manufactured pet foods and is of particular importance to cat food products, having long been viewed as a gold standard in terms of feeding performance. There are many fish species within the group classed as tuna, all of which belong to the family *Scombridae*. Some of these species are currently being fished in an unsustainable manner while pressure on other species is increasing (Majkowski, 2007). The ability to determine which species a sample of fresh or processed tuna meat belongs to is a quality control measure for pet food products. DNA sequencing is an effective method for species confirmation but processed tuna meat contains heavily fragmented DNA that can prove difficult to amplify. This work tested two DNA extraction procedures and three published primer sets for tuna identification. Amplification conditions were optimised for processed tuna enabling the identification of several species of tuna from both fresh and processed samples.

Material and methods Samples of fresh and processed tuna were acquired from local retail outlets. One fresh sample was obtained, labelled as Yellowfin tuna (*Thunnus albacares*). Three processed samples were collected on two separate occasions, one labelled as Skipjack chunks in brine (*Katsuwonus pelamis*), one labelled as Yellowfin steaks in olive oil and one labelled as Albacore steaks in olive oil (*Thunnus alalunga*). DNA extraction was attempted with the Qiagen DNeasy Blood and Tissue Kit (Qiagen 69504). Also a phenol/chloroform/isoamyl alcohol method was used. DNA concentration was measured with a Nanodrop 1000 spectrophotometer. For the PCR 3 sets of previously published primers were used. Set 1 (AD/AR, Terol *et al,* 2002) amplified a 171bp fragment while Set 2 and 3 (BD/BD1-R, BD2-D/BRmod, Espiñeira *et al*, 2009) produced smaller overlapping amplicons. PCR reaction conditions were as follows (final concentrations): 5X Phusion HF buffer (1X, Finnzymes), dNTPs (0.8mM, 0.2mM each, Applied Biosystems), forward primer (0.5µM, Eurofins MWG Operon), reverse primer (0.5µM, Eurofins MWG Operon), Phusion DNA polymerase (1Unit, Finnzymes), template DNA (10µl for processed samples, 1µl for fresh samples), nuclease free water (to final volume, Qiagen). Total reaction volume was 50µl. Optimal annealing temperatures were defined by running a temperature gradient for all primer pairs and selecting the best result. For primer Set 3 there was evidence of some non-specific fragment amplification. This was minimised by using the higher annealing temperature of 61°C. Cycling parameters were 30sec at 98°C followed by 40 cycles of 10sec at 98°C, 30sec at the annealing temperature (Set 1 50°C, Set 2 52°C, Set 3 61°C) and 1min at 72°C. The PCR product was purified using the Qiagen PCR Purification Kit (Qiagen 28104) prior to being sent for sequencing. The sequences were then aligned with reference sequences taken from Genbank. The resulting alignments were used to generate neighbour-joining trees. The reliability of the groups was assessed with a bootstrap test using 1000 iterations and a seed value of 111.



Results The Qiagen extraction method proved problematic for the processed tissue samples. Incomplete lysis of the tissue resulted in blocking of the extraction columns and poor DNA yield. The phenol/chloroform/isoamyl alcohol method showed better performance and had the advantage that the extraction could be scaled up easily to increase yield. The 171bp PCR showed inconsistent performance with the processed tuna samples. In the case of the Albacore sample this reaction did not work at all. The smaller fragments from primer Set 2 and 3 (147bp and 142bp respectively) worked more consistently and gave better yields of PCR product. Once sequences were checked and contiguous segments assembled the sequences were aligned using ClustalX and neighbour-joining trees were generated and viewed in NJplot (Figure 1).

Figure 1 Neighbour-joining tree of genetic relationships from the species studied carried out with 196bp sequences generated with two overlapping fragments using the primer sets 2 and 3. Reference sequences are from Genbank and accession numbers are shown in the parenthesis. *D. maruadsi* was included as an outgroup. Bootstrap values are represented as percentages on each branch. Branch length indicates divergence according to the scale given. All samples clustered correctly with their Genbank reference sequences.

Conclusions The primer sets 2 and 3 worked more consistently across species and gave higher yields of PCR product. This may be related to the shorter target amplicons and to the degenerate bases used in these primers. When combined these primers generate a 196bp amplicon (with primers removed) for use in phylogenetic analysis. This has been shown previously to be sufficient to reliably differentiate between the *Thunnus*, *Sarda*, *Auxis*, *Euthynnus*, *Decapterus* and *Scomberomorus* genera (Espiñeira *et al*, 2009). The methodology used here worked well and is a useful way of identifying the species of fresh and processed samples of tuna meat.

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021

The effects of flavoured rope additives on commercial pen-based oral fluid sampling in pigs

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Introduction Pigs are naturally attracted to chew absorbent cotton rope, thereby depositing mechanically stimulated oral fluids which can then be extracted and utilised for diagnostic purposes (Prickett and Zimmerman, 2010). Salivary flow can also be stimulated chemically, the most potent example of such a stimulant demonstrated in human research is citric acid (Streckfus and Bigler, 2002). The current study aimed to investigate salivary stimulation in the pig by measuring the effects of using fruit juice or sucrose solution dried onto cotton ropes on the volume of oral fluid that could be collected from commercial pens of weaned and finishing pigs.

Material and methods This study comprised a replicated Latin Square design, testing 4 flavour treatments across two replicates of 4 pens of pigs in each of two age groups; weaned pigs aged 8 weeks and finishing pigs aged 18 weeks. Commercial pens of cross-bred ((Large White x Landrace) x (Duroc x Pietrain)) pigs of mixed gender were used in the study. Weaned pig pens measured 3.42m x 1.84m (n=25 pigs/pen) and finishing pens measured 3.06m x 3.94m (n=17 pigs/pen). All pens were fully slatted, with a suspended plastic enrichment toy. Pure natural cotton rope of 18mm diameter (Outhwaites Ropemakers, Hawes, UK) was cut into 60cm lengths for finishers, and 80cm lengths for weaners. One of each length of rope was soaked for 60 minutes in apple juice, pineapple juice, 10% sucrose solution or water (control ropes with no flavour added) and then allowed to dry overnight. On each sample collection day, one of these ropes was presented, according to the Latin Square design, to each pen in the replicate for a 30 minute period between 08:00 and 11:00 AM. Following presentation, ropes were sealed into labelled plastic bags and the oral fluids extracted initially by hand, and then the residue by centrifugation for 10 minutes at 1500 x g. The volume of oral fluid extracted from each rope was measured using a pipette and recorded. Analysis of Variance (ANOVA) was performed using Minitab version 16. OF volumes, in millilitres, for weaners and finishers were selected as dependant variables, with rope treatment, pen number and trial day as fixed factors. A two sample t test using pen means from the 4 collection days was used to assess the effect of pig age on the volume of OF collected.

Results Around 20ml of fluid was obtained from each pen of weaned and finishing pigs upon each collection, with little variation. Of this, 13.5 ± 0.6 ml could be extracted by hand, with the residual 6.3 ± 0.4 ml requiring centrifugation. Rope flavour treatment had no significant effect on the volume of OF produced by weaner ($F_{(3,22)}$ =0.01, P=0.998) or finisher ($F_{(3,22)}$=0.53, P=0.668) pigs (Fig. 1). No significant effect of pig age on OF yield was found (T=0.1, P=0.925).

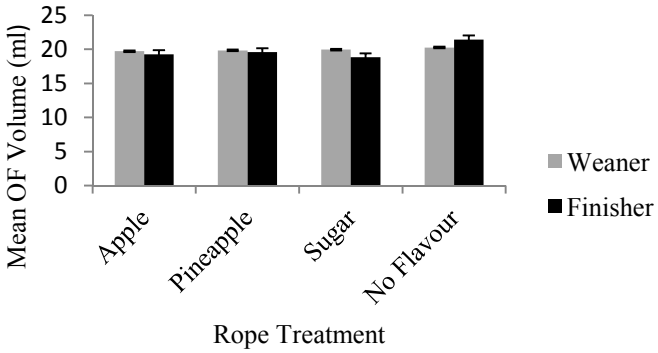

Figure 1 The mean total OF volume ± SEM collected from weaner and finisher pigs according to rope treatment.

Conclusions The addition of flavours to ropes for porcine OF sampling did not affect OF yield via greater attraction or salivary stimulation**.** The volume of oral fluid that can be easily extracted by hand from a pooled sample rope was sufficient in all cases for diagnostic testing, although the extent to which the composition of oral fluid may differ according to extraction method i.e. hand or centrifuge is not clear.

Acknowledgements We thank the staff at Cockle Park Pig Unit (Newcastle University) for their assistance and BPEX for funding this studentship.

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The impact of grazing by Irish Moiled and Dexter cattle on soft rush (*Juncus effusus*)

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Introduction Although a certain level of soft rush (*Juncus effusus*) cover can be beneficial to wildlife for breeding waders in certain habitat types, when not managed, dense cover can affect the agricultural quality of land and eligibility for agri-environment schemes (where 30% cover is recommended). Cutting, herbicide application, fertiliser application and grazing management have all been used to control rushes in pasture (Lazenby, 1956; McCarthy, 1971). Merchant (1995) found that cutting rushes to ground level twice during the growing season for at least two consecutive years was the best control option, and grazing goats (Merchant, 1996) gave control. There are few accounts of the potential of cattle, (Bullock *et al*, 1998) especially rare and traditional breeds to control rushes in pasture. This project reports a trial on the effects of grazing density on soft rush using two native Irish breeds.

Material and methods Seven plots (0.5ha each) were fenced on an area of Species Rich Wet Grassland with low forage quality and high soft rush infestation. The site, near Lough Neagh, N Ireland, is managed under the Northern Ireland Countryside Management Scheme. Cattle grazed plots 1 – 6 over varying periods at one of 3 stock densities (2 replicates each): - High - 6 Irish Moiled (IM) cattle; Medium - 4 Irish Moiled cattle; Low - 2 Dexter (D) cattle. The seventh plot was pre-mown before grazing by 6 Irish Moiled cattle. Grazing period was dependent on forage availability assessed weekly as sward height. Botanical composition (SR), sward structure, trampling and defoliation of rushes was assessed before and after grazing. Grazing treatments were allocated at random during the period July to October. Experimental stock comprised of cattle of varying ages including lactating and non-lactating cows, heifers and castrated males. Cows nursing calves were used but calves were not counted in the experiment.

Results Results from plots 1 – 6 were subjected to analysis of variance between treatments. After one season grazing, all grazing treatments reduced rush (R) heights (cm) and IM cattle reduced soft rush cover (%) to approximately 35%. There was no significant interactions between treatment and soft rush height or percentage (%) cover. Soft rush (SR) and grass (G) utilisation within plots followed a similar pattern, as % of grass grazed increased (i.e. forage depleted), so too did the % of soft rush grazed. At high grazing density rushes were trampled more than low and medium grazing density. Species richness was unaffected by grazing. Soft rush heights (plot 7) directly after cutting was 14cm ± 0.71, after 3 weeks regrowth was 46cm ±1.43, and after grazing 18cm ±1.10.

Table 1 Effect of grazing by IM and D cattle on a range of pasture and soft rush variables.

Treatment	G % before - G % after	R % before	R % after	R % after - R % before	Trampled R	SR % pre Grazing	SR post grazing	S.R after - S.R before
6 IM	-21.5	54.5	34.8	-22	47	8.8	0.5	-8.3
4 IM	-11	34	34.3	-2	20.5	15.3	4.3	-11
2 D	-4.5	34	36.8	2	4	19.8	14.7	-5.1
e.s.e	4.88	3.97	3.52	4.51	4.73	4.33	2.54	4.03
Sig	0.19	0.05	0.86	0.06	0.02	0.336	0.06	0.63
	NS	*	NS	NS	*	NS	NS	NS

Conclusions Native cattle can be used to significantly reduce soft rush infestations on a seasonal level, although the final rush density reached (35%) was just above the level acceptable for agri-environment scheme requirements. It is likely that grazing in subsequent seasons would reduce rush infestation even more. A combination of control methods such as the inclusion of cutting may achieve greater long-term success rates of rush eradication in pasture. Irish Moiled cattle consumed soft rush regrowth with no apparent ill effects though this requires further testing.

Acknowledgements The author wishes to gratefully thank the Rare Breeds Survival Trust for funding through the Grazing Animal Project and Craigavon Borough Council for facilitating the project; Dr Jim Mc Adam, Dr Melanie Flexen and Dr Sally Watson of Agri-Food and Biosciences Institute for their advice and assistance throughout the study.

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023

The performance of ruminant livestock and the quality of their meat produced under intensive and extensive rearing methods – a review

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Introduction With a loss of biodiversity within our rural landscapes since the beginning of the industrial revolution, there is a need for more sustainably managed livestock production, in order to stabilise what flora and fauna populations remain. There are already some nature conservation orientated grazing systems in place, but barriers to the expansion of such systems (i.e. low economic output) within our countryside are prevalent. The aim of the present study was to review existing knowledge concerning the differences in ruminant livestock performance and the quality of meat between extensive, semi-natural grazing systems, and the more intensive rearing methods. Within this overview, a similar evaluation of meat quality from rare/native livestock breeds, compared with improved varieties of livestock, under the comparative management schemes mentioned, was also undertaken**.**

Animal Performance and Carcass Quality When considered cumulatively, animal live weight gain is greater under the more intensive systems of rearing, especially since improved pastures have been shown to hold greater densities of livestock. Conversely, no clear pattern seems to emerge from the literature concerning individual live weight gains, and the reasons behind this are unclear. From the few studies reviewed, livestock fed concentrates tend to have greater carcass weights, confirmation scores and fatness levels than those on pasture. On the other hand, studies investigating such on improved and unimproved pastures depict less of a clear picture. No clear differences between rare/native breeds and improved/continental breeds seem to exist, except for carcass fatness, which has been found to be greater in the slower growing, early maturing rare/native breeds (Richardson *et al*., 2008)

Meat Quality The Polyunsaturated Fatty Acid (PUFA):Saturated Fatty Acid ratio was more favourable in meat produced under extensive rearing systems (Richardson *et al*., 2008; Fraser *et al*., 2009), and this could be attributed to the lower intramuscular fat content associated with such (Hoehne *et al*., 2012). Much of the scientific literature suggests livestock on a grazed grass/grass silage diet, will produce meat with a greater proportion of n-3 PUFA and a lower proportion of n-6 PUFA in the intramuscular fat (IMF), corresponding to a lower n-6PUFA:n-3PUFA ratio, in comparison to those on concentrate diets. On the more bio-diverse pastures however, the n-6PUFA:n-3PUFA ratio was found to be higher in the livestock meat, when compared with meat from improved pastures (Richardson *et al*., 2008; Fraser *et al*., 2009). Reasons behind the greater proportion of n-6 PUFA in the former have yet to be outlined. No clear pattern between extensive and intensive pastures exists for the conjugated linoleic acid. Furthermore, no key differences between breed type, or any breed x rearing method interactions were noted for any of these nutritional quality traits.
Some differences in tenderness, juiciness and flavour do exist between extensive and intensive pastures. Although these may not be consistent across the research, any sensory qualities highlighted for meat from extensive pastures could be used to promote local or regional products, as suggested by Ådøny *et al*. (2005). Differences also exist in the sensory qualities of meat between rare/native breeds and improved/continental breeds. No clear pattern exists in the research between intensive and extensive rearing methods for meat colour, or between rare/native breeds and improved/continental breeds. Indeed, there are many possible factors that influence such and until these are quantified in meat from unimproved systems, little understanding behind the different outcomes between studies will become available. The oxidative stability of meat fat and colour, from animals reared on extensive systems, is greater than intensive concentrate-based systems, due to the higher vitamin E content of forages (Warren *et al*., 2008). There may also be differences between intensive and extensive pastures, in favour of the latter (Fraser *et al*., 2009).

Conclusion It is clear that pasture grazing produces different results in animal performance and meat quality when compared to concentrate-fed livestock. Differences between improved and unimproved pastures have also been noted, but these are less clear as a primary consequence of the paucity of research covering animal performance and meat quality on/from extensive pastures. There is therefore a great need for more research. In an ideal world, such investigations would quantify the parameters discussed in this review under different extensive and intensive rearing methods, using different breeds on different vegetation communities, in different geographical locations, and in different years, as these were some of the factors found to possibly create unclear patterns between studies. It is appreciated however that such a task would be colossal, but as research progresses in this area, more defined patterns may emerge to provide more focus for future investigations.

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024

The impact of Beulah Speckled-face and Herdwick grazing on a chalk grassland site on Chipstead Downs

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Introduction Conservation grazing on chalk grassland requires an efficient and knowledge-based approach to maintain this fragile habitat which, if poorly managed, can take years to restore (English Nature, 2005). Scrub regrowth can transform chalk grassland into dense woodland and with the decline in commercial grazing, open chalk grassland has disappeared across much of the Surrey area. The Downlands Project is a conservation organisation which uses two breeds of sheep to restore and maintain chalk grassland: Herdwick and Beulah Speckled-face. This study compared the grazing behaviour of the two breeds at the Stagbury A site on Chipstead Downs, Surrey.

Material and methods Initially Stagbury A was split into three plots, one for each of the Herdwick and Beulah groups and a control site with no livestock present. Four sheep of each breed were introduced and the grazing preferences of the two breeds were recorded over five weeks to determine the short-term impact on the vegetation. Only four individuals were used to be proportional to the size of the enclosures, also three sheep are considered viable to study grazing behaviour (Penning *et al*. 1993). Eight individual scrub plants were chosen within each of the three areas, with at least one individual of each scrub species present within each area. Other scrub plants were randomly selected along a transect on the sites. The plots were separated using electric fencing which was erected the day before the sheep arrived. An initial vegetation data collection was taken the day before the sheep were introduced. Sward height measurements were taken at 20 random points along a transect using a drop-disc measure.. Photographs were taken of individual scrub plants once a week and rated on a nominal scale of grazing intensity. Additional observations were made to monitor any changes across the areas including trampling effect. Sward heights were analysed using a single factor ANOVA To determine whether there was any significant difference of the impact of grazing on sward height between the sheep breeds, a student t test was performed

Results From the ANOVA comparing the three treatments $F = 9.595$, $p < 0.001$ (2 d.f.)
Therefore the null hypothesis that the presence of grazing animals has no significant difference on sward height between the three sites measured was rejected.
The calculated t value (0.78, 10 d.f.) was not significant; therefore the null hypothesis that Herdwick and Beulah grazing does not produce significantly different sward height values was accepted.
The t-test showed no significant difference between breeds but the ANOVA demonstrated that the sward differences between plots was between the grazed and ungrazed sites.
Additional factors could be clearly seen to affect sward height. For example the trampling effect from sheep walking across the plot or resting. The measurement of scrub grazing shows that some species were grazed to a higher intensity than others, this also differed between the sites, with the Herdwick group grazing Ash more intensely whilst the Beulah had a preference for Elder. Both breeds were found to graze Hawthorn and Dogwood to a similar intensity. Whilst field maple was only present in the Beulah site, it remained largely ungrazed until the last few weeks of the data collection once other species such as Dogwood had been stripped of foliage. Other varying factors included the age of scrub growth affecting grazing intensity, with younger growth preferred by both breeds

Discussion No significant difference in the sward grazing of Beulah and Herdwick was found and therefore in setting up a grazing plan for chalk grassland, the use of either Beulah or Herdwick within a flock will not make a significant difference to the biomass of vegetation removed through grazing behaviour. This would allow for breed selection to be based on other factors e.g. economic and practical, including return from market sales and general breed temperament.
However, there was an observed difference in scrub grazing preferences between the two breeds along with observed results of grazing preference and other effects of the livestock presence. This could help with management plans to determine how hard to push certain breeds and the succession of which they will graze particular species of scrub. Further research would shed more light on this issue, as well as a greater understanding of dietary preferences between sheep breeds which may allow a more tailored grazing management based on the type of grassland and scrub species present and on the conservation objectives for the site.

Acknowledgements The author would like to thank GAP, RBST and the Downlands Project staff and volunteers for their support and funding as well as the guidance of Dr Richard Small.

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The influence of sire effects on methane emissions from finishing beef cattle in an experiment using divergent sire breeds and diets

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Introduction Methane arising from microbial digestion in the rumen of beef cattle is an important contributor to greenhouse gas emissions emitted from farm animals as indicated by a substantially larger carbon footprint per unit of meat from beef cattle in comparison to monogastric animals. To mitigate methane emissions, genetic selection would be highly cost-effective, because genetic improvement is permanent, cumulative and has the potential to be disseminated worldwide. However, there is lack of knowledge on whether the microbial methane production is influenced by the genetics of the host (animal). To obtain an insight into the potential influence of the host genetics on methane production by the ruminal microbes, an experiment was carried out using different breed types with structured sire groups within breed. The objective of the study was to analyse the differences between breeds and among sire groups within breed, as an indication for genetic effects. In addition, the interactions of breed (sire) effects and diets on methane emissions were estimated.

Material and methods The data was obtained from a 2x2 factorial experimental design of 72 steers from a two-breed rotational cross between Aberdeen Angus (AA) and Limousin (LIM) at the Beef Research Centre of Scotland's Rural College (SRUC). The same number of experimental animals was sired by purebred AA and LIM. Depending on the sire used, the expected additive genetic contributions were 2/3 and 1/3 of each of the two breeds. The progenies were from 5 AA and 4 LIM sires. The average number (range) of progenies per sire were 7 (2 to 12) and 9 (6 to 14) for AA and LIM, respectively. Two diets were used, a predominately concentrate-based and a mixed forage-concentrate-based diet with a forage:concentrate ratio (dry matter (DM) basis) of 8:92 and 48:52, respectively. Methane emissions were individually measured for 48h within 6 respiration chambers. The animals were allocated to the respiration chambers in a randomised block design with 3 replicates. Data on 4 animals could not be considered due to health issues and an air leak of the respiration chamber. Least Squares Means (LSM) were estimated using the GLM procedure of SAS based on a model including breed (or sire within breed) effects along with diet, respiration chamber and a randomised block effect.

Results The LSM for daily methane emissions were significantly different ($P<0.05$) at 184 g/d and 164 g/d for the breeds AA and LIM respectively, but not significant for methane emissions per kg DM intake (DMI). Estimates of LSM for daily methane emissions from each sire group showed significant differences ranging from 135 to 205 g/d (Figure 1). In contrast to the breed effects, there were also significant differences between LSM of sire group effects in methane emissions per kg DMI (Figure 2). Slightly different rankings of sires based on methane emissions per day in comparison to those based on per kg DMI are likely due to differences in feed intake among sire groups. There were non-significant ($P>0.05$) interactions between breed (or sire) and diet.

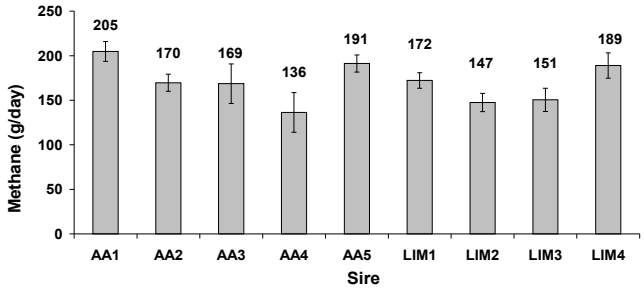

Figure 1 LSM of sire groups in methane emissions in g/day

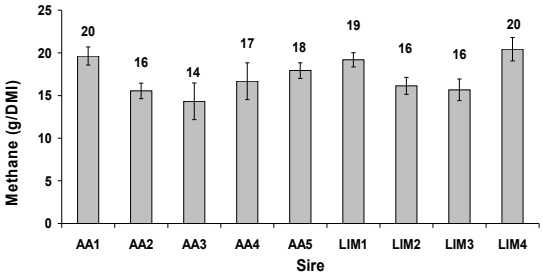

Figure 2 LSM of sire groups in methane emissions in g/ kg DMI

Conclusions The results indicate that the significant difference between breeds in daily methane emissions is due to higher feed intake of AA compared to LIM, as there were no significant differences between breeds for methane emissions per kg DMI. Substantially higher variation of LSM among sire groups were obtained than the significant differences between breeds, indicating that there is most likely a substantial genetic influence of the host on the rumen microbial production of methane. The variation of LSM among sire groups was still present, when methane emissions are expressed per kg DMI, which suggests that there is a direct influence of the host on the rumen microbial methane production independent of the amount of feed consumed. For the implementation of selction for methane mitigation within genetic improvement programmes it is important that the ranking of sires for methane emissions is not changed by interactions with diets.

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026

Methane emissions from finishing cattle fed either a forage-based or high concentrate diet

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Introduction Methane (CH_4) produced by fermentation of feed organic matter within the rumen is a major greenhouse gas from ruminant livestock production. Mitigation strategies to reduce CH_4 emissions may be based on manipulation of the rumen microflora, selection of animals that naturally emit less CH_4 or nutritional manipulation. One nutritional manipulation is to increase proportionately the amount of concentrates in the diet which reduces CH_4 production as metabolic hydrogen is diverted to propionic acid rather than CH_4. However, there is little information that allows comparison of mitigation strategies within production systems for finishing cattle. Here, a large-scale comparison of CH_4 emissions from forage-based or high concentrate (barley beef) beef finishing systems is reported.

Material and methods Aberdeen Angus x Limousin (AA x LIM) or Limousin x Aberdeen Angus (LIM x AA) cross-bred steers (n=36 per breed cross) were fed complete diets (g/kg, dry matter (DM) basis) consisting of either 480 forage: 520 concentrate (whole crop barley silage, 272; grass silage, 212; barley, 322; maize dark grains, 182; minerals and vitamins, 11) or 75 forage: 925 concentrate (barley, 689; maize dark grains, 204; barley straw, 75; molasses, 17; minerals and vitamins, 12) respectively. Diets were offered *ad libitum* to steers once daily. The experiment was therefore a 2 x 2 factorial arrangement of breed and diet. Prior to chamber measurements, feed intake and live-weight gain (LWG) had been measured for a minimum of 8 weeks. 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. Within the chambers (76 m^3), steers were loose-housed in 4 x 3 m pens. Samples of inlet air were also taken for measurement of ambient CH_4 concentration. CH_4 concentrations (10 per h for each chamber and ambient air) were measured by infrared absorption (MGA3000, ADC Ltd., Hoddesdon, UK). Dry air flow, corrected to standard temperature and pressure was calculated for each individual record of CH_4 concentration, Daily CH_4 production was calculated as the average of individual values and converted to a mass basis. Measurements were not made on one steer and data were rejected from three steers because an air leak rendered data unreliable. Data were analysed using Genstat using linear mixed models where the factors were the 2 x 2 arrangement of breed and diet, block, chamber and week of experiment. Data are reported as means and standard error of difference.

Results Cattle offered the forage-based diet consumed less feed (kg DM/day, Table 1) compared with the concentrate-based diet. Dry matter intakes were also significantly greater for AA x LIM steers than LIM x AA steers. Whether expressed on a g/day, g/ kg DM intake or kJ/MJ gross energy intake (GEI) basis, steers fed the concentrate based diet produced less CH_4 than the forage-fed steers (P<0.001). AA x LIM steers produced more CH_4 on a daily basis (P<0.05) but the effect disappeared when CH_4 production was expressed per kg DM intake or per MJ GEI.

Table 1 Dry matter intakes (DMI, kg/d) and CH_4 production by cattle fed either forage or concentrate based diets

Diet	Concentrate		Forage			Significance		
Breed	AA x Lim	Lim x AA	AA x Lim	Lim x AA	SED	Breed	Diet	Breed x Diet
DMI intake	11.4	10.0	10.2	8.7	0.52	***	**	NS
CH_4 (g/d)	152	135	216	194	12.6	*	***	NS
CH_4 (g/kg DMI)	13.5	13.6	21.3	22.3	1.22	NS	***	NS
CH_4 (kJ/MJ GEI)	39.0	39.0	61.7	64.7	3.5	NS	***	NS

GEI, gross energy intake. ***, P<0.001; **, P<0.01; *, P<0.05

Conclusions As expected there was a substantial reduction in CH_4 production from steers fed the high concentrate diet compared to the forage-based diet with a mean reduction of 8.2 g/kg DMI. There was also considerable animal to animal variation in responses with interquartile ranges of 4.8 and 5.6 g/kg DMI for the concentrate and forage-based diets respectively. Thus while increasing the concentrate proportion of the diet has substantial mitigation potential, exploiting animal to animal variation in methane production will also be important (Roehe *et al*. 2013).

Acknowledgments The authors are grateful for the funding from the Scottish Government (RESAS) and DEFRA and for excellent technical support at SRUC.

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027

The effect of dietary starch and lipid source on the performance, methane production and milk fatty acid profile of Holstein dairy cows

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Introduction Methane production by cattle represents a loss of dietary energy and is a source of concern due its impact on climate change (Moss *et al*. 2000). A Review study (Grainger and Beauchemin 2011) indicated that dietary starch and lipid supplementation are effective strategies to reduce ruminant methanogenesis. However, the effects of the interaction between starch and lipid source on ruminal methane production are unclear. The objective of the study was to investigate the effect of starch and lipid source on the performance, methane production, and milk fatty acid profile of dairy cows.

Material and methods Sixteen multiparous dairy cows that were approximately 100 d in lactation were used in 4 x 4 Latin square design with four dietary treatments and four 28 d periods. The dietary treatments were; maize based concentrate and a saturated fat source (Megalac, Volac UK Ltd.); wheat based concentrate and a saturated fat; maize based concentrate and sunflower oil; wheat based concentrate and sunflower oil. The concentrates were formulated to contain a starch content of 290 g/kg DM and were fed at the rate of 7.8 kg/ day/cow in 3 equal meals from out of parlour feeders. The oils were added to the total mixed ration (TMR) at 25 g oil /kg DM, and the TMR was offered once daily at 1.05 of *ad libitum* intake. Methane output was measured using the SF_6 tracer technique (Johnson *et al*., 1995). Milk production and feed intake was recorded daily during the final 5 d of each period, with milk samples being collected on four occasions and analysed for fat and protein. Additional samples were collected on the final day of each period for subsequent fatty acid analysis by GC. Data was analysed in Genstat version 14.1 (VSN International Ltd, Hemel, Hempstead, Hertfordshire, UK) with main effects of starch source (S), oil source (O) and their interaction (S x O) determined.

Results Dry matter intake was 2.6 kg/d higher ($P < 0.001$) in cows when offered diets containing Megalac compared to Sunflower oil (Table 1). Milk yield averaged 33.2 kg/d and was unaffected by treatment. In contrast, milk fat content was lower ($P < 0.05$) in cows when fed Sunflower oil, with the consequence that fat adjusted milk yield was 2.3 kg/d lower in cows receiving this treatment. Cows fed the maize starch based concentrate gained more body condition ($P < 0.05$) and produced 19 g/d less methane ($P < 0.05$) compared to animals receiving the wheat based concentrate. When methane output was expressed per kg DM intake, cows receiving Sunflower oil had a higher output (20.3g/kg DM intake) compared to those receiving Megalac (18 g/kg DM intake; $P < 0.001$). When expressed per unit of milk yield, cows receiving the wheat based concentrates produced a higher ($P < 0.05$) methane output (12.3g/kg milk yield) compared to those receiving the maize based concentrate (11.3g/kg milk yield). Compared to Megalac, Sunflower oil supplementation reduced the concentration of C16:0 in milk fat (26.8 vs. 30.7 g/100g for Sunflower and Megalac respectively) and increased the concentration of C18 fatty acids, in particular C18:0 C18:1 *cis*-9 ($P < 0.05$).

Table 1 Intake, performance and methane production of cows offered concentrates containing wheat or maize starch and supplemented with either a saturated fat source (Megalac) or Sunflower oil

Starch in concentrate	Wheat		Maize			Significance		
Oil	Megalac	Sunflower	Megalac	Sunflower	s.e.d.	Starch (S)	Oil (O)	S x O
Total DM intake, kg/d	21.3	18.4	20.8	18.6	0.559	0.670	<0.001	0.324
Milk yield, kg/d	32.6	32.9	33.6	33.6	0.761	0.111	0.793	0.760
Fat correct milk yield, kg/d	30.5	27.9	28.9	27.0	1.46	0.248	0.037	0.776
Milk fat, g/kg	37.4	34.3	35.1	32.3	0.151	0.051	0.008	0.881
Milk protein, g/kg	32.1	32.3	32.5	32.1	0.034	0.840	0.612	0.214
Body condition change	-0.08	-0.05	0.09	0.10	0.090	0.019	0.798	0.830
CH_4, g/d	386	375	362	362	11.3	0.027	0.485	0.456
CH_4, g/kg DM intake	18.3	20.7	17.7	19.8	0.69	0.127	<0.001	0.744
CH_4, g/kg milk yield	12.7	11.8	11.3	11.3	0.549	0.023	0.231	0.267
C16:0, g/100g	30.9	27.2	30.4	26.4	0.500	0.103	<0.001	0.696
C18:0, g/100g	10.4	11.0	10.0	11.4	0.245	0.903	<0.001	0.021
C18:1 *cis*-9, g/100g	24.8	26.0	25.6	26.1	0.536	0.196	0.026	0.379

Conclusions Maize compared to wheat based concentrates reduced methane output when expressed as g/day or g/kg milk yield. The inclusion of sunflower oil reduced DM intake and milk fat content and was ineffective in reducing methane output, but reduced C16:0 in milk fat.

Acknowledgement This work was supported by Mole Valley Feed Solutions, Somerset, UK

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028

Characteristics of mastitis and effect on carbon foot print of conventional and organic dairy production systems

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Introduction Achieving food security will be a major challenge; in a recent report FAO (2010) the average global carbon footprint was 2.4 kg of CO_2 eq. per kilogram of milk, of which 93% was placed on the farm. Overall, large variations between conventional and organic dairy production systems has shown no major systematic differences, with the lower milk yields in organic systems being compensated by greater system efficiency (Cederberg and Flysjö, 2004; Thomassen *et al*., 2008; Kristensen *et al*., 2011). Organic farming systems are thought to produce safe and healthy food with consideration to the animal welfare and environment (Hansson, Hamilton, Ekman, & Forslund, 2000) with minimal or no antibiotic use, however little controlled systems research has been completed over time in order to assess the effect that animal health and longevity has on the carbon foot print of milk production systems.

Material and methods The experiment was completed at the dairy cattle research unit of Massey University, Palmerston North, New Zealand, between August 2005 and May 2011. There were 46 organic and 51 conventional lactating Holstein Friesian and Friesian x Jersey cross bred dairy cattle to be managed on 41.7 ha of useable land, which was subdivided into matched farmlets of 25 organically and 25 conventionally managed paddocks (mean 0.83 ha, ranging from 0.08 to 1.53 ha). The dairy cattle grazed all year around, on pastures composing of a mixture of perennial ryegrass (*Lolium perenne)*, while clover (*Trifolium repens)* and other weed grasses species (poa annual), in a 15 to 25 d rotational grazing system managed in accordance with national (AgriQuality) and international (USDA) organic standards. Milk samples were collected from all quarters of all cows at 1, 7, 14, 130 and 260 days in milk and transferred directly into a cool box and then to the pathology laboratory at Massey University, which is (ISO) accredited for mastitis pathogen identification and this was completed according to standard procedures for each pathogen type. The data was found to be normally distributed, with the exception of geometric mean somatic cell counts, and analyzed using a pro GLM MIX procedure in SAS 9.2 with management (organic or conventional), number of lactations, number of days in milk, individual quarter sampled and the interaction between management and lactation (herd x lactation) were included in the model as fixed effects, while individual animal was included as a random effect to account for repeated measures, while differences between means were assessed using pairwise by least significant difference test, using individual errors and a confidence interval of 95%.

Results The conventionally managed animals had significatly higher milk yields and stocking rates compared with organically manged animals. While somatic cell count levels did not differ, organically manged cows had higher levels of *S. Uberis* in early lactation and more cows were culled and replaced due to mastitis compared with conventionally managed aniamls. Overall organically managed animals emitted significantly higher levels of CO_2-eq per kg of ECM, per ha and more land to be required per kg of ECM, compared with conventionally managed animals.

Table 1 Mean energy corrected milk (ECM) yield, somatic cell count and CO_2-eq emissions from organically and conventionally managed dairy cattle

	Organic	Conventional	SEM	Sig
ECM yield kg/hd/d	13.1	16.2	0.12	<0.001
Stocking rate (lactating cows/ha)	2.27	2.34	0.011	0. 049
Somatic cell count, 000 cells/ml	158	142	11.0	0.832
CO_2-eq kg / kg energy corrected yield	1.30	1.19	0.091	0.037
CO_2-eq t/ha	11.3	10.9	0.10	0.042
Land use m^2/kg of ECM	1.18	1.06	0.070	0.031

Conclusions Organic dairying had significantly higher CO_2-eq emissions, which were due to lower milk yield and stocking rates for lactating cows, along with increased levels of land required for rearing replacement livestock, which was mainly due to culling of relatively young cows due to *S. Uberis* related mastitis, especially in early lactation, as opposed to infertility or lameness.

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029

The influence of forage mixtures on methane emissions from growing dairy heifers

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Introduction The primary objective of commercial livestock farming is the production of edible animal products, but there is also increasing emphasis on reducing carbon emissions, including methane per unit of food produced. For UK farmers, this presents a challenge as high production costs, especially higher feed prices impact on profit margins. However, the strong global demand for meat and milk products presents opportunities for alternative farming practices, including the use of grass-based systems which potentially have the lowest feed input costs. The production of methane by ruminants is influenced by the type of carbohydrate available for fermentation and therefore has a direct relationship with forage type and quality. In addition, plant secondary compounds such as condensed tannins have also shown inhibitory effects on methanogenesis (Woodward *et al*., 2001). The objective of this study was to measure methane emissions from growing dairy heifers fed four diverse forage mixtures.

Material and methods Four blocks of land each measuring 1.1 ha were established with four different forage mixtures in September 2009 at the University of Reading Centre for Dairy Research. Conventional techniques for establishing the forages were followed and the four blocks consisted of perennial ryegrass (*Lolium perenne*; ryegrass) which acted as the control, ryegrass/red clover (*Trifolium pratense*), ryegrass/birdsfoot trefoil (*Lotus corniculatus*; trefoil), and ryegrass/wild flowers (predominant species sorrel, ox eye daisy, yarrow, knapweed and plantain). The forage mixtures were harvested as small bale wrapped haylage. Four Holstein-Friesian dairy heifers aged 24 months of initial live weight 339 kg were fed twice daily each of the four forage mixtures in a balanced 4x4 Latin Square design experiment with five-week periods. The forages were fed at a level to achieve a growth rate of 0.75 kg/d based on the estimated metabolisable energy concentration of the ryegrass haylage, including a 7.5% safety and activity allowance, and were adjusted weekly. Heifers were incrementally introduced to forage treatments over a four-day period. Thereafter, the first four weeks of each period were used for adaptation to forages followed by six days in open-circuit respiration chambers for measurement of dry matter intake (DMI), methane production, and energy and nitrogen balance as described by Cammell *et al.* (1986). Data were analysed using the Mixed Procedure of SAS® and a model tested fixed effects of forage type (3 df), and random effects of heifer (3 df) and period (3 df). When significant effects occurred treatment means were separated using Dunnett's comparisons.

Results There was an effect of forage treatment on methane emissions, as well as DMI and dry matter digestibility (DMD) (Table 1), however no effect on animal live weight. Methane yields, expressed as g CH_4/kg DMI, were lower for ryegrass/wild flowers compared to control. Forage treatment effects on methane yield appeared to be partly attributed to differences in DMD, which was lower (P = 0.008) for ryegrass/wild flowers and ryegrass/trefoil compared to the more digestible ryegrass control and ryegrass/red clover mixture. However, forage treatment rankings for methane yield as g CH_4/kg DMI and g CH_4/kg digestible DMI (DDMI) were not the same, suggesting that plant chemical composition, including secondary compounds, may be a factor.

Table 1 Dry matter intake, digestibility and methane emissions in growing dairy heifers fed four forage mixtures.

	Forage treatment					
	Ryegrass	Ryegrass/red clover	Ryegrass/trefoil	Ryegrass/wild flowers	s.e.m	P<
Live weight (kg)	401	402	397	389	12.81	0.273
DMI, kg/d	8.06	7.06[b]	7.55[d]	7.47[c]	0.254	0.039
DMD, g/kg	713	689	648[b]	585[a]	15.11	0.008
CH_4, g/d	230	200[b]	218	190[b]	8.980	0.025
CH_4, g/kg DMI	28.4	28.0	28.9	25.6[b]	0.693	0.026
CH_4, g/kg DDMI	39.1	42.5[d]	46.1[c]	41.6[d]	0.855	0.073

[a, b, c, d] denote means differ from ryegrass control (P<0.001, P<0.01, P<0.05, P<0.10, respectively)

Conclusions Growing dairy heifers fed a ryegrass/wild flower forage mixture emitted less methane per day and per unit of DMI compared to ryegrass on its own or as a mixture with red clover or trefoil, but methane per unit DDMI was lowest for ryegrass. Concentrations of plant secondary compounds may have contributed to the effects of wild flowers on methane emission, as well as the digestibility and fermentation of forage components in the rumen.

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030

Analysis of ruminal digesta *post mortem* by qPCR targeting archaeal and bacterial 16S rRNA genes enables the *post hoc* estimation of methane emissions from beef cattle

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Introduction Methane emissions from cattle form a large part of the environmental footprint from animal agriculture. Most of the methane emanates from ruminal fermentation, where methanogenic archaea convert H_2 and CO_2 to methane, which is then lost to the atmosphere by eructation and exhalation. Although it seems intuitive that the numbers of ruminal archaea in samples of ruminal digesta from live animals should be related to methane emissions from the animal, several studies have found little evidence of such a relation. The first objective of the present study was undertaken because it is often difficult to obtain samples of ruminal digesta from live animals, particularly for studies in the field. This aim was to compare archaeal numbers in digesta taken from live beef cattle and in digesta recovered at slaughter, in order to determine if *post-mortem* samples could be used instead of the more difficult live-animal samples. The second aim of the study was to investigate how total numbers of ruminal archaea in each type of sample varied with methane emissions from the same animals as measured in respiration chambers.

Material and methods Thirty-six Aberdeen Angus and 36 Limousin steers were housed at the Beef Research Centre of SRUC, Edinburgh. The steers received two diets, one mainly concentrate-based and the other a forage-concentrate-based diet, with forage:concentrate ratios (DM basis) of 8:92 and 48:52, respectively. Eighteen animals of each breed received each diet. Methane emissions were measured individually for 48 h in 6 respiration chambers. The animals were allocated to the chambers in a randomised block design with 3 replicates. Measurements from 4 animals were discarded due to health issues and an air leak from the respiration chamber. Samples of ruminal digesta were recovered both by stomach tube immediately after animals left the chambers or within two weeks at slaughter. Digesta were strained immediately, mixed with glycerol/buffer solution as cryoprotectant and stored at -20 °C. Samples were subsequently thawed and DNA was extracted by the RBB+C method. Bacterial 16S rRNA genes were analyzed by qPCR using primers UniF and UniR and a BioRad iQ5 analyser. Archaea were amplified using the universal archaeal primers Met630F and Met803R. The analysis of data was performed using the GLM procedure of SAS, fitting different models (e.g. including breed, diet), with the main emphasis to identify the regression of methane emissions on ruminal digesta from individual animals.

Results The ratio of archaea:bacteria (A:B) varied more than ten-fold in digesta taken from individual animals when in the respiration chamber or subsequently at slaughter. When the two types of sample were compared, a correlation of R^2 = 0.352 was found (Figure 1). The volumes of methane produced per kg DMI varied more than 4-fold, and a general correlation was found between methane emissions from individual animals and A:B ratio, with the correlation being better for the *post-mortem* samples (Figure 2). Although there seems to be a nonlinear increase between A:B ratios and methane emissions, further analysis showed that this nonlinearity is due mainly to differences in diet. Within diet, the regressions were linear.

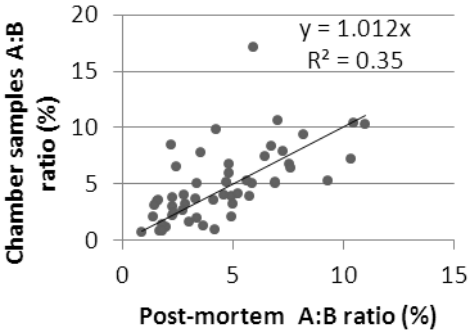

Figure 1 Comparison of the archaea:bacteria ratio (A:B) in ruminal digesta samples taken from live animals and at slaughter

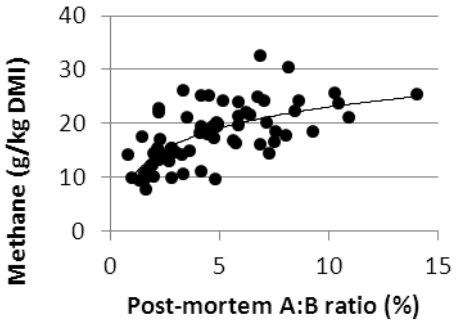

Figure 2 Relation between methane emissions of steers in respiration chambers and the A:B ratio in ruminal digesta at slaughter

Conclusions The archaea:bacteria ratio of ruminal digesta at slaughter was found to be a reasonable proxy for the ratio in samples taken previously from live animals. The ratio varied according to the methane emissions of individual animals, though the relation was variable between diets. This means that experimental procedures on live animals participating in methane investigations may become more mild, because digesta sampling may not be necessary, and that estimations of on-farm methane emissions may be made from *post-mortem* digesta samples. Further refinement of the microbial analysis will be required for more accurate *post hoc* estimation of methane emissions from farm animals, but the simple ratio of archaea:bacteria will be useful for many purposes.

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Prioritising measures to mitigate agricultural greenhouse gas emissions: A best-worst scaling survey of expert and farmer opinion in the sheep industry

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Introduction Rearing sheep produces the potent greenhouse gases (GHGs) methane and nitrous oxide. Multiple, well documented, opportunities exist for reducing and offsetting ruminant emissions on farm (Eckard *et al*., 2010). However, prioritising mitigation measures is problematic. Considerable uncertainties exist regarding the mitigation potential of many measures due to limitations in the scientific community's understanding of underlying biological processes, and temporal and geographical variation in mitigation potential across agricultural systems (Smith *et al*., 2007). In the absence of a complete systematic evidence base, this study aimed to assess the effectiveness and practicality of sheep farm GHG mitigation measures based on current expert and farmer opinion.

Material and methods The most effective mitigation measures (26) were shortlisted from the scientific literature by an expert panel. Best-Worst Scaling (a discrete choice survey technique) was used to elicit expert and farmer opinion on the shortlisted mitigation measures. Respondents were shown repeated sets of five different measures. Experts were asked to select the most and least effective for reducing emissions, and farmers the most and least practical to implement in each set. Mean effectiveness and mean practicality scores across the sample of respondents were estimated using a choice model. Experts in agricultural land management or livestock management with knowledge of GHG mitigation were drawn from academia, government and industry (n=55). Farmer practicality surveys were completed with an interviewer at agricultural shows across England and Wales (n=225), using a convenience sampling approach.

Results The estimated mean expert effectiveness and farmer practicality scores were zero-centred and plotted in an effectiveness and practicality 2 x 2 space (Figure 1). Measures in the upper right quadrant scored higher for both effectiveness and practicality whereas those located in the lower left-hand quadrant were lower scoring for both criteria. Practical and effective measures included three targeting flock productivity (increase lamb growth rates for earlier finishing (8), improve ewe nutrition in late gestation to increase lamb survival (14) and lamb as yearlings (19)); two relating to pasture management (include legumes in pasture reseed mixes (7) and select pasture plants bred to minimise dietary nitrogen losses e.g. high sugar grasses (26)); and one relating to fertiliser management (reduce mineral fertiliser use (16)).

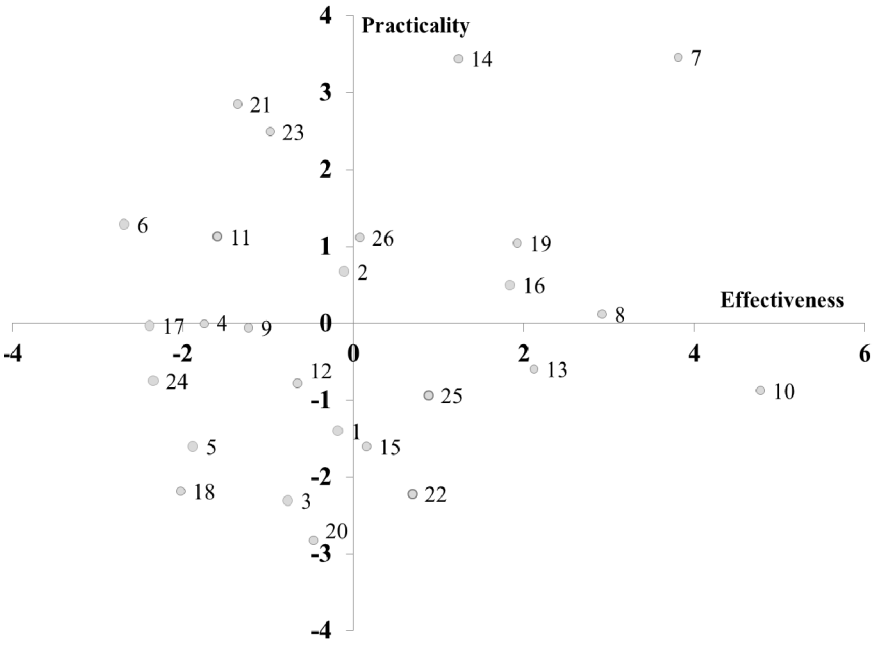

Figure 1 Zero-centred scatter plot of mean effectiveness and practicality scores for the 26 shortlisted mitigation measures.

Conclusions Effective and practical measures are priority candidates for inclusion in sheep farm GHG reduction strategies. Measures outside the upper right quadrant of the effectiveness-practicality plot pose interesting trade-offs and considerations for policy decision makers. Practical, ineffective measures may achieve wider adoption with limited regulation or support whilst less practical, effective measures may require greater regulation or support to ensure their adoption.

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Fatty acid profile and gene expression in canine lipoma – a promising treatment target?

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Introduction Canine lipomas are common benign cancers composed of lobules of well differentiated adipocytes with high incidence in older and obese dogs. Adipose tissue (AT) is a source of several bioactive peptides or "adipokines" and is regulated by several hormones including glucocorticoids (GCs), principally cortisol. GCs act primarily at the GC receptor (GR) influencing fat storage and metabolism(Mattsson and Olsson, 2007). Key enzymes involved in the regulation of fatty acids in AT include fatty acid synthase (FASN) which generates long chain fatty acids and Stearoyl-CoA desaturase-1 (SCD1) which regulates synthesis of unsaturated fatty acids.
Current recommended treatment for canine lipoma is surgical excision, which has an inherent risk to the animal and cost implications for the owner. Human research has described the use of intra-lesional administration of combined GC and local anaesthetic in the non-surgical treatment of lipoma, despite previous work suggesting an absence of the GR in lipomas(Chaudhuri *et al.*, 1985). Little is known about the molecular or fatty acid profile of canine lipoma, limiting future clinical research into this non-invasive treatment. The aim of this pilot project was to examine the fatty acid profiles and expression of key lipid metabolising genes and GR in canine AT and lipoma.

Material and methods Uncomplicated lipoma (n=7), infiltrative/fast growing lipoma (n=3) and subcutaneous AT (n=5) samples were collected from dogs undergoing routine surgery at local and affiliate veterinary practices following local ethical approval and full informed owner consent. Tissue samples were stored and transported in RNAlater prior to laboratory processing. Real time PCR with optimised canine primers was used to measure RNA expression of SCD1, GR, FAN and RPS5 (housekeeping gene). Lipid was extracted from tissues using the Folch method(Folch *et al.*, 1957), methylated and subjected to gas chromatography for fatty acid profiling. Statistical analyses were carried out using PASW Statistics (SPSS) (version 21.0).

Results SCD1 was significantly lower in AT and infiltrative lipoma samples compared to uncomplicated lipoma (Figure 1). Using real time PCR the presence of GR in canine lipoma was demonstrated, although there were no differences in expression between groups. Several fatty acids displayed significant differences in profiles between groups, see Figure 2 for details.

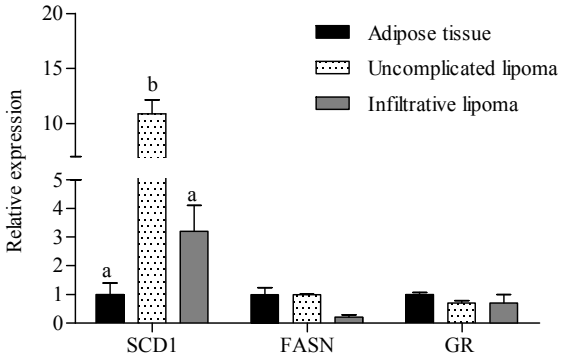

Figure 1 Gene expression in AT and lipoma samples. Different subscripts denote statistical significance (P<0.05).

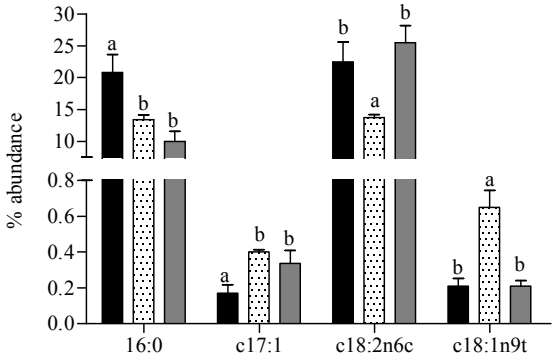

Figure 2 Percentage abundance of palmitic acid (c16:0), heptadecanoic acid (c17:1), linoleic acid (c18:2n6c) an elaidic acid (c18:1n9t) in AT and lipoma samples. Different subscripts denote statistical significance (P<0.05).

Conclusion We have demonstrated the presence of the GR in canine lipoma tissue, opening new research avenues for non-invasive lipoma treatments. Although only a pilot, the differences in SCD1 expression between lipoma groups suggest a reduction in unsaturated fatty acid production in the infiltrative lipomas. Not all of the fatty acid results are consistent with this, however, suggesting complex metabolic alterations.

Acknowledgements The authors wish to thank the School of Veterinary Medicine and Science for funding and the veterinary practices and owners who provided tissue samples.

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Cerebrospinal fluid clusterin is a potential biomarker of canine neurodegenerative disorders

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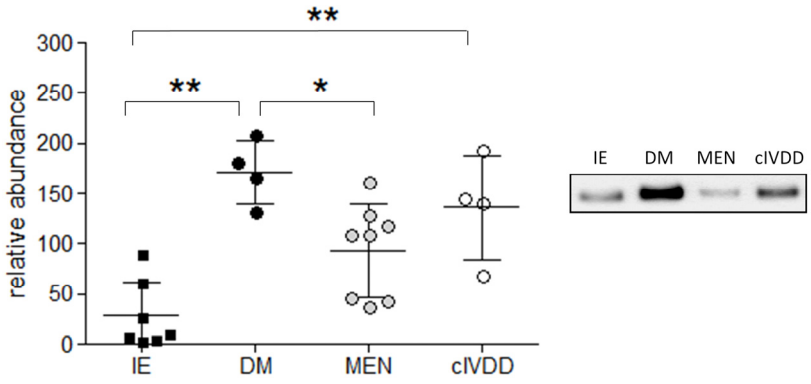
Introduction The diagnosis of canine neurodegenerative disorders relies on a combination of clinical evaluation, imaging modalities and, if available, genetic assessment and often requires neuropathological examination for confirmation. Despite the continual improvement in the range of diagnostic tests, confirmation of specific diagnosis for canine neurodegenerative disorders such as degenerative myelopathy (DM) can be elusive in the clinic, complicating decision-making and therapeutic management. A recent study has revealed a mutation in the canine superoxide dismutase 1 (*Sod*1) gene (Awano *et al*. 2009), implying DM is orthologous to a form of mutation in human familial amyotrophic lateral sclerosis (fALS). However, in DM some dogs homozygous for the *Sod*1 mutation do not develop DM and similar variation in disease penetrance is observed in fALS. Thus, though identification of the *Sod*1 mutation raises the index of suspicion it is not specifically diagnostic. The identification of potential biomarkers for DM would aid clinical diagnosis. The objective of this study was to explore the potential of canine cerebrospinal fluid (CSF) as a source of biomarkers for supporting clinical diagnosis of DM.

Material and methods Clinical material for this study was derived from cases undergoing routine neurological investigation at the University of Glasgow Small Animal Hospital, with ethical approval. All cases were diagnosed using routine techniques including haematology, biochemistry, CSF analysis and advanced imaging. Controls CSF were sourced from idiopathic epilepsy (IE, n=7), meningoencephalitis (MEN, n=8) and chronic intervertebral disk disease (cIVDD, n=4). Additionally, all cases were genotyped as to the presence of the *Sod*1 mutation that is associated with DM; using an in house protocol. For the DM group, only cases that were homozygous for the *Sod*1 mutant gene were selected. One-dimensional electrophoresis (1-DGE) was performed comparing protein profiles of IE (n=4) and DM CSF (n=4). A protein band that was more intense in the DM samples was excised and the protein(s) identified by MALDI-TOF MS. Validation studies of potential candidate(s) as DM (n=4) biomarker were performed using Western blotting to compare the protein levels in DM with other neurological conditions. The mRNA expression was also conducted using spinal cord material obtained from control (n=4) and DM (n=4) archive spinal cord tissue. CSF samples were not available for the archive cases that were used in the mRNA studies. Clusterin expression in spinal cord tissue in control (n=4) and DM (n=5) was also assessed by immunohistochemistry.



Results CSF protein profiling by 1-DGEs detected a protein band in DM CSF at ~38kDa that was more intense in DM CSF compared to IE. MALDI-TOF mass spectrometry analysis identified clusterin or apolipoprotein J as a major component of this band. A comparison across a range of neurological conditions found that clusterin was elevated in DM (n=4) and chronic intervertebral disk disease (cIVDD) compared to IE (DM vs. IE, $p<0.001$; cIVDD vs. IE, $p<0.05$) and meningitis (DM vs. meningitis, $p<0.01$; cIVDD vs. meningoencephalitis, $p>0.05$). There was no significant difference in CSF clusterin between DM and cIVDD. Clusterin mRNA level in the thoracic spinal cord of DM and control cases (dogs unaffected by neurological diseases) was compared by RT-PCR. The mean mRNA level was elevated by 42% in DM (n=4) compared to control cases (n=4) using cyclophilin as a house keeping control mRNA. This difference bordered on statistical significance ($p=0.05$). Immunostaining of clusterin demonstrated that this protein is strongly expressed in both control and DM-affected group.

Conclusions We have established that canine CSF is a valuable source of biomarkers for canine neurodegenerative disorders. mRNA levels in DM indicates that the elevation of clusterin in CSF may originate from CNS parenchyma suggesting clusterin is a potential biomarker for canine neurodegenerative conditions, indicative of neuronal dysfunction and death. Clusterin may therefore represent one component in a panel of emerging biomarkers that may combine to distinguish specific neurodegenerative disorders such as DM in the clinic.

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The effect of a dietary addition of short and medium chain fatty acids on sow production parameters

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Introduction The modern sow is highly prolific, producing large litters which are lean and grow rapidly (Rosero *et al.,* 2012). However this can have a negative impact on the production performance of individual pigs. Short and medium chain fatty acids have important roles in promoting and regulating pig gut health (Dierick *et al.,* 2002); improved gut health elevates production performance. The aim of this study was to investigate the effect of a dietary addition of short and medium chain fatty acids to dry and lactation sow diets on sow production parameters, including piglet performance, numbers born and milk quality.

Material and methods Forty multiparous Large White × Landrace sows on a commercial unit (600 sow unit, birth to bacon) were offered one of two treatments; Treatment 1: Control - Standard dry sow and lactation sow rations; Treatment 2: Addition of fatty acids- Standard dry sow ration with 0.5kg/T of fatty acid mixture and Lactation sow ration with 1.0kg/T fatty acid mixture. Twenty sows were allocated to each treatment, sows were fed the dry sow ration from service to the end of the dry period and the lactation ration was fed throughout the lactation period. The mix comprised of short and medium chain fatty acids (C_4, C_6, C_8, C_{10}, C_{12} and C_{16}), encapsulated salt of volatile and essential fatty acids. Colostrum samples were collected at farrowing and milk samples were collected on day 7 post farrowing. Sow parameters including number of piglets born, piglet mortality and individual piglet birth weights were recorded. From each litter, four piglets nearest the average weight of the litter (two male and two female) were selected as focal animals and tagged. Focal piglets were weighed at fortnightly intervals until 8 weeks of age. Focal piglets were also blood sampled on 5 occasions at fortnightly intervals from 2 weeks of age. Sow parameters (numbers born, born alive, born dead and individual piglet birth weight), colostrum and milk parameters (protein, lactose, fat) and Immunoglobulin levels in colostrum and milk also analysed using a one-way ANOVA. Individual sows represent an experimental unit all variation in ANOVA is expressed as the standard error of the mean (S.E.M.). Sera immunoglobulin levels were also analysed using REML fitting a covariance structure over time to take account of the repeated measures (sample 1, 2, 3, 4, 5). All data were analysed using GenStat release 14.2 (2011, VSN International Ltd).

Results Sow parameters revealed no treatment effects ($P>0.05$). There was no significant treatment difference between focal piglet weights at week 0, however focal piglets from the Treatment 2 group were significantly heavier at 2, 4, 6, and 8 weeks of age (Table 1). Treatment 2 sows showed significantly increased levels of lactose ($P<0.001$) and fat ($P<0.05$) in their colostrum and lactose levels ($P<0.05$) were elevated in treatment 2 sow milk. In treatment 2 sow colostrum there was an increase in oleic acid (C18:1c9) ($P<0.001$) and a decrease in Palmitic acid (C16:0) ($P<0.001$). The immunoglobulin profile in colostrum and milk was altered; IgG was proportional greater than IgM in both treatment colostrum ($P< 0.05$) and milk ($P< 0.05$) whereas control sows demonstrated proportionately higher levels of IgM compared to IgG in both colostrum and milk. There were no significant interactions between treatments over the five bleeds on piglet sera immunoglobulin levels ($P> 0.05$).

Table 1 The influence of the addition of a short and medium chain fatty acid mixture on focal piglet weights

Age (weeks)	Treatment 1 weight (kg)	Treatment 2 weight (kg)	SEM	$F_{(1,79)}$	P-value
0	1.58	1.54	0.036	0.31	>0.05
2	4.65	5.54	0.110	32.04	<0.001
4	8.87	9.50	0.168	7.05	<0.005
6	11.34	12.87	0.023	21.77	<0.001
8	18.36	19.84	0.391	6.95	<0.05

Conclusions The addition of a mixture of fatty acids to sow dry and lactation diets improved sow milk quality through increased levels of fat, lactose and IgG in colostrum. This provided the newborn piglets with an improved energy intake and passive immunity which may explain the increased piglet weights as such changes should promote piglet vitality and growth (Quesnel *et al.,* 2012).

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High throughput sequencing of 83 candidate genes of the GH-IGF1 axis in DNA pools from dairy cattle divergent for somatic cell count

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Introduction The GH-IGF1 axis, also known as the somatotrophic axis, is central to post natal growth and development in mammals and influences quantitative traits in livestock species including milk production, size, body composition, fertility and the immune response (Lucy 2008). Studies have reported associations between DNA variants within, and QTLs overlapping, chromosomal regions harbouring the genes constituting this axis and performance in cattle (Mullen *et al.* 2010; 2011; Waters *et al.* 2012). However, few causal mutations affecting quantitative traits have been identified in cattle and livestock in general, largely due to the polygenic nature of quantitative traits requiring dense genome wide marker or sequence analysis on large populations of animals with accurate phenotypic data to identify and accurately estimate small effects especially on lowly heritable traits. Even with the rapid developments in genomics, sequencing of large numbers of individual genomes remains prohibitively expensive. Consequently, the objective of the current study was, using a target enrichment, DNA pooling and high-throughput sequencing approach, to characterise the genetic variation and estimated allele frequencies in DNA pools of cattle divergent for somatic cell count.

Material and methods DNA samples for 150 artificial insemination Holstein-Friesian (HF) bulls were divided into two groups divergent for genetic merit for somatic cell count (SCC) while also simultaneously minimising the co-ancestry within each group. For both sample groups, DNA was pooled using equimolar DNA quantities from each individual animal. The Agilent SureSelect Target Enrichment System was used to selectively capture whole gene and regulatory DNA sequences for 83 somatotrophic axis genes, followed by sequencing using the Illumina GAII platform. All DNA sequence data were aligned using the UMD 3.1 reference genome and the BWA aligner software package. DNA sequence polymorphisms were identified using the SAMtools package. A minimum of four non reference allele reads was required to identify polymorphisms across both groups. A two tailed Fishers exact test was used to compare allele frequencies between the high and low SCC DNA pools. Nominal P-values were adjusted for multiple testing using a FDR cut off of $P<0.01$.

Results In total, ~ 4 million reads spanning ~ 2 Megabases (Mb) of sequence data with, on average, ~ 200-fold coverage per base was obtained. A total of 4,278 SNPs (n=1,288 putatively novel) spanning the 83 genes were identified. Thirty-six percent (n=1,532) of SNPs identified were located within putative regulatory regions in the 5' and 3' UTR. Fifty-eight percent (n=2,475) were intronic and the remaining 6% (n=271) were exonic, of which approximately half (n=124) were non-synonymous (NS) substitutions. In total, 376 SNPs showed a significant (adj. $P<0.01$) allele frequency differential between the low and high SCC cattle groups. Table 1 shows eight examples of this SNP category.

Table 1 Eight SNPs in Holstein-Friesian cattle divergent for somatic cell count (the frequency of the second allele is shown for each SNP)

Entrez Gene ID	Chr.	Location within gene	SNP	High SCC freq	Low SCC freq
IGF1	5	Intron	G / T	0.58	0.00
GHR	20	Intergenic	A / G	0.32	0.00
GH1	19	Intergenic	G / T	0.00	0.21
SIRT6	7	Exon (NS)	G / C	0.49	0.25
IRS1	2	Exon (NS)	T / G	0.00	0.79
GH1	19	Exon (NS)	C / T	0.00	0.41
GHR	20	Intronic	A / C	0.14	0.42
STAT5B	19	Intronic	A / C	0.79	0.26

We previously identified independent associations between somatic cell score and variants in *GHR*, *GH1* and *IGF1* (Waters *et al.* 2012), however, these studies used sequence analysis of only small regions of each gene.
In contrast, polymorphisms reported herein were identified from sequencing entire genes and regulatory regions. The accuracy of allele frequency estimates generated using the DNA pooling approach has recently been demonstrated (Mullen *et al.* 2012) and it is plausible a subset of these polymorphisms underlie heritable variation in resistance to udder infection.

Conclusions This study represents an initial step in the identification of candidate causal polymorphisms and followed by genotyping across large panels of cattle with accurate SCC phenotypes, association analysis and functional biology has the potential to reveal novel causal polymorphisms affecting udder health in cattle.

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Harvest yields from six biomass willow varieties grown for three years and irrigated with farmyard dirty water over two growing seasons

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Introduction Farmyard dirty water (FDW) on Northern Ireland farms is a problematic effluent due to its nutrient and relatively high biochemical oxygen demand (BOD) contents, all factors that potentially carry a high pollution threat to surface waters (Foy *et al.* 2008). This has environmental and financial issues for dairy farms where there is a statuary requirement to have storage capacity if ground and weather conditions prevent land spreading, the common method of disposal of FDW. Short rotation coppice (SRC) willows for energy crops have relatively high transpiration rates and may also uptake nutrients, reducing drainage and nutrient leaching from soil, thus ameliorating polluting effluents, though effects can be variety specific (Weih and Nordh, 2002). The aim of the study was to investigate the effects of different rates of FDW irrigation on yields of selected willow varieties and water quality.

Material and methods Six varieties of willows, Beagle, Endeavour, Olaf, Sven, Terra Nova and Tora, all developed for biomass energy cropping, were selected for planting on a 5.0 ha trial site at AFBI Hillsborough during 2007. In a completely randomised factorial design of 4 irrigation treatments x 2 replications, each treatment area (57m x 90m) contained mono-plots of each variety planted in three double (standard spacing) rows x 90m long and a mixture plot of 6 double rows x 90m with all varieties randomly planted. Irrigation period was 1st April to 30th October in 2010 and 2011. Treatments (*T*) irrigation rates, based on FDW nitrogen (N) content, were; *T4* 250 kg N /ha, *T3* 170 kg/ha , *T2* 90 kg /ha and *T1* (control) nil/ha, with volume (m^3) equivalents of 22, 15 and 8m^3/d applied, set to potential evapo-transpiration of 4.4mm/day. FDW contained dairy parlour washings, urine and faeces from walkways and yards. FDW components (Table 1), were analysed by standard laboratory methods, BOD by 5-day Oxi-top method.

Table 1 Components (3yr means) of FDW applied in treatments (standard deviations (±) are shown in parentheses)

pH (mg/1)	Conductivity (mS cm-1)	BOD_5 (mg/l)	N (mg/l)	P (mg/l)	K (mg/l)
5.93 (± 0.7)	4.39 (± 1.67)	2700 (± 452)	177.5 (± 79.2)	55.94 (± 23.8)	541.67 (± 288.4)

Surveys of post-planting survival were made in spring 2009 after the first year growth cut-back in February. Irrigation system was controlled by a dedicated program (Laqua; model:LWIS) and volumes recorded by an in-line flow meter (Magwa; model: MJK11). Local drainage and ground waters were grab sampled (n = 14) fortnightly for standard laboratory analysis of the parameters in Table 1. The site was harvested in March 2012 after 3 years growth with a CLAAS self-propelled chip harvester in the standard commercial method. Whole plots were harvested individually, the chip blown into pre-tared 12m^3 capacity silage trailers drawn by 100hp tractors to a certified weighbridge for gross fresh weight (Fwt) yield before unloading. Chips were grab sampled (min 1.0 kg x 5 per load) oven dried at 80º C x 48hrs for dry matter (DM) determination. Yield estimations (DM t/ha) were calculated from: ((plot yield (kg) * % DM) ÷ (plot area (m^2)) x 10000 (m^2)) ÷ 1,000. Results were examined with analysis of variance (ANOVA) for significance at the 0.05 probability level using Genstat 8.

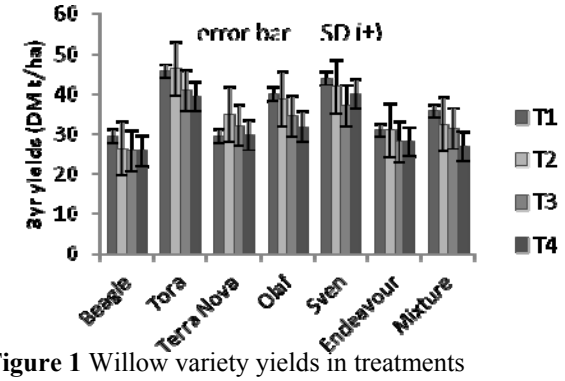

Figure 1 Willow variety yields in treatments

Table 2 Irrigation site drainage water component concentrations (± SD)

pH (mg/1)	Cond'y (mS cm-1)	BOD_5 (mg/l)	N (mg/l)	P (mg/l)	K (mg/l)
7.86 (0.3)	401 (96.8)	4.62 (3.3)	2.22 (1.7)	0.09(0.06)	5.17(1.4)

Results Post planting survival of all species was relatively even, averaging 83.9 % ± 6.3 (SD). Terra Nova had the lowest and a significantly different ($P<0.05$) DM content. Yields were significantly different ($P<0.05$) between treatments and varieties but no interaction of treatment x variety was observed. Yields (DM) in the highest FDW rate (*T4*) were significantly lower ($P<0.05$) than in the other treatments (Figure 1). Tora produced the highest yields 42.9 t/ha ± 3.46 (SD), Beagle the lowest at 26.86 t/ha ± 1.68 (SD). Mixed plot yields were inter-mediate at 31.51 t/ha ± 3.6 (SD). FDW application varied during the two years, restricted by rainfall and soil saturation. Total FDW (m^3) for 2010 and 2011 were *T2,*1088.8, *T3,* 2056.6, *T4,* 2661.5 m^3, providing kg/ha equivalent 85.50, 181.69 and 235.12 kg N; 26.94, 57.26 and 74.10 kg P and 260.92, 544.45 and 717.52 kg K per treatment respectively. Results from drainage and ground water samples showed that concentrations of nutrients and BOD_5 (Table 2) were not significantly different (NS) from pre- FDW application water but were substantially lower (>95%) compared to the applied FDW.

Conclusions Results indicated that FDW applied at the highest rate may have had a suppressing effect on yields. Ground and surface water results did not show higher than normal levels of nutrients and BOD which suggested that a bioremediation effect did pertain.

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Characteristics of recent new entrant dairy farmers to the Irish dairy industry

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Introduction In advance of EU milk quota abolition in 2015, the Irish government has decided to allocate ¼ of the annual 1% increase in milk quota between 2009 and 2015 on a permanent basis to new entrants to dairying. Two hundred and thirty new entrants have successfully received 200,000 litres of milk quota in the initial 3 years of the scheme. The objective of this study was to describe the characteristics of new entrant dairy farmers, and their farm businesses developing within the Irish dairy industry in terms of geographical distribution, planned production system characteristics, intended operational scale and expected profitability based on an analysis of successful applications to the Irish New Entrant Dairy Scheme over a three year period (DAFF, 2009).

Material and methods A total of 230 applications and business plans of entrants who received up to 200,000 litres of milk quota through the New Entrant Scheme from 2009-2011, were divided into 50 key variables and analysed to determine the regional, age, experience, educational and off-farm income effects on overall business plan expectations. The collated information was analysed using chi-square (PROC FREQ) and generalized linear model (PROC GLM) procedures (SAS, 2006). Address data of the entrants was geocoded to enable GIS mapping of the geographical distribution of new entrants.

Results and discussion The results of this study indicate that a young and highly educated group of new farmers (Table 1) are using the New Entrant Scheme to enter the Irish dairy industry with the majority converting from beef and mixed enterprise farms. Ninety-three percent of new dairy entrants have at least two years of formal 3rd level agricultural education and intend to become relatively large scale and efficient milk producer's post EU milk quota abolition.

Table 1 Characteristics, intentions and expectations of new entrants to the Irish dairy industry (2009-2011)

	Average	s.e
Age	36	
Total Land (ha)	58.08	
Cow numbers	70.2	1.55
Stocking Rate (LU/ha)	1.74	0.039
Milk solids/ha (kg MS/ha)	655	17.6
SFP received (€)	18,576	1128.4
Capital Borrowed (€)	88,165	5651.3
Total Expenditure (€)	188,681	8417.1
Expected profit per litre (€)	0.05	0.007
Exp. profit per hectare (€)	422	50.7

There was no significant effect of region, dairy experience or education on the expectations and intentions of new entrants. In contrast, applicant age has a significant impact on the equity available to invest in the dairy farm and expectations, as younger entrants have less owned resources, are increasingly reliant on additional borrowing and have significantly increased expectations for the productive capacity of their potential farm businesses when compared to older entrants. With 81% of new entrants to dairying located in the south of Ireland, quota abolition is likely to result in a further concentration of milk production in the south of Ireland.

Conclusion Applicant age and other income have a significant impact on business plans and expectations of new dairy farms. The results provide a further indication that quota abolition is likely to result in an increased intensity of milk production focused in traditionally intensive milk producing areas in the south of Ireland.

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Influence of three protein sources (soyabean meal, groundnut meal and blood meal) with or without fishmeal, on performance of growing greater cane rats (grasscutters)

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Introduction Backyard greater cane rats (*Thryonomys swinderianus*) rearing provides income for smallholder farmers and upgrades the diet of poor rural and urban households. Cane rat rearing fits well into household production as investment and labour costs are low. Being herbivores they do not compete with humans for their food. The economic potential of their meat is high within the West Africa sub-region as it is preferred to that of every other kind of domestic livestock or commercially available game. It has an excellent taste and high nutritional value (low fat, low cholesterol, rich in protein, vitamins and minerals) (FAO, 2001). The aim of the study was to identify protein sources that would improve performance as well as cut down on production cost.

Material and methods A total of 54 greater cane rats of nine weeks of age with a mean body weight of 3570±2.1 g and treated for both internal and external parasites were used in a completely randomized 3x2 factorial design feeding trial over an eight week period. The factors were 3 protein sources (soya bean meal, SBM, groundnut meal, GNC and blood meal, BM) and 2 levels of fishmeal (0 and 1%). There were 6 dietary treatments and 3 replicates per treatment with 3 animals each. The treatments were designated T_1, T_2, T_3; for SBM, GNC and BM with 0% fishmeal and T_4, T_5, T_6; for SBM, GNC and BM with 1% fishmeal, respectively. The cane rats were allowed one-week adaptation to both the feed and cage. The body weight of each animal was taken prior to the commencement of the experiment and at the start and end of each sampling period 12 hours after withdrawal of feed. Nutrient digestibility and nitrogen balance were determined by collection of faeces and urine from days 15 – 21. Total daily collection was done for the faecal matter and the urine. About 10 % of the total faecal matter and the urine were retained daily. The urine was stored in a refrigerator (-20 °C) and the faecal matter dried at 60 °C in a hot air oven for 24 hours and stored until required. The data obtained from the intake and digestibility measurements were subjected to analysis of variance (ANOVA) using Genstat (2008) version 8 for windows 7 and differences between means were detected using the Least Significance Differences (LSD) test.

Results The results of the study are shown in Table 1. Feed intake was numerically higher for the 1% supplemented fishmeal diets T_4, T_5 and T_6 as compared to those without fishmeal; T_1, T_2 and T_3. Intake was significantly ($P<0.05$) higher for the blood meal diets T_3 and T_6 than the soya bean meal diets, T_1 and T_4. T_2 and T_4. The average daily gain was significantly ($P<0.05$) higher for T_3, blood meal without fishmeal compared to T_2 and T_5 and similar with T_4 and T_6. The mean daily weight gain for T_4 and T_6 were not significantly ($P>0.05$) different from each other, however T_2 and T_5 were similar and significantly ($P<0.05$) lower. The FCR was significantly ($P<0.05$) higher for groundnut meal than soya bean meal. Feed cost was marginally lowest for T_3, blood meal without fishmeal. Retained nitrogen was similar and significantly ($P<0.05$) higher for T_2 and T_5 than T_1 and T_4. Nitrogen digestibility was significantly ($P<0.05$) higher for T_1 and T_4 than T_2, T_3, T_5 and T_6 which were all similar to one another.

Table 1 Effects of 3-protein sources with or without fish meal on growth performance of cane rats (grasscutters)

	0% FISHMEAL			1% FISHMEAL			
PARAMETERS	T_1	T_2	T_3	T_4	T_5	T_6	SEM
Mean Daily Feed Intake (g)	22.0^{c}	23.9^{bc}	28.4^{ab}	22.3^{c}	27.9^{ab}	29.1^{a}	4.56
Mean Daily Weight gain (g)	11.6^{ab}	8.9^{b}	13.0^{a}	12.4^{ab}	9.5^{b}	12.7^{ab}	2.24
FCR (feed/gain)	1.9^{de}	2.7^{ab}	2.2^{cd}	1.8^{e}	2.9^{a}	2.3^{bc}	0.40
Feed Cost/kg (GH ¢)	0.41	0.40	0.39	0.43	0.41	0.41	-
Nitrogen Digestibility (%)	95.7^{a}	76.5^{b}	79.0^{b}	91.8^{a}	77.4^{b}	79.0^{b}	6.12
Nitrogen Balance (g N/kg))	3.8^{d}	7.2^{a}	6.3^{bc}	6.0^{c}	7.1^{ab}	5.7^{c}	0.9

SEM- Standard Error of Means: $^{a, b, c, d}$means within rows with different superscripts differ significantly ($p<0.05$).

Conclusions From the study, the inclusion of soya bean meal and blood meal in the diet for growing captive cane rats with or without 1 % fishmeal could improve performance over than when fed groundnut meal. Feeding blood meal in the diet without fish meal was cheapest GH¢ 0.39 (US$ 0.195)/kg of diet.

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Evaluation of six feed resources developed from agro-industrial by products

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Introduction According to FAO (2012), in developing countries and countries in transition, locally available feed resources are often under-utilised due to lack of information on their chemical composition and nutritive value. The knowledge about these unconventional feed resources can contribute to the development and use of innovative and appropriate feeding options and strategies (FAO, 2012). A substantial amount of agro-industrial by products such as abattoir wastes (Adeniji and Balogun, 2008), brewers' wet grain (Olagbaju, 2012) and hatchery wastes (Abiola *et al* 2009) could be generated daily in Nigeria. Recycling of these agro-industrial by products possess an advantage of providing alternative feed resources for livestock and reduction in environmental pollution due to disposal problems associated with these wastes in Nigeria. The objective of this study was to evaluate the nutritional potentials of six feed resources developed from abattoir wastes, brewery waste and hatchery waste.

Material and methods Six feed resources were developed from brewers' dried grain, bovine blood, bovine rumen content and hatchery waste. Product 1was brewers' dried grain with bovine blood (BDGBB) mixed in ratio 1:1. Product 2 was bovine rumen content with bovine blood (BRCBB) mixed in ratio 1:1. Product 3 was brewers' dried grain with bovine rumen content and bovine blood (BDGBRCBB) mixed in ratio 1:1:1 (Olagbaju, 2012). Product 4 was brewers' dried grain with processed hatchery waste (BDGPHW) mixed together in ratio 1:1. Product 5 was bovine rumen content with processed hatchery waste (BRCPHW) mixed together in ratio 1:1. Product 6 was brewers' dried grain with bovine rumen content and processed hatchery waste (BDGBRCPHW) mixed together in ratio 1:1:1. All the mixings in the six products were for weight for weight. The processed hatchery waste was obtained by boiling homogenised freshly collected hatchery waste for 30 minutes to reduce the moisture content and possible pathogenic microbes. These developed feed resources were later sun-dried to about 10% moisture content. The proximate composition of the developed feed resources was determined as outlined by AOAC (1995). 24 growing pigs (Large White X Hampshire) with initial mean weight of 14.59±0.23kg were used for the digestibility trial which lasted for 7 days for each of the six feed resources developed.

Results The proximate composition of the developed feed resources is shown in Table 1. The feed resources were high in crude protein (32.25-40.25%) and with moderate fibre level (8.69-12.72%). The apparent nutrient digestibility of the proximate fraction of the feed resources (Table 2) showed a significant difference ($p<0.05$) in all the parameters monitored. None of the parameter monitored was below 50% which may indicate that the nutrients in them could be well digested by growing pigs.

Table 1 Proximate composition of feed resources developed from agro-industrial by products

Parameters (%)	BDGBB	BRCBB	BDGBRCBB	BDGPHW	BRCPHW	BDGBRCPHW
Dry Matter	89.22	90.47	88.04	94.52	93.44	89.9
Crude Protein	34.65	36.78	35.47	40.25	38.56	32.25
Crude Fibre	9.29	10.09	8.54	8.69	10.41	12.72
Ether Extract	8.15	9.03	7.04	6.04	6.54	6.62
Ash	12.26	19.30	18.43	21.48	23.05	18.77
NFE	24.87	15.07	18.56	18.06	14.44	19.54

Table 2 Apparent nutrient digestibility of feed resources developed from agro-industrial by products

Parameters (%)	BDGBB	BRCBB	BDGBRCBB	BDGPHW	BRCPHW	BDGBRCPHW	PROB
Dry Matter	76.02^{a}±2.8	53.51^{c}±2.1	71.41^{b}±1.3	76.17^{a}±2.8	61.01^{c}±4.7	62.88^{bc}±1.7	0.006
Crude Protein	80.67^{a}±1.5	55.41^{b}±0.7	75.42^{a}±3.2	74.05^{a}±1.8	55.78^{b}±4.7	53.94^{b}±1.5	0.001
Crude Fibre	68.22^{a}±1.0	50.72^{b}±3.5	67.17^{a}±1.5	67.16^{a}±7.1	56.74^{ab}±2.5	55.95^{ab}±3.5	0.06
Ether Extract	73.72^{a}±1.6	59.32^{bc}±1.9	69.98^{b}±1.3	67.60^{ab}±3.2	53.15^{c}±2.9	66.51^{ab}±7.5	0.06
NFE	77.80^{a}±2.0	58.92^{b}±0.7	76.72^{a}±4.56	80.16^{a}±5.4	68.51^{ab}±2.8	72.88^{a}±4.3	0.05

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

Conclusion Findings from this study showed that protein feed resources could be developed from agro-industrial by products and these feed resources if incorporated into the diet of growing pig could be well utilised.

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Simple procedure for converting abattoir and brewery wastes into animal feed using wheat offal

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Introduction In Nigeria, agro-industrial by-products/wastes such as brewers' wet grains, rumen contents and bovine blood are readily available sources for the development of alternative feedstuffs, which may contribute to sustainability of livestock production and supplementation of conventional livestock feedstuffs (Makinde and Sonaiya, 2010). Despite availability of such by-products in various abattoirs and breweries, they remain largely underutilized mainly because of difficulty in drying, lack of appropriate technology and high cost of equipment for conversion into feedstuffs. Therefore, most are dumped to the environment causing pollution because the processing available is inefficient involving long drying (3-14 days) and resulting in mostly unpalatable products of variable quality. Wheat offal, a standard and conventional feedstuff, possesses a high capacity to absorb water resulting in quicker drying (4 hours) of wet feed resources (Makinde and Sonaiya, 2010). The aim of the present study was to develop a simple procedure for converting brewers' wet grains, rumen contents and bovine blood into animal feed using wheat offal as an absorbent.

Material and methods Fresh bovine blood (BB) and rumen contents (RC) were collected from an abattoir into clean plastic containers. Table salt was added to blood (18 g salt/kg blood) as anti-coagulant (Makinde and Sonaiya, 2010). Brewers' wet grains (BWG) was collected from a major brewery and wheat offal (WO) purchased from a reputable feed mill. Blood and RC were hand-mixed at ratio 1:3 (w/w) and the mixture mixed with wheat offal at increasing concentration (Table 1). Brewers' wet grains, BB and WO were hand-mixed by holding BWG constant and increasing the concentrations of BB and WO to obtain nine different combinations (Table 1). The mixtures were sun-dried (in the dry season) by spreading thinly on black polythene sheets in two replicates each on a concrete surface. Ambient and surface drying temperatures were recorded and the mixtures ground with a plate (burr) mill after drying to obtain rumen contents with blood and wheat offal meal (RBW) and brewers' wet grains with blood and wheat offal meal (BBW). The surface area (m^2) covered by the mixtures during drying was measured to enable estimation of sun drying rates (as $kg/m^2/h$). The difference between the initial and the final weights (after 4 h drying) of the mixtures gave their wetness. The mixtures were turned once in the first hour of drying, which involved rubbing handfuls together and spreading again. Data on sun drying rates, moisture content, wetness and moisture loss (wetness/initial weight) were arranged as a completely randomized block design with mixtures as the main treatment effect and replicates as blocks. Data was analysed with the 2-way analysis of variance using the General Linear Models procedure of SAS (2000). Mixtures from BBW and RBW that had the highest BB and lowest WO contents with ≤ 10 - 12% moisture content in 4 h were analysed for gross energy and crude protein contents.

Results Both RBW (2:1) and BBW (1:2:1) had high gross energy contents and greater than 20% CP contents (Table 1) and expectedly, moisture contents, wetness and drying rate of mixtures increased ($P < 0.05$) as the concentration of wet materials increased. Coversely, moisture contents, wetness and drying rate of mixtures decreased as the concentration of WO increased and most mixtures (75%) dried to < 12% moisture content in 4 h. Similarly, moisture loss increased as WO content increased.

Table 1 Gross energy, crude protein and sun drying characteristics of mixtures of rumen contents, brewers' dried grains, bovine blood and wheat offal

RBW	1:1	2:1	3:1							SEM	P
GE, kcal/g	ND	4338	ND								
CP, g/kg	ND	2.6	ND								
Moisture, g/kg	0.87^{c}	1.1^{b}	1.3^{a}							0.02	0.002
Wetness, g	100^{a}	175^{b}	245^{a}							3.33	0.0003
Drying rate, $kg/m^2/h$	0.42	0.37	0.39							0.08	n.s.
Moisture loss, g/kg	62^{a}	57.4^{b}	49^{c}							0.23	0.0001
BBW	1:1:1	1:1:2	1:1:3	1:2:1	1:2:2	1:2:3	1:3:1	1:3:2	1:3:3		
GE, kcal/kg	ND	ND	ND	4283	ND	ND	ND	ND	ND		
CP, g/kg	ND	ND	ND	43.8	ND	ND	ND	ND	ND		
Moisture, g/kg	0.8^{f}	0.7^{h}	0.55^{i}	1.1^{c}	0.85^{e}	0.75^{g}	1.6^{a}	1.3^{b}	1^{d}	0.03	0.0001
Wetness, g	187.5^{g}	167.5^{h}	160^{i}	257.5^{d}	252^{e}	247.5^{f}	332.5^{a}	327.5^{b}	287.5^{c}	3.61	0.0001
Drying rate, $kg/m^2/h$	0.22^{f}	0.20^{h}	0.06^{i}	0.38^{c}	0.29^{e}	0.21^{g}	0.71^{a}	0.44^{b}	0.33^{d}	0.03	0.0002
Moisture loss, g/kg	3.6^{i}	4.2^{g}	5.3^{d}	4.1^{h}	5.1^{e}	6^{b}	4.7^{f}	5.4^{c}	6.5^{a}	0.04	0.0001

ND = Not determined; RBW = Rumen contents, blood and wheat offal; BBW = Brewers' wet grains, blood and wheat offal

Conclusions These results show that wheat offal can efficiently convert wet feed resources such as brewers' wet grains, rumen contents and bovine blood into alternative energy and protein animal feedstuffs using a low technology process. Similar use of wheat offal during the wet season when sunshine is limited and nutrient digestibility of developed feedstuffs merit further investigation.

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Effect of essential oil compounds on metabolism of dietary polyunsaturated fatty acids by ruminal microbes *in vitro*

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Introduction *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) provide various human health benefits including prevention of cardiovascular diseases (Abeywardena and Patten, 2011). Ruminant products, including meat and milk, contain a high proportion of saturated fats, however, due to the extensive biohydrogenation of PUFA in the rumen. The potential of essential oils compounds (EOCs) to modify rumen fermentation is well documented (Benchaar *et al*. 2008; Calsamiglia *et al*. 2007; Hart *et al*. 2008). However, few studies have investigated their effects on fatty acid metabolism. The objective of this study was to screen 20 pure EOCs for their potential to protect dietary *n*-3 PUFA from biohydrogenation *in vitro*.

Material and methods A basal diet ground through 1 mm sieve size was formulated using 70:30 *Lolium perenne* hay and concentrate (45.9% wheat feed, 14.8% sunflower, 10% palm kernel, 10% malt nuts, 7% rape seed meal, 6% molasses, 4.6% mineral and vitamins, 1.7% blended fat). The basal diet was supplemented with 30 g oil/kg DM from a mixture of fish oil and ground linseed to a attain 50 g/kg DM lipid. A total of 504 serum bottles with 1 g substrate, 80 ml buffer, 20 ml inoculum and 300 mg/l EOC were incubated for 24 h in two runs. Treatments were replicated four times: CON (no EOCs), CAM ((+)-camphene), CAVA (carvacrol), CAVO (L-carvone), CIS-3 (*cis*-3hexen-1-ol), CITR (*cis/trans*-citral), EUG (eugenol), FENC (L-fenchone), GERN (geraniol), GERC (geranyl acetate), GUA (guaiacol), LIN (linalool), LINA (linalyl acetate), MEN (L-menthone), NERO (nerol), PIN ((+)-(α)-pinene), PUL ((R)-(+)-pulegone), TER (terpinene-4-ol), TERP (α-terpineol), THY (thymol) and VEBE ((1S)-(-)-verbenone). Ruminal fluid was collected from three Suffolk cross ewes on same basal diet. Samples were frozen at -20 °C for determination of fatty acid profiles. Data were analysed using one way ANOVA, with experimental runs used as blocking factor in GenStat 13th edition. Duncan's Multiple Range test was used to make multiple comparisons.

Results Fatty acid analysis focussed on linoleic acid (LA; C18:2*n*-6) and LNA disappearance and the appearance of conjugated linoleic acid (CLA) and the more saturated products, vaccenic acid (VA; *trans*-11-C18:1) and stearic acid (SA; C18:0), as well as the fish oil fatty acids, EPA and DHA (Table 1). EOCs that had no major effect on fatty acid proportions relative to CON were not included in table for readability. The phenolic EOCs, CAVA and THY, maintained the highest concentrations of these fatty acids after 24-h incubation compared to the CON (P<0.001). This was consistent with the substantial reduction of SA and VA relative to CON. Other phenolic compounds, EUG and GUA, resulted in higher LNA concentrations relative to CON (P<0.001), their effect being lower than the other phenolics. Related unsaturated alcohols, NERO and GERN, with their parent compound, CITR, resulted in more than 5-fold higher LA and LNA remaining compared to the CON (P<0.001). LINA, MEN and PUL also increased LNA by 60% whilst PIN increased concentrations by 70% (P<0.001). CAVO, FENC and LIN also lowered biohydrogenation of LA and LNA, resulting in lower SA and more VA compared to the CON (P<0.001). The EOCs also had variable effects on EPA and DHA metabolism: the remaining concentrations ranged from 1.6-3.9 and 1.3-2.8 g/100 g total fatty acids respectively. The remaining EPA concentration was more than double that of the CON for CAVA, CITR, GERN, LINA, MEN, NERO, PIN, PUL and THY (P<0.001). Similarly, DHA concentration for these compounds was >45% relative to CON (P<0.001). CAVO and GUA increased EPA by 44% relative to CON (P<0.001), while for FENC, EUG and LINA the proportions were 40% higher than the CON (P<0.001). Most compounds had marginal effect on CLA, however, CITR, GERN, NERO and PIN resulted in higher values compared to CON.

Table 1: Effects of essential oil compounds on proportions of PUFA and biohydrogenation products (g/100 g total fatty acids) at 24 h incubation *in vitro*

	TREATMENTS																
	CON	CAVA	CAVO	CITR	EUG	FENC	GERN	GUA	LIN	LINA	MEN	NERO	PIN	PUL	THY	s.e.d.	P-value
LA	1.76^{a}	7.90^{o}	3.29^{f}	4.30^{k}	3.14^{e}	3.33^{fg}	4.51^{l}	3.16^{e}	3.39^{g}	3.74^{i}	4.10^{j}	5.04^{m}	5.02^{m}	3.63^{h}	7.59^{n}	0.047	0.001
LNA	2.18^{a}	9.82^{p}	4.79^{i}	6.04^{l}	4.32^{g}	3.98^{f}	6.08^{l}	4.31^{g}	4.54^{h}	5.49^{j}	5.81^{k}	6.78^{m}	7.20^{n}	5.40^{j}	9.13^{o}	0.059	0.001
EPA	1.61^{a}	3.88^{k}	2.86^{g}	3.68^{j}	2.91^{g}	2.70^{f}	3.85^{k}	2.88^{g}	3.11^{h}	3.81^{k}	3.63^{ij}	3.82^{k}	3.88^{k}	3.58^{i}	3.69^{j}	0.040	0.001
DHA	1.29^{a}	2.71^{j}	1.98^{d}	2.71^{j}	2.48^{gh}	2.24^{f}	2.75^{j}	2.27^{f}	2.07^{e}	2.72^{j}	2.63^{i}	2.73^{j}	2.43^{g}	2.53^{h}	2.77^{j}	0.035	0.001
SA	17.68^{b}	5.86^{q}	10.93^{h}	8.26^{n}	11.16^{g}	10.66^{i}	8.35^{mn}	11.19^{g}	9.88^{j}	9.14^{k}	8.79^{l}	7.97^{o}	8.39^{m}	9.08^{k}	6.17^{n}	0.061	0.001
VA	6.62^{f}	2.99^{a}	8.48^{l}	6.99^{h}	9.21^{o}	9.40^{p}	7.83^{k}	9.21^{o}	8.95^{m}	9.39^{p}	9.08^{n}	7.80^{k}	7.52^{j}	9.55^{q}	3.99^{b}	0.034	0.001
[1]CLA	0.102^{a}	0.108^{abcd}	0.115^{de}	0.216^{i}	0.111^{bcd}	0.113^{cd}	0.148^{g}	0.104^{ab}	0.131^{f}	0.131^{f}	0.109^{abcd}	0.186^{h}	0.142^{g}	0.134^{f}	0.103^{a}	0.0034	0.001

$^{a-o}$ Within row, means without a common superscript letter are different at 5% probability level [1]conjugated linoleic acid, CLA C18:2 *cis*-9, *trans*-11

Conclusions Of the 20 EOCs screened, carvacrol, thymol, linalyl acetate, L-menthone, (+)-(α)-pinene and (R)-(+)-pulegone were identified as the most effective at inhibiting biohydrogenation. However, carvacrol and thymol highly reduced total volatile fatty acids production ≥60% relative to the control (not shown), indicating a general toxicity to microbial fermentation. Linalyl acetate, L-menthone, (+)-(α)-pinene and (R)-(+)-pulegone warrant further investigation to ascertain optimum inclusion rates *in vitro* and *in vivo*.

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042

Performance of rabbits fed graded levels of roasted pigeon pea meal

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Introduction The pigeon pea plant as a whole has been found to be a useful feed source. Iorgyer *et al.* (2008) reported that boiled pigeon pea can be incorporated in diets for feeding rabbits without any deleterious effects on productive performance. There is dearth of information about the use of roasted pigeon pea as a protein source for rabbits; hence this study aims at looking at the effect of incorporating different levels of roasted pigeon pea meal in rabbit diet.

Material and methods Forty male and female Dutch x Chinchilla rabbits aged 5 - 7 weeks, with an average initial live weight of 725g were used in the study. The rabbits were assigned to four dietary treatments, balancing for breed, sex and weight. Each treatment was replicated five times with each replicate having two rabbits. The experimental design was the completely randomized design (CRD). The experiment was carried out at the Rabbit Research House of the Abubakar Tafawa Balewa University, Bauchi. Bauchi town is located at latitude 13^{o} 30'N and longitude 11^{o} 50'E in the Northern Guinea and Sudan Savanna zones of Nigeria. Roasting of pigeon pea at about 80^{0}C took 3 - 5 minutes. The seeds were allowed to cool and then milled in a hammer mill. The heat treated pigeon pea meal (PPM) was used in compounding the experimental diets. Treatment 1 (control) was maize-soybean based diet with 0% PPM while treatments 2, 3 and 4 contained 10, 20 and 30% PPM in the diets respectively. Diets were iso-nitrogenous and iso-caloric. The rabbits were housed in a single tier rabbit cage located. The feeding trial lasted for five weeks during which data were recorded for feed intake and body weight. Data obtained from performance parameters were subjected to the analysis of variance (Steel and Torrie, 1980).

Results There were no significant effects of dietary treatments on all the performance parameters examined (Table 1). The results obtained in this study for Daily feed intake (DFI), daily weight gain (DWG), feed conversion ratios (FCR) and final live weight (FLW) agree with the findings of Iheukwumere *et al*. (2008) who reported that the DFI, DWG, FCR and FLW of rabbits fed diets containing boiled pigeon pea meal were not significantly different from the control diet.

Table 1 Effect of graded dietary levels of roasted pigeon pea meal on performance of rabbits

Parameters	Dietary levels of PPM (%)				SEM
	0	10	20	30	
Initial live weight (g)	865	788	875	748	56.0^{NS}
Final live weight (g)	1389	1435	1420	1284	96.0^{NS}
Daily feed intake (g)	54	58	54	42	2.2^{NS}
Daily weight gain (g)	14	16	13	12	1.1^{NS}
Feed conversion ratio	4	4	5	3	0.2^{NS}

SEM = Standard error of mean
NS = Not significant

Conclusion The results obtained from the experiment indicate that PPM could be included up to 30% in the diets of rabbits without negatively influencing performance of rabbits.

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Pre-weaning calf growth in spring born, Limousin sired suckling steer and heifer calves and changes in cow liveweight and body condition score in their Aberdeen Angus crossbred dams

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Introduction Pre-weaning changes in suckled calf and cow liveweight (LW), liveweight gain (LWG) and body condition score (BCS) are seldom published for different stages of growth during the summer months due to the inconvenience associated with measurement of these parameters whilst cattle graze outdoors during the mating period. However, an improved understanding of both calf and cow performance parameters during this period would assist in management decision making under practical farming conditions. As part of a wide ranging programme monitoring cow oestrus behaviour, the objective of this study was to determine changes in both calf and cow performance at key stages of the suckling period when managed within a spring calving suckler herd.

Material and methods A total of 48 spring calving Aberdeen Angus crossbred suckler cows (AAx) and their Limousin sired crossbred calves (LIMx) were used in a continuous design experiment during the suckling period from birth to weaning in the spring-summer of 2012. Cows were calved indoors from mid-March to early May when calf LW at birth was recorded. Shortly after calving both cows and calves were turned out to grass at approximately 3.1 livestock units (LSU) per ha until 31st August when stocking rate was reduced to approximately 2.2 LSU/ha. Cows had 24 steer and 24 heifer calves and were grazed and managed as one group throughout the summer grazing period. Calves had access to a barley based creep feed on an *ad libitum* basis from 1st September until weaning. Calf LW, cow LW and cow BCS were determined on 29th May, 31st August and at weaning on 24th November. Calf LWG, cow LWG and cow BCS change (BCS Δ) were determined by difference over the respective suckling periods whilst calf 100 and 200 day weights were calculated from LWGs during the relevant periods. Calf output was determined as calf 200 day LW (kg) per 100 kg cow LW when measured at weaning. To examine the influence that sex of calf may have on both calf and particularly cow performance, all parameters were statistically analysed for differences in performance between the sex of the calf (steers and heifers) using the residual maximum likelihood (REML) facility in Genstat 15.

Results Both calf and cow performance parameters during the suckling period are given in Table 1 when compared by sex of calf. Male calves were significantly (P<0.001) heavier at birth than heifer calves. However, by the end of May and thereafter, steer calves were not significantly heavier than heifer calves at any stage. Cows lost BCS during the late summer period whilst they had been gaining BCS between May and late August. Sex of calf had no influence on the LW or BCS changes occurring in the cows at any time throughout the summer months.

Table 1 Pre-weaning calf LW (kg), LWG (kg/d) and changes in cow LW (kg) and BCS (BCS Δ) during the summer months

	Calf sex					Calf sex			
Calf performance	Steer	Heifer	s.e.d.	Sig.	Cow performance	Steer	Heifer	s.e.d.	Sig.
LW at birth	47	40	1.21	<0.001	LW on 29th May	641	636	22.35	
LW on 29th May	100	99	5.59		LW on 31st Aug	671	674	21.44	
LW on 31st Aug	206	202	9.13		LW on 24th Nov	675	657	23.51	
LW on 24th Nov	277	275	13.44		LWG (May-Aug)	0.31	0.41	0.112	
100 day LW	159	148	6.72		LWG (Aug-Nov)	0.05	-0.20	0.132	
200 day LW	254	243	11.11						
LWG (birth-May)	1.09	1.04	0.077		BCS on 29th May	3.01	3.08	0.137	
LWG (May-Aug)	1.14	1.13	0.067		BCS on 31st Aug	3.38	3.30	0.193	
LWG (Aug-Nov)	0.82	0.81	0.111		BCS on 24th Nov	2.91	2.95	0.112	
Calf output	38.3	37.3	2.11		BCS Δ (May-Aug)	0.37	0.22	0.150	
(kg/100 kg cow LW)					BCS Δ (Aug-Nov)	-0.47	-0.35	0.143	

Conclusions Generally, the results indicate that both steer and heifer calves performed similarly throughout the summer but that late summer growth was slower at approximately 0.8 kg/d whereas early summer growth was approximately 1.1 kg/d. Sex of calf had no effect on cow performance parameters in these multiparous spring calving suckler cows. However, as with the calves, their performance was lower during late compared with early summer months. Calf LWG parameters are similar to that reported in AA sired (approximately 1.1 kg/d) but slightly lower than Charolais sired (approximately 1.2 kg/d) suckled calves during the pre-weaning period within an autumn calving suckler herd (Hyslop *et al*, 2003).

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Dry matter and crude protein digestibility of WAD sheep fed *Newbouldia laevis*, *Ficus thonnigii* and *Mangifera indica* as supplement to *Panicum maximum* during the dry season

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Introduction In the tropics the natural pasture which supply the bulk of ruminants' feed becomes dry and of low nutritive value during the dry season leading to a marked decrease in voluntary intake and digestibility. Browse plants provide adequate protein and can act as energy supplements for ruminants [Van Soest, 1995, Aregheore, 1999b.] most especially in the dry season when grasses become dry and poor in quality. The focus of this research was therefore designed to assess the nutritive value of some indigenous plants consumed by ruminants in humid and sub humid region of Nigeria.

Material and methods The study was carried out at the small ruminant section (sheep unit) of the Teaching and Research Farms, University of Agriculture, Abeokuta, Ogun State. Nigeria. Browse plants were harvested around the premises of the institution and known quantity of each plant was fed as leaf additive in the concentrate to the animals, *Panicum maximum* was fed as basal diet. Twenty female growing West African dwarf Sheep, 5 to 7 months of age weighing between 10.50 – 13.0 kg of average weight were randomly assigned to four dietary treatments having five replicates per diet in a completely randomized 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 period of the experiment was 84 days (12 weeks).
Digestibility studies were carried out by the total faecal and urine collection (McDonald *et al*., 1987) from all the animals for 7days, during the last seven days, faeces voided by each animal were collected; weighed and 10% was kept for analyses. To avoid ammonia losses, urine was collected into bottles with few drops of H_2SO_4. Weights of the goats were measured at the beginning and at the end of the collection period. Faecal samples collected were analysed for crude protein and dried matter (AOAC, 1990) protocol.

Results *Ficus thoniigii* 18.073% had highest (P< 0.05) CP content. Similar (P>0.05) CP content was recorded for *Mangifera indica* (13.210%) and Newbouldia laevis (12.637% while *Panicum maximum* (9.770%) had least (P<0.05) value. Sheep fed *Newbouldia laevis, Ficus thonnigii* and *Mangifera indica* as supplement to grass (*Panicum maximum*) had higher (P<0.05) DM intake than those fed grass alone. Highest value (P<0.05) of CP intake was recorded for sheep that consumed *Mangifera indica* leaves (2.435kg/day) as supplement to grass, while lower values were recorded for sheep that consumed *Newbouldia laevis* (2.300kg/day) and *Ficus thonnigii* (2.130kg/day). Sheep on grass alone (0.195kg/day) had least CP consumption. Similar trend was observed in DM digestibility, however, apart from *Mangifera indica* that had highest (P<0.05) CP digestibility value (94.605%), the CP in *Panicum maximum* grass had better CP digestibility value than that obtained in *Newbouldia laevis* and *Ficus thonnigii*.

Table 1 Dry matter and Crude Protein component, intake and digestibility of sheep fed *P. maximum* supplemented with browse plants

Parameters	*P.maximum* only	*P.maximum* + *M.indica*	*P.maximum* + *N.laevis*	*P.maximum* + *F. thonningi*	SEM
DM content (%)	91.78	90.84	90.90	91.87	0.20
CP content (%)	9.77^{b}	13.21^{ab}	12.64^{ab}	18.07^{a}	1.13
DM intake (kg/day)	1.44^{b}	1.70^{a}	1.61^{ab}	1.56^{ab}	0.04
CP intake (kg/day)	0.20^{d}	2.44^{a}	2.30^{b}	2.13^{c}	0.04
DM digestibility (%)	93.35^{c}	94.71^{a}	94.53^{ab}	94.35^{b}	0.20
CP digestibility (%)	90.84^{b}	94.61^{a}	85.71^{c}	85.88^{b}	1.42

Conclusions This result indicated that *Ficus thonnigii, Mangifera indica* and *Newbouldia laevis* could serve as browse supplement to *Panicum maximum* in sheep production during dry season.

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Comparison of antibiotic resistance exhibited by some gram-ve bacteria isolated from untreated and antibiotic treated bovine ejaculate

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Introduction Artificial Insemination is an important technology worldwide for managed reproduction in farm animals. The international trade in bull semen is huge, with an estimated 243 million doses of semen produced annually (Thibier *et al*, 2000). The transmission of disease in semen is controlled through the European Commission Council Directive 88/407, amended in 2003 which describes the specific antibiotics and their minimum final concentration which must be added, prior to freezing, to control bacterial contaminants. This could lead to antibiotic resistance occurring in commensal bacteria and opportunistic pathogens found in the genital tract of cattle in response to this selection pressure (O'Brien 1997). This paper describes the bacterial profile of ejaculates collected from bulls standing in a cattle breeding centre, before and after processing. For some of the Gram –ve bacteria isolated and identified, their resistance to the antibiotics added routinely during semen processing was investigated.

Material and methods Bacterial load in untreated and antibiotic-treated semen was determined by colony counts from semen samples cultured on brain heart infusion and nutrient agar plates. The bacteria were tested for resistance against selected antibiotics. Initial sensitivity testing was performed using the standard solution concentration used in the AI centre, (referred to as GSLT as it comprised gentamicin, spectinomycin, lincomycin and tylosin (Minitube Inc: Verona, USA)) and separate 30mg disks of tylosin and gentamycin (Mast Diagnostics Ltd). Resistance was assessed by measuring the diameter of bacterial growth inhibition zone around these disks. The resistance of isolated bacteria was further tested against increasing concentrations of the GSLT solution (M5-M2 disks). Representative antibiotic-resistant bacterial isolates were identified using PCR.

Results A mixed bacterial growth was cultured from both untreated and treated semen samples. Cultures from untreated semen exhibited high levels of sensitivity to initial testing therefore no further testing was undertaken. For treated semen a full resistance profile against M5-M2 disks and final identification of representative colonies is summarised in Table 1.

Table 1 Resistance (R) and sensitivity (S) with diameter of growth inhibition (mm) to four different concentrations of gentamycin, spectinomycin, lincomycin and tylosin in seven bacterial colonies cultured from treated bull semen samples.

Bacterial colony number	Bacterial Species	Antibiotic sensitivity (S) and growth inhibition diameter (mm) or resistance (R)			
		M2-disk	M3-disk	M4-disk	M5-disk
2	*Stenotrophomonas sp.*	R	R	R	R
4	unidentified	R	R	R	R
5	unidentified	R	R	R	R
6	*Stenotrophomonas sp.*	R	R	R	R
7	unidentified	R	R	R	R
8	*Sphingomonas sp.*	S – 11	R	R	R
9	*Pseudomonas sp.*	S – 24	S - 22	S - 19	S - 17

Conclusions The isolation of Gammaproteobacteria with broad antibiotic resistance, in this study, suggests that treated semen used for AI is a potential source of antibiotic-resistance genes within the bovine reproductive tract. The virulence factors exhibited by these bacteria isolated from treated semen, whilst a concern in its own right, may also be capable of transfer between bacterial species through DNA gene transfer systems. The fact that *S. maltophilia* associated resistance plasmid and transponson carriage has been demonstrated in *Escherichia coli* (De Gelder *et al.* 2008) supports this hypothesis. This may impact adversely on the antibiotic treatment of the metritis/endometritis syndrome in cattle and reproductive efficiency as *S. Maltophilia* isolated from bovine semen can adversely affect sperm motility and suppress early embryonic development (Bielanski *et al.* 2003). Whilst this study has not attempted to identify the source of the bacteria isolated, the results suggest that either the procedures for washing teaser bulls, preparing the artificial vagina before semen collection or the quality of distilled water used within the laboratory could have introduced the bacteria isolated from the batches of semen.

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In vitro validation of the antiparasitic properties of medicinal Ethiopian plant extracts

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Introduction Medicinal plants play an important role in parasite control in developing countries, and are of growing interest in developed countries where drug resistance increasingly hinders chemoprophylaxis. However, despite wide ethno-veterinary use, scientific evidence on plant preparation efficacy and side effects is scarce, which hampers establishing phytomedicine as a reliable parasite control strategy. Here, we present the validation on the antiparasitic properties of five medicinal plant preparations originating from Ethiopia. The selection of plants was based on an ethno-botanical survey (Tolossa *et al*., unpublished), which consisted of a detailed review of existing, anecdotal information on medicinal plants use for endo- and ecto-parasite control in Ethiopia, combined with a series of detailed interviews with smallholder farmers and traditional healers in selected areas of Ethiopia.

Material and methods Choko, Harsaa, Pa'I, Chawli and Cheketa plants were collected from South Omo Zone, Ethiopia. Selection of parts collected for chemical extraction (e.g. leaves, roots, bark) was based on information derived from the traditional healers interviewed. The extraction solvent used was 70% methanol (MeOH), chosen for its medium polarity. The extraction was performed at room temperature for 12h. The dried extract residues were reconstituted in aqueous 1% DMSO at 50 g/l. The extracts were tested against: a) the ovine nematode *Teladorsagia circumcincta,* one of the species responsible for parasitic gastroenteritis, b) the ovine mite *Psoroptes ovis*, responsible for sheep scab and c) the bovine tick *Rhipicephalus appendiculatus* responsible for the transmission of tick-borne diseases. A larval motility assay (n=3) was adapted from Smout *et al* (2010) to quantify the anthelmintic activity of the plant extracts. Mite and tick larval immersion tests (n=3) were adapted from Borges *et al* (2003) and used to quantify activity against *P. ovis* and *R. appendiculatus* respectively. ANOVA was used to compare between plant extracts and appropriate controls.

Results Choko extract resulted in a significant reduction in *T. circumcincta* larval motility ($P<0.05$), whereas Harsaa extract did not affect motility (Figure 1; $P<0.01$). Although extracts from Pa'I and Chawli are routinely used by traditional healers to control endo-parasites, our results did not confirm this activity. Extracts from Harsaa and Cheketa showed a significant activity against ecto-parasites (Figure 2; $P<0.01$). Cheketa was more active than Harsaa, with mortality higher than 80% in both species, whereas Harsaa resulted in 60% mortality. *P. ovis* was susceptible to Choko extract ($P<0.05$), while *R. appendiculatus* was not susceptible.

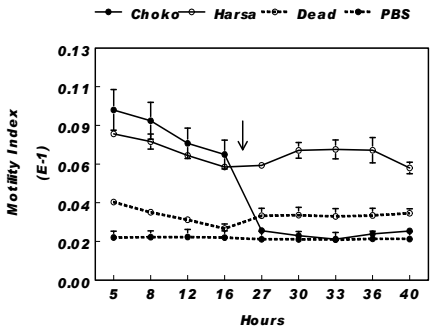

Figure 1 Motility of *T.circumcinta* L3 following the addition of Harsaa and Choko extracts. The arrow indicates the timing of the addition of extracts to the L3 cultures.

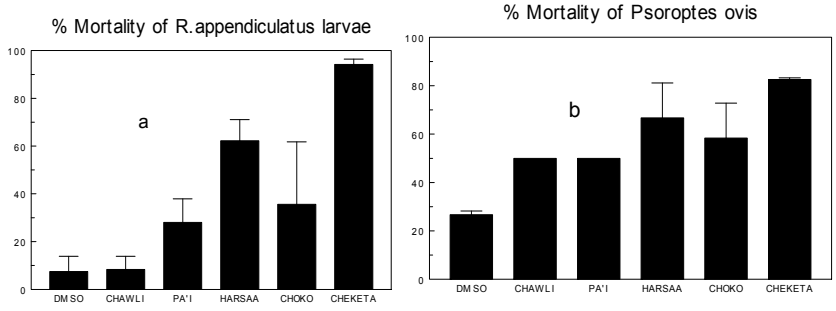

Figure 2 Mortality of (a) *R.appendiculatus* larvae or (b) *P.ovis* population following incubation in the extracts of five medicinal plants.

Conclusions Of the five plants tested in the current study, three are traditionally used against endo-parasites and two against ecto-parasites. Our results confirmed this activity for the extracts used against ecto-parasites (Harsaa and Cheketa), but for only one of the extracts used against endo-parasites (Choko). It cannot be excluded that the procedure used in the study did not result in the extraction of the active compounds from these plants, as the use of 70% MeOH will result in extraction of phytochemical groups of a certain polarity. Further extractions are currently in process with the use of solvents of different polarity and future planned experiments will test these for additional activity. *In vitro* validations as presented here will be followed by *in vivo* testing of active and selected inactive extracts to confirm biological activity.

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Evaluation of the glucose tolerance, insulin sensitivity and combined glucose and insulin tolerance tests for the assessment of glucose metabolism in Holstein dairy calves

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Introduction In man and other mammalian species, prenatal and neonatal nutrition impact on health and metabolism in adult life. Metabolic disease is an important source of economic loss in the dairy cow (Singh *et al*, 2010). Evaluating the efficiency of carbohydrate metabolism in neonates may be useful to identify animals likely to be susceptible to metabolic disease during adulthood. Such specific predictors of future performance could be economically beneficial by facilitating animal selection. We tested the hypothesis that *ad libitum,* as opposed to restricted access to milk replacer from birth, would have a measurable impact on glucose/insulin dynamics by 2 weeks of age. A secondary hypothesis, that combined glucose and insulin tolerance tests (CGIT, Eiler *et al*, 2005) offer a simple, integrated evaluation of insulin sensitivity and glucose tolerance was tested by comparing changes in plasma glucose concentrations for all calves in response to separate CGIT, insulin sensitivity (IST) and glucose tolerance tests (GTT).

Material and methods Twelve Holstein heifers, randomly assigned to one of 2 contrasting dietary groups at birth were tested when animals were 11.8 ± 0.4 days old; Group 1 *n*=6; body mass (BM); 43.7±0.9kg; body condition score (BCS) (Edmonson, *et al,* 1989), 2.7±0.1: Group 2 *n*=6; BM, 42.9±2.5kg; BCS, 2.7±0.1. All calves had *ad libitum* access to water, coarse mix (18% CP, 3.5% Crude Fat, 8% Crude Fibre, 1.92% Ca, 0.5% P) and hay throughout. Milk replacer (MR; 125g/L, 23% CP, 18% Fat, 7.5% Ash) was restricted to 2.5L twice daily for animals in Group 1, while calves in Group 2 had *ad libitum* access via a computerised teat feeder. Concentrate intakes were <200g/day for Group 1 and negligible for Group 2. Intravenous (i.v.) tests were performed at 3 day intervals using previously implanted jugular catheters with the order of test administration being randomised for each calf. Tests were preceded by 12 h fasts. On each occasion, 3 basal samples were collected prior to i.v. infusion of either glucose, 150mg/kg (GTT), insulin, 0.05U/kg (IST) or glucose, 150mg/kg and insulin, 0.05U/kg (CGIT). Blood samples were collected at 1, 5, 10, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135& 150 min post-infusion. Timings of samples were initially clustered closely to measure the immediate response i.e. the size and timing of either the positive (GTT) or negative (IST) phase or both (CGIT), and later more extended points to measure the time taken to return to baseline plasma glucose concentrations. Heparinised blood samples were stored on ice and plasma was stored at -20°C pending glucose analysis by the hexokinase method (Kone30i). Area under the glucose curve (AUCg) was calculated using the trapezoidal method and data were analysed using ANOVA and 2 sample T-tests (STATA 12, StataCorp LP, U.S.A.).

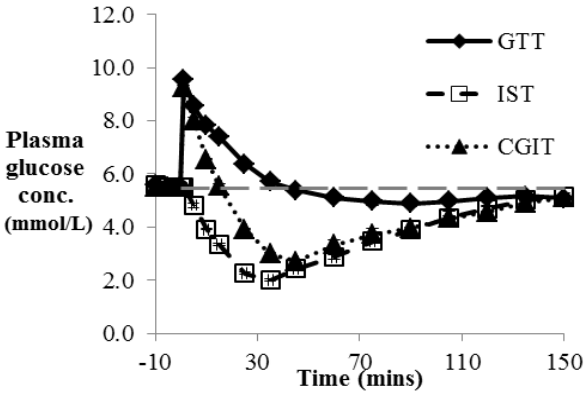

Figure 1 Mean (± s.e.) plasma glucose concentrations for all calves (*n*=12) at each time point during the GTT (solid line, filled points), IST (dashed line, open points) and CGIT (dotted line, solid points).

Results For each test, changes in mean plasma glucose concentrations were similar post-infusion for all calves. Where no differences were noted between dietary groups (P<0.05) data were pooled (*n* =12, Fig. 1). The IST resulted in a 66.6 ±2.4% decrease in circulating glucose concentrations within 35 ±2 min of insulin administration but only 4/12 had returned to baseline values by the final sample time. For the GTT, glucose concentrations returned to baseline 60 ±5 min post-infusion. Changes in plasma glucose following combined glucose/insulin infusion (CGIT) were intermediate to the independent tests and described a biphasic response with time. The time to the start of the negative phase was relatively consistent (22 ±3 min) but only 3 animals in Group 2 returned to baseline glucose concentrations by the final sample time. This difference was evident when mean AUCg were evaluated for the CGIT (Group 1 [limit fed], 566.6 ±35.1: Group 2, [*ad libitum* fed] 734.2 ±71.0 mmol/L/min) (P=0.04).

Conclusions Evaluation of the insulin secretion response (GTT) and tissue sensitivity to circulating insulin (IST) in pre-ruminant calves suggested that when independently evaluated, these elements of glucose homeostasis were not affected by the dietary differences used in this study. The CGIT was simple to perform, well tolerated and allowed concurrent appraisal of the dynamic changes associated with both elements of the homeostatic response. Between-group differences in AUCg suggested that the concurrent challenges of the CGIT may reveal otherwise obscured differences in the combined physiological response. This warrants further study to evaluate associated changes in insulin and non-esterified fatty acid concentrations. The CGIT offers a simple and useful test for field evaluation in cattle but remains to be tested in growing and adult lactating cattle where greater differences in carbohydrate metabolism are to be expected.

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Characterisation of Asinine Pulmonary Fibrosis and similarities to an emerging human interstitial lung disease

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Introduction Pleuroparenchymal Fibroelastosis (PPFE) is an emerging, idiopathic and likely under diagnosed condition that does not fall within any of the current classifications of human interstitial lung diseases (Frankel *et al,* 2004). Key features include an upper zone predominance of pleural fibrosis with associated intra-alveolar fibrosis and elastosis of alveolar walls (Frankel *et al,* 2004; Reddy *et al*., 2012). The objective of our study was to examine *ex vivo* lung tissue from a small cohort of aged donkeys with a high prevalence (35%) of fibrosing interstitial lung disease termed Asinine Pulmonary Fibrosis (APF;Morrow *et al*, 2010). APF is a potentially debilitating and untreatable syndrome of donkeys that is poorly understood and rarely documented. We hypothesise that some cases of APF share several key gross and histopathological features of PPFE and propose that both can be linked to recurrent respiratory infection.

Material and methods Whole asinine lungs were collected from 30 aged donkeys at routine necropsy examination at two UK donkey sanctuaries between June 2009 and September 2012. 19 'APF affected' donkeys had evidence of pulmonary fibrosis on gross examination while 11 'control' animals had grossly normal lungs. Lungs were manually inflated prior to clamping of the trachea and gross images were photographed with a digital camera. 16 whole inflated *ex vivo* lungs (11 APF, 5 controls) were then imaged with high resolution computed tomography (HRCT). Tissue samples were collected from each lung into 10% buffered formalin according to a standard protocol before undergoing routine processing to paraffin blocks. Sections were routinely stained with haematoxylin and eosin (H&E), elastic van Gieson (EVG) and Masson's trichrome (MT). HRCT images and histology sections were reviewed independently and blindly by a radiologist and pathologist respectively from both medical and veterinary fields. Sections and HRCT images were categorised as 'definite', 'consistent with' or 'inconsistent with' with regard to PPFE using criteria described by Reddy *et al* (2012). Cases were categorised as 'definite' on either CT or histology if there was pleural thickening with associated subpleural fibrosis either concentrated in upper or dorsal lung lobes (with respect to CT evaluation) or demonstrating intra-alveolar fibrosis with alveolar septal elastosis (with respect to histological evaluation of EVG sections). CT images were categorised as 'consistent with' if there was dorsal lobe pleural thickening and associated subpleural fibrosis but the distribution of fibrosis was not concentrated in the dorsal lung lobes or there was evidence of coexistent lung disease elsewhere. Histology sections were categorised as 'consistent with' if intra-alveolar fibrosis was present but either not associated with pleural fibrosis, not predominantly subpleural or not in a dorsal lobe biopsy. 'Inconsistent with' was assigned to cases that lacked the aforementioned features either on CT or histology.

Results Ages of 'APF affected' (median 31 years, range 14-53) and 'control' (median 28 years, range 4-36) donkeys at the time of death were not significantly different (Mann Whitney, $p>0.05$). The donkeys comprised 11 geldings and 19 entire jennies. 10/19 APF affected cases were euthanased on humane grounds due to respiratory disease, while 9 were euthanased on humane grounds for other reasons and pulmonary fibrosis was an incidental *post mortem* finding.
10/19 APF affected cases were categorised as either 'definite' or 'consistent with' PPFE on histological evaluation, while 9 showed histological evidence of pleuroparenchymal fibrosis but this did not have an intra-alveolar distribution. 8/11 APF affected cases were categorised as either 'definite' or 'consistent with' PPFE on evaluation of HRCT images. Two of the remaining 3 cases showed pleural and subpleural fibrosis concentrated in the ventral lung lobes while one demonstrated diffuse ground glass opacity with minimal pleural fibrosis. Histological evaluation of these three cases also resulted in an 'inconsistent with' classification. All control cases were classified as 'inconsistent with' on both HRCT and histology.

Conclusions APF is a common yet rarely diagnosed and apparently untreatable syndrome of aged donkeys. This study is the first to combine HRCT and histological data to characterise and document pathological features of APF. We conclude that the majority of cases of APF share key pathological features with human PPFE. Further study of APF may yield valuable information to help elucidate the aetiopathogenesis of this emerging human disease.

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049

Effect of spent tea leaves on *in vitro* total gas production from rice straw-based ruminant diets

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Introduction Spent tea leaves (STL) are a waste product from the tea beverage industries. STL are high in crude protein (CP) and secondary metabolites such as tannins (Ramdani *et al.,* 2012). Ramdani *et al.,* (2013) have observed improved *in vitro* rumen degradability and reduced ammonia production for rice straw (RS) based diets in response to STL additions. However, the presence of tannins in association with lower ammonia production may diminish the rumen microbial activity and ultimately total gas production. This study examined the effect of adding green or black STL (SGTL or SBTL) to partly substitute RS (variety, IR50) in ruminant diets on total gas production from these diets.

Material and methods Five different diets, in triplicate, all containing 70% concentrate with 30% RS (T0), 20% RS + 10% SGTL (SGTL10), 10% RS + 20% SGTL (SGTL20), 20% RS + 10% SBTL (SBTL10) and 10% RS + 20% SBTL (SBTL20) were used to measure *in vitro* total gas production over 48h. Both SGTL and SBTL were obtained as reported by Ramdani *et al.,* (2012) by boiling about 2.8 g tea leaves in 300 ml of water. Rumen fluid (RF) was obtained from a freshly slaughtered grass-fed Texel cross lamb in an abattoir, filtered under CO_2 with a two-layered cheesecloth into a warm thermos flask. The RF was mixed with McDoughall buffer, flushed with CO_2 and stored in a dark bottle in a water bath at 39^0C. About 200 mg sample of each test diet was transferred into a 50ml glass syringe (SAMCO, UK), lubricated with Vaseline and fitted with a 4 way-male-slip stopcock (Cole Palmer Instrument, UK) before 20 ml buffered RF were added and the syringes placed in a shaking water-bath at 39^0C. Total gas produced in each syringe was measured every two hour for up to 48h. pH was measured at the end of 48h incubation by using a calibrated pH meter (Hanna Instrument, Portugal). The one-way ANOVA on Minitab 16 was used to statistically compare the 5 different diets for total gas production at only 24h and 48h as well as the pH at the end of the incubation.

Results Table 1 shows that the total gas production was increased as the incubation times raised. Across the diets, the most significant increase in total gas production was within 24h, particularly from 6h (4.4 - 5.0 ml) to 20h (23.4 - 27.3 ml). After that, the total gas production tended to rise slowly reaching between 33.1 and 35.3 ml at 48h. At 24h, all STL-containing diets had significantly ($P<0.05$) higher total gas production than the control (T0). A similar trend was also found at 48h although it was not statistically different for all diets. Among STL-containing diets, the SGTL additions seemed to result in higher gas production than SBTL. In addition, there was no difference among diets for pH even though the SGTL-containing diets tended to have lower pH than SBTL-containing diets.

Table 1 The effect of SGTL and SBTL addition on total gas production (ml) and pH of rice straw based ruminant diets during various hours of incubation

Diets	0h	2h	4h	6h	20h	22h	24h	26h	28h	30h	44h	46h	48h	pH
T0	0	0.5	3.1	4.4	23.4	25.6	26.5[b]	27.1	27.8	28.5	32.4	32.9	33.1	6.79
SGTL10	0	0.5	2.5	5.0	27.0	28.7	29.8[a]	30.3	30.8	31.5	34.7	35.2	35.3	6.78
SGTL20	0	0.7	2.7	4.0	25.0	27.3	29.3[a]	29.7	30.7	31.5	34.3	35.0	35.2	6.77
SBTL10	0	0.7	2.3	5.0	25.0	26.2	27.8[a]	28.5	29.7	30.2	33.2	33.8	34.0	6.81
SBTL20	0	0.7	3.0	4.5	25.7	27.3	28.7[a]	30.2	30.7	31.3	33.8	34.5	34.7	6.82
SEM							0.68						0.66	0.05
P-value							P<0.05						P>0.05	P>0.05

Means with different letters in the same column are significantly different; SEM=standard error of mean.

Conclusion STL addition to partly replace RS in ruminant diets may result in increased total gas production. This increase is in line with the improvement of *in vitro* degradability but it was not affected by the reduced rumen ammonia production or no change in pH. Further experiments are on-going to observe possible changes in methane and CO_2 production in response to STL additions.

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A preliminary investigation into the effect of using ICAR 305 day lactation figures as a parameter for breed selection

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Introduction Recent studies have reported an adverse correlation between high yields and reproductive performance in dairy cattle (Mackey, Gordon, McCoy, Verner, & Mayne, 2007), (Wicks & Leaver, 2004). It is estimated that infertility costs the UK dairy industry £500M annually, the consequences being both reduced income for farmers and increased cost to consumers where milk contract prices are based on cost of production. This study investigates whether the use of 305 day lactation total as a selection parameter may have led to selective breeding from less fertile animals. It is suggested that the volume production element of the breeding values discriminates against shorter periods between calving and conception resulting in the selection process being biased against the most fertile animals over multiple generations.

Material and methods Milk recording data (n=500) from five of the UK's leading herds was gathered. Herds were selected on the basis of peer recognition for technical standards, data quality and a herd average 305 day yield > 11000kg. Within the selected herds second lactation production was extracted from animals currently in their third and fourth lactation, this ensured that only animals which remained viable after the data was gathered were selected. Secondary data was used so it is assumed that the management and genetics were similar to achieve these high levels of production. Data was normally distributed; analysis was undertaken via linear regression. The independent variable, pre-service yield measured between days 20 – 50 was compared to dependent variables, days in milk at conception and 305 day lactation total. Further analysis of a sub-group (n=152) defined by pre-service yield mean ±2.5KG was performed. Linear regression analysis was carried out to investigate the effect of pre-service yield on days in milk at conception and the effects of both pre-service yield and the days in milk at conception on 305 day lactation total.

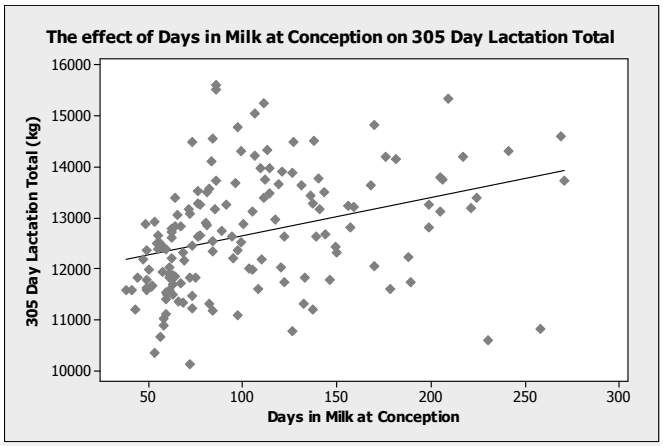

Figure 1 The regression analysis of 305 day lactation total against days in milk completed at conception

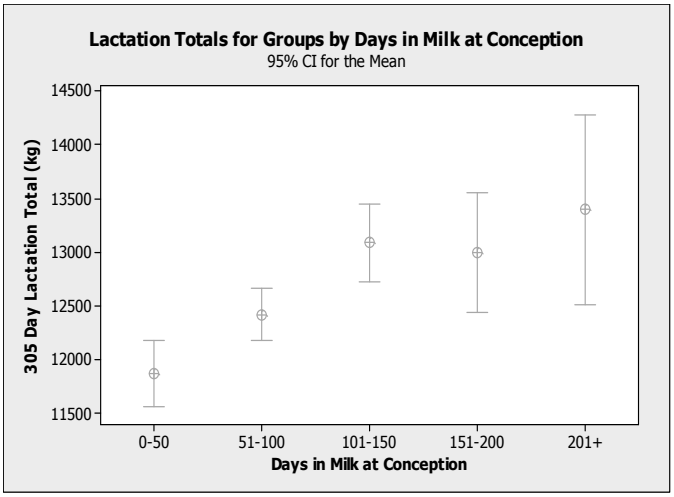

Figure 2 Interval analysis of 305 day lactation total against days in milk completed at conception

Results The analysis was carried out on a subgroup of 152 animals which had pre service yields in the range 47–52 kg. The effect of the pre-service yield on days in milk at conception and gave a regression line of 176.9days–1.46 days per kg pre-service yield which indicated that the highest yielding cows conceived earlier. Had higher yielding cows conceived later, the implication would be that the higher production totals attributed to later conception would be due to the relative potential of the animals, this result challenges that assertion.
Figure 1 shows the regression analysis of 305 day lactation total against days in milk at conception. The results being 11906kg + 7.42kg per DIM conceived.
Figure 2 shows the same results as above as an interval plot. The number of animals in each sector (commencing at 0-50 days pp) are 11, 76, 39, 14 and 12 with mean 305 day yields 11868, 12419, 13088, 12988 and 13397 kg respectively.

Conclusion The results for the effect of pre-service yield on days post partum at conception indicate that higher yields were not significant in delaying conception within the study group. The figures demonstrate the effect of later conception on 305 day yield. Lower lactation yields are partly driven by cows which conceive at less than 76 days post partum failing to complete a 305 day lactation, however the continuation of increasing yields beyond this threshold indicates a potential cost in production of early conception. As infertility has increased, shorter voluntary waiting periods have become normal, impacting further on the 305 day lactation figures for the most fertile animals. The results from this study are based on a relatively small population, further work on a large study group is needed.

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A validation exercise of digital Brix refractometer for on-farm quality assessment of Jersey colostrum

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Introduction Immunoglobulins are globular proteins and have many antimicrobial and other protective properties that aid in establishing passive immunity. This is extremely important as the calf is born almost completely agammaglobulinemic, the neonate is therefore entirely dependant on passive immunity to protect it from disease until its own immune system has matured, which can be up to 4 months postpartum. The success of passive immunity is dependent on the calf receiving adequate Ig from colostrum within 24 hours. After this time 'closure' occurs, whereby the intestine of the calf is no longer permeable to the Ig macromolecules. Radial immunodiffusion (RID) is the gold standard for colostrum quality assessment. This assay can take up to 24 hours to yield results and is also costly, therefore unsuitable for quick on-farm assessment of colostrum (Bielmann *et al.,* 2010). Brix refractometry has been used successfully for Ig assessment in mares (Chavatte *et al.*, 1998) and Holstein cows (Bielmann *et al.,*2008), however to date has not been used on Jersey colostrum. The aim of this study was to validate the use of a digital Brix refractometer to assess Jersey colostrum quality by comparing readings with those obtained from RID.

Material and methods 22 samples of frozen Jersey colostrum were used. The colostrum samples, along with PBS, 2% acetic acid and SRID buffer (IDBiotech, France) were all brought to room temperature before use.
Each colostrum sample was first analysed 3 times using a digital Brix refractometer (Atago PAL-1, Tokyo), and an average obtained.
For RID analysis, the colostrum samples were centrifuged at 1865rpm for 15 minutes, and the fat layer removed. The dilution factor was adjusted to compensate for the difference in consistency, and to ensure precipitate rings were within the range of the IDRing Viewer (IDBiotech, France) The colostrum samples were therefore initially diluted 1:4 with PBS, then 50µl of the dilution 1 was then further diluted 1:100 with 4950µl of PBS. These were then diluted by a further factor of 10, with 50µl of dilution 2 and 450µl of SRID buffer 1X. They were manually homogenized before each dilution.
Wells 1, 2, 3 and 4 of the IDRing test plates were filled with 15µl of standard 200µg/ml, 100µg/ml, 50µg/ml and 25µg/ml respectively. Wells 5-10 of the test plates were filled with 15µl of the samples. Any unused wells were filled with 15µl of deionised water. The test plates were placed in humid boxes and incubated at 35˚C ± 5˚C for 20 hours. The test plates were removed from the humid boxes and filled with 2% acetic acid solution (approximately 5ml per plate), and incubated at room temperature for 1 minute. The plates were drained and the agar gel rinsed twice, then filled with distilled water before incubating at room temperature for a further 15 minutes. The plates were drained and the wells filled with distilled water to enable ease in measurement of the precipitate rings on the IDRing Viewer. The diameters of the precipitate rings formed around each well were measured using a ruler, including those of the standards. To avoid parallax error, one eye was closed for measurement.

Results Pearson's correlation was used to measure the strength of the relationship between the two methods (Figure.1), and the correlation was found to be significant to $P<0.01$ ($r = 0.668$).

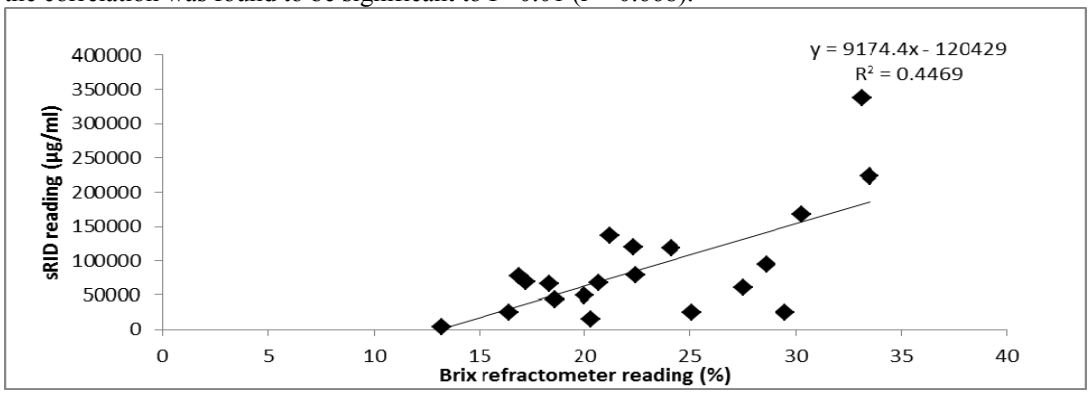

Figure 1 Relationship between sRID readings and Brix refractometer readings

Conclusions There was a relationship between results using both methods. Further research should be undertaken to confirm this relationship and validate the Brix method for use on Jersey colostrum. This method requires minimal amounts of colostrum to provide results, while also being both rapid and relatively low cost; providing a quick and easy method of assessing colostrum quality.

Acknowledgements The authors gratefully acknowledge funding from BBSRC and the farm which provided the colostrum samples

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Performance and carcass characteristics of African giant land snails fed *Alchornea cordifolia* leaf meal in replacement for soyabean meal

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Introduction Snail farming is environmentally sustainable venture and can be done with little skill (Akinnusi 1998). The protein content of snail meat is between 16 and 18% which compares well with other livestock's products like mutton, beef and pork (FAO 1986). There are recent research findings on the use of pawpaw leaf and fruit, cocoyam tubers and mango fruits for feeding snails (Awah, 1992) but there is a paucity of information on the use of the *Alchornea cordifolia* leaf meal (ACLM) in the diet of snails despite its nutrient content (Alikwe and Timibite, 2012). Hence this study was conducted to investigate growth and carcass analysis of snails fed ACLM

Material and methods The experiment was carried out at the Snail unit of the Livestock Production Technology Department of Niger Delta University, Wilberforce Island Bayelsa State located on Latitude 4° 15' North, 5° 23' South and longitude 4° 22' West 6° 45' East in the humid zone of South- South Nigeria**.** 150 growing snails **(***Archachatina marginata*) of mean weight 62.20g (range: 53.23 to 68.07g) were used for the feeding trial. A completely randomized design was used for the study. The trial had 5 treatments:0% ACLM, which served as control, 3, 6, 9, 12% ACLM replacing soybean Each treatment was replicated three times with 10 snails per treatment / replicate. The feeding trial lasted for 56 days. The snails were reared in a cage with 0.25 x 0.25 x 0.5m^3 compartments. The bottom of the cage was filled with moist sandy loam soil to a depth of 15cm and the top was covered with mosquito net reinforced with wire netting for aeration. The ACLM was prepared by harvesting *Alchornea cordifolia* leaves on the University campus then chopped air dried, milled and sieved to obtain the powdery meal. The feed intake was taken on a daily basis by subtracting the left over from the feed offered. The weight gain, shell length, shell thickness and mortality were recorded on a weekly basis. At the end of the feeding trial, 6 snails were randomly selected from each treatment for carcass analysis. The snails were killed by breaking the shell. The foot (edible portion), the shell and the visceral material were weighed separately. Data on performance and carcass characteristics were subjected to Analysis of Variance (ANOVA) in SPSS Program Version 16 software were calculated as Standard Errors of the Mean (SEM), while Duncan's Multiple Range Test (DMRT) was used in assessing the significant differences among the treatment means. Significant was accepted at 0.5% level of probability.

Results The summary of the performance of snails fed different levels of ACLM. Snails fed 12% ACLM diet recorded loss in mean weight (-07g) of whole snail. The weight of whole snail consistently decreased with increase in ACLM level of the concentrate diets. This suggested that higher inclusion of ACLM in the concentrate diets fed to snails did not enhance ($P>0.05$) weight of whole snails. Inconsistent growth response was observed in snails' performance in terms of the weight of edible snail meat, feed intake, and feed conversion ratio. Snails did not differ ($P>0.05$) in these performance characteristics except in feed intake ($P<0.05$). Snails fed 0% ACLM gained the highest weight of edible snail meat

Table 1 Carcass Characteristics of African Giant Land Snail fed with ACLM supplement.

Parameters	0%ACLM	3%ACLM	6%ACLM	9%ACLM	12%ACLM
Feed intake (g/snail)	6.05 ± 0.56^{d}	4.25 ± 0.31^{c}	2.97 ± 0.24^{b}	2.00 ± 0.12^{a}	1.26 ± 0.06^{a}
Final weight (g/snail)	68.62 ± 1.11^{b}	67.75 ± 5.13^{b}	64.27 ± 0.67^{a}	63.47 ± 0.87^{a}	62.47 ± 1.31^{a}
Initial weight (g/snail)	62.63±8.5	60.83±6.7	57.23±5.6	53.23±3.0	68.07±6.0
Weight gain(g/wk)	0.75 ± 0.01^{c}	0.87 ± 0.02^{b}	0.88 ± 0.01^{b}	1.28 ± 0.04^{a}	-0.7 ± 0.01^{d}
Carcass weight (g/snail)	21.10±1.2	19.30±2.4	19.27±1.3	16.27±1.8	14.20±0.4
Shell thickness (mm)	0.26 ± 0.004^{b}	0.28 ± 0.003^{cd}	0.29 ± 0.007^{d}	0.23 ± 0.009^{a}	0.27 ± 0.001^{bc}
Shell length (cm)	$4.38\pm0.022a^{b}$	4.39 ± 0.03^{b}	4.37 ± 0.05^{b}	4.31 ± 0.025^{ab}	4.23±0.03a
GIT (g/snail)	0.70±0.3	0.60±0.1	0.83±0.2	0.60±0.1	1.17±0.4
DM feed conversion	8.07^{a}	4.90^{b}	3.40^{c}	1.56^{d}	1.8^{d}

^{abc} means with different superscript along rows are significantly different ($p<0.05$).

Conclusion The results obtained in the study indicated that whole snail weight decreased consistently with increasing ACLM content. Snails fed 3-6%ACLM concentrate diet had the highest mean weight gain. Snail's growth response to weight of edible meat, shell, feed intake, and feed conversion ratio were inconsistent in pattern. So we conclude that ACLM should be used as a feed additive not as a meal supplement due to its high content of bioactive/anti-nutritive content though well tolerated by Ruminants

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Energy utilization by growing rabbits fed diets containing maize, wheat and sorghum or their combinations with or without enzyme supplementation

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Introduction A major problem of rabbit production in Nigeria and other African nations is the acute shortage of maize because of stiff competition for its use in the industry and for human consumption. Renewed efforts are neccessary to explore and popularise the use of alternatives like sorghum and wheat in cheap diet formulations for rabbits which are not totally new, but practitioners are not just familiar and thus, not so comfortable with their use, coupled with the potential problem of non starch polysaccharides in wheat and presence of tannin in red sorghum variety FAO (1981). The present study therefore determined energy utilisation by growing rabbits fed diets containing maize, wheat and sorghum or their combinations with or without enzyme supplementation.

Material and methods Six-week old cross bred growing rabbits with weight range of 500 to 600g were allotted to eight experimental diets with eight rabbits per diet. The rabbits were raised individually in cages with separate feeders and drinkers and with devises for separate collection of faeces and urine. D_1 had maize as the major energy source, D_2 and D_3 consisted of wheat and sorghum completely replacing maize respectively while D_4 had 50% of wheat and 50% of sorghum replacing maize. D_1 to D_4 were without enzyme while D_5 to D_8 are of the same composition with D_1 to D_4 but with enzyme supplementation {which contains endo-xylanase (EC 3.2.1.8) produced from the *Bacillus subtilis* at the rate of 100g/1000Kg Feed}. Metabolic study was carried out on individual rabbits in which the rabbits were acclimatised for seven days, followed by another seven days of urine and faecal collection when the animals were given known weights of respective experimental diets. The faeces so collected were dried (65^0C) until a uniform minimum moisture content (< 10%) was achieved. The well labelled, dried samples of feed and faeces were analysed for gross energy (GE) (Gallenkamp adiabatic bomb calorimeter). The GE intake, faecal GE, digestible enregy (DE) and metabolisable energy (ME) were calculated. The expermental design was a 2 (with or without enzyme) by 4 (maize, wheat, sorghum and wheat + sorghum inclusion) factorial and was analysed as such using ANOVA. Significant means were separated using Tukey's comparison test at $P<0.05$).

Results Rabbits fed un-supplemented sorghum diet and xylanase supplemented maize diet had the highest ($p<0.05$) GE intake (MJ/day), DE (MJ/day) and ME (MJ/day) values, followed by the group fed xylanase supplemented wheat + sorghum diet and also in all the measurements above. Other measurements, such as feed intake, diet GE, daily faecal output, faecal GE (both MJ/Kg and MJ/day), DE (MJ/Kg), ME (MJ/Kg), DE:GE, ME:DE, and ME:GE were not significantly influenced ($p>0.05$) by the interaction of energy sources and enzyme supplementation.

Table 1 Effect of energy concentrates and xylanase supplementation on energy retention of rabbits fed experimental diets

	Without Enzyme				With Enzyme				
Parameters	Maize	Wheat	Sorghum	Wheat + Sorghum	Maize	Wheat	Sorghum	Wheat + Sorghum	SEM
Feed Intake g.DM	39.95	38.58	53.37	30.08	52.23	24.15	22.91	45.29	4.18
Diet GE MJ/Kg	11.99	12.07	11.75	11.88	11.99	12.07	11.75	11.88	0.11
GE Intake MJ/day	0.48^{c}	0.47^{c}	0.63^{a}	0.36^{d}	0.63^{a}	0.29^{de}	0.27^{e}	0.54^{b}	0.05
Daily Faecal g.DM	14.18	11.69	16.53	14.78	18.92	10.90	5.38	14.59	1.45
Faecal GE MJ/Kg	1.07	1.06	1.06	1.05	1.06	1.05	1.04	1.05	0.03
Faecal GE MJ/day	0.15	0.12	0.18	0.16	0.20	0.11	0.06	0.16	0.02
DE MJ/day	0.33^{c}	0.35^{c}	0.45^{a}	0.20^{d}	0.43^{a}	0.18^{de}	0.21^{d}	0.38^{b}	0.03
DE MJ/Kg	10.93	11.01	10.69	10.83	10.93	11.02	10.71	10.83	0.04
ME MJ/day	0.32^{c}	0.34^{c}	0.44^{a}	0.19^{d}	0.42^{a}	0.17^{de}	0.20^{d}	0.37^{b}	0.05
ME MJ/Kg	10.09	10.21	9.76	9.93	10.05	10.12	9.69	9.83	0.07
DE:GE %	68.75	74.47	71.43	55.56	68.25	62.07	77.78	70.37	2.47
ME:DE %	96.97	97.14	97.78	95.00	97.67	94.44	95.24	97.37	0.47
ME:GE %	66.67	72.37	69.84	52.78	66.67	58.62	74.07	68.52	2.51

^{abcd} Means on the same row having different superscript are significantly different ($P<0.05$); SEM= Standard error of means

Conclusions The main indices of energy utilisation (GE intake, DE and ME) showed that the rabbits utilised supplemented maize and unsupplemented sorghum diets better than wheat-containing diets. It is therefore advisable to use appropriate enzyme (Xylanase) when maize is to be employed as energy source. On the alternative, sorghum grains can be used effectively without addition of xylanase. Wheat appeared to be a poor substitute for maize irrespective of xylanase inclusion.

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Influence of manure type and time of sampling on proximate composition and *in vitro* gas production post incubation parameters of *Panicum maximum* (Ntchisi) hay

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Introduction In livestock enterprises, one of the most important factors determining profitability is to achieve optimal levels of feeding. This aim is most problematic during the dry season when available feed is scarce and of low quality. A "staircase" growth pattern is observed when animals are not adequately fed during the dry season. Therefore, livestock farmers are facing their biggest challenge during the dry season (Ikhatua and Adu 1984). Hay has been considered the mainstay diet for ruminant livestock during dry season in Nigeria, as there are usually excess forages during the rainy season and to have these ruminant animals in maintain their body weight in this season of the year effort have to be put in place to conserve the excess for the period when the forages especially the natural pasture will be deficient in quality and quantity. This study is aimed at evaluating the proximate composition and *in vitro* post incubation parameters as influenced by animal manure and sampling time.

Material and methods The experiment was conducted at the Organic Research farm and laboratory of the Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Nigeria to evaluate the effect of animal manures and sampling time on the proximate composition and *in vitro* gas production post incubation parameters of *P. maximum* (Ntchisi). The forage (*Panicum maximum* var. Ntchisi) was sourced from the Organic Research Site of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria, five manure types (cattle, swine, poultry, goat and control) was used in the experiment with four replicates. The forage was harvested after 8 weeks of re-growth at about 15cm above ground level. The forage was chopped to about 10cm and air dried to reduce the moisture content and stored. Storage duration (0, 6 and 12 weeks) was also considered and the hay was milled at the respective weeks for the proximate analysis and *in vitro* digestibility. The DM content of silage samples was determined by drying in a forced-air oven at 60°C until constant weight was achieved. After drying, samples were ground to pass a 2 mm sieve. The crude protein (CP), ether extract (EE), and ash were determined according to AOAC. The *in vitro* gas production to estimate the digestibility of the grasses with different manure was determined and metabolizable energy (ME) and organic matter digestibility (OMD) according to the procedure of Menke and Steingass (1988) while short chain fatty acid (SCFA) was estimated according to Getachew *et al.* (2004). In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe. A 5x3 factorial experiment measured the effect of these five manures and three sampling times. Statistical evaluation was accomplished by Analysis of Variance (ANOVA), Duncan Multiple Range Test, on 0.05 probability values using SAS ® 9.0 version

Results All the parameters determined and estimated are significantly (P<0.05) different except for the ash content. The hay produced from swine fertilized grasses had the highest CP value (93.5 g/kg) and had the least value (4.5 ml) of methane produced. The CP of the hay decrease significantly (P<0.05) with increase in sampling time and the methane produced also followed same trend.

Table 1 Effect of manure type and time of sampling on the proximate composition (g/kg DM) and *in vitro* post incubation parameters of *P. maximum* (Ntchisi) hay

	Dry Matter	Crude Protein	Ether Extract	Ash	GV ml/200mgDM	ME MJ/kg	SCFA µmol	OMD g/kgDM	DMD g/kgDM	Methane ml
Effect of manure type										
Control	943.3^{b}	78.4^{b}	38.9^{ab}	81.8	32.3^{a}	5.73^{ab}	0.5^{ab}	470.4^{ab}	479.1^{c}	7.1^{a}
Cattle	946.7^{b}	91.6^{a}	47.4^{a}	80.6	30.0^{ab}	6.33^{ab}	0.7^{a}	510.1^{a}	607.2^{a}	4.7^{b}
Poultry	950.0^{ab}	88.4^{ab}	31.3^{b}	95.3	19.1^{c}	4.85^{b}	0.4^{b}	421.0^{b}	527.4^{b}	4.6^{b}
Goat	951.7^{ab}	87.5^{ab}	37.8^{ab}	80.8	25.6^{b}	6.64^{a}	0.7^{a}	524.1^{a}	592.7^{ab}	4.9^{b}
Swine	956.7^{a}	93.5^{a}	37.6^{ab}	84.4	31.0^{ab}	6.46^{ab}	0.6^{ab}	518.8^{a}	610.3^{a}	4.5^{b}
SEM	4.0	3.1	6.16	7.1	1.74	0.78	0.1	48.8	31.9	0.9
Effect of sampling time										
0	958.0^{a}	114.0^{a}	41.0^{b}	86.2	13.3^{c}	4.08^{c}	0.26^{c}	574.8^{c}	623.1^{a}	6.3^{a}
6	946.0^{b}	87.5^{b}	52.8^{a}	88.0	41.6^{a}	7.90^{a}	0.93^{a}	611.2^{a}	590.2^{ab}	4.9^{b}
12	945.0^{b}	71.2^{b}	21.9^{c}	79.5	27.9^{b}	6.02^{b}	0.61^{b}	480.3^{c}	500.1^{b}	4.6^{b}
SEM	3.0	7.3	3.3	5.3	3.21	0.44	0.08	27.5	29.0	0.7

$^{a-c}$: Means in the same column with different superscripts are significantly (P<0.05) different; GV=gas volume at 24 hours

Conclusion Hay produced from swine manure fertilized grasses have been found to have better quality in terms of the crude protein and produced the least methane gas making it a superior forage resource with less energy loss.

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Subclinical mastitis and estimated relative economic losses in dairy herds in Greece

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Introduction Despite many years of concerted efforts, mastitis remains the main health issue of dairy cows, worldwide (Ruegg 2012). However, as the frequency of its clinical form has largely subsided, the main objective today is to reduce the prevalence of subclinical mastitis which causes considerable economic losses by reducing both milk production and milk prices (Biggs 2009; Blowey and Edmondson 2010). Somatic cell counts (SCC) are valuable in this respect and data produced by official milk recording schemes are very effective tools in every-day management of the problem (Rhoda and Pantoja 2012). The objective of this study was to evaluate the extent of subclinical mastitis problem in Greek dairy herds and estimate the economic losses associated with it.

Material and methods Official individual cow monthly milk (ICMM) records (quantity, fat and protein percentage and SCC) from 24 randomly selected dairy herds (out of a total of 135), keeping 1920 Holsteins, in Thessaly, Central Greece, were used in this study. Testing period was January to December 2009; herd size ranged from 30 to 300 cows and mean milk production was 8,000 kg per cow per year (3.8% fat, 3.2% protein). The official SCC threshold (400,000) and a reasonable industry target of 150,000 (Ruegg 2012) were used for comparisons. ICMM records were classified in the following four SCC clusters: a) <400,000, b) 400,000-800,000, c) 800,000-1,600,000 and d) > 1,600,000; then, the size (expressed as % of total cows) and the contribution of each cluster in bulk tank SCC were calculated. Four 6-herd clusters were formed based on mean annual SCC: a) "low", b) "average", c) "marginal" and d) "high". Analysis of variance was used to detect any relationships of individual monthly SCC cow classifications with milk production and fat and protein percentage and herd SCC clusters with herd size, milk production and fat and protein percentage. Economic losses were calculated as follows: a) Based on published data (Biggs 2009; Blowey and Edmondson 2010; Philpot and Nickerson 1991), milk production losses were considered to be 175 kg per cow per year at a mean annual SCC of 200,000 and then increasing linearly by 70 kg for every 50,000 increase of SCC. b) Local 2009 milk and feed prices were used; half the nutrients needed for the extra milk production were assumed to be provided from extra feed (300 € per ton of home-mixed concentrates) and half were considered to be "saved" by reducing udder inflammation; milk price was set at 380 € per 1,000 of milk. c) A provisional penalty of 5 € and 10 € per 1000 kg of milk for herds with a mean annual SCC between 400,000 and 650,000 and >650,000, respectively, was also applied. Actual SCC of milk delivered to processors and actual penalties were difficult to assess; most of the farmers used high SCC milk for feeding calves. No quality incentives for low SCC milk and high solids milk were applied at the time.

Results Farm level analysis showed that only 15 herds had a mean annual SCC below the official threshold of 400,000 (mean 405,000). ICMM records of the four SCC clusters were 78.3%, 10.2%, 6.2% and 5.3%, respectively. Cluster (d) (>1,600,000) contributed about 4.5% of total milk but around 40% of total somatic cells in the bulk tank. These are records from either chronically/heavily infected cows or undetected (no forestripping) mild clinical mastitis cases. If milk from these cows was excluded from the bulk tank, all herds would be below the SCC threshold of 400,000 (mean SCC of 218,000). An ICMM record with a SCC > 1,600,000 was also negatively correlated with milk fat percentage (-0.14%, $P<0.005$). Mean annual SCC in "low", "average", "marginal" and "high" herds were 220,000, 307,000, 423,000 and 657,000, respectively. No statistically significant differences ($P>0.05$) were found regarding herd size, milk and fat and protein percentages, probably due to the small sample size at herd level. Milk production of herds classified as SCC "high" was, on average, 2.3 kg per cow per day lower than that of herds classified in the other 3 clusters ($P<0.05$). Mean economic losses for the four herd clusters are shown in Table 1, based: a) on a target SCC of 150,000 and b) on mean SCC of herds classified as "low". Losses can be significant and comparable to those of low reproductive efficiency, especially for "marginal" and "high" herds. With 2012 milk and feed prices losses reported in Table 1 would be about 5% higher.

Table 1 Economic losses (€ per cow per year) due to subclinical mastitis is Greek dairy herds relative to if mean annual SCC were 150,000 or 220,000.

	Herd clusters based on mean annual SCC			
	Low	Average	Marginal	High
Mean annual SCC of 150,000	44	85	146	318
Mean annual SCC of 220,000 ("low" cluster)	-	41	102	274

Conclusion Subclinical mastitis was a significant problem for 9 out of 24 herds (37.5%) in this study. Excluding high SCC milk (>1,600,000) from the bulk tank seems to be an effective short term measure. Wider adoption of the well-known 5- and 10-step prevention plans would be highly beneficial to dairy farmers.

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The effect of *Samanea saman* and *Stylosanthes hamata* supplementation on intake and microbial nitrogen production in Djallonké sheep fed/offered Nerica 1 rice straw basal diet

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Introduction Rice straws in general have low nutritive value; thus the need to improve them is imperative as they are an abundant crop residue. Harnessing legume/MPTs like *Samanea saman* and *Stylosanthes hamata* to provide the needed energy, some minerals, vitamins and protein can improve microbial action and promote growth. Absorbed nucleic acid purines are degraded and excreted in the urine as their derivatives, hypoxanthine, xanthine, uric acid and allantoin and could be used to estimate the supply of microbial protein from the rumen to the intestine (Chen *et al*., 1990; FAO/IAEA, 1997). The aim of this study was to determine the intake level and microbial protein supply when *Samanea saman* and *Stylosanthes hamata* are fed to sheep.

Material and methods Four rumen fistulated Djallonké rams, with an average weight of 22.5 kg, and 30 months of age, were used for the studies in a Randomised Complete Block Design (RCBD), (4 treatments) in a 2 x 2 factorial arrangement of treatments. The factors were 2 types of foliage and 2 levels of offer with the periods (four 7-day periods) as blocks. Two levels each (360 and 480 g) of the foliages *Samanea saman* and the *Stylosanthes hamata* were offered as supplements to create four rumen environments. The treatments were designated T_{SA360}, T_{SA480}, T_{ST360} and T_{ST480} for 360 g *Samanea saman*, 480 g *Samanea saman*, 360 g *Stylosathes hamata* and 480 g *Stylosanthes hamata*, respectively. An initial 10 days were allowed the animals to adjust to the diet followed by four 7-day periods, which were used for the measurement of feed intake and collection of urine. The rams were housed in individual metabolism cages (0.7 m x 1.2 m). The cages were provided with plastic buckets for water and had wooden feeding troughs. Microbial nitrogen yield was calculated according to the equation of Chen and Gomes (1995) in the sheep. DM intake, excretion of urinary PD and microbial N supply data were analysed as a Randomised Complete Block Design using analysis of variance and the PROC general linear model (GLM) of SAS (2002).

Results Microbial nitrogen reaching the duodenum was significantly ($P<0.05$) different between the two foliages and also between the two levels of offer for both. MN production was significantly ($P<0.01$) higher (18.5 g d^{-1}) for T_{SA480} and significantly ($P<0.01$) lower (15.6 g d^{-1}) for T_{ST360}. The EMNS was similar ($P>0.05$) for the two levels of offer for *Stylosanthes hamata* and significantly ($P<0.05$) higher for the lower level of offer of *Samanea saman*. MN production was higher (18.5 g d^{-1}) for T_{SA480} due to higher efficiency of microbial protein production leading to higher urinary PD excretion by the sheep. This could also be due to the higher dry matter intake, which was expected to induce higher microbial synthesis (Makkar *et al*., 1988).

Table 1 Urinary excretion of purine derivatives in sheep fed the experimental diets.

	T_{SA360}	T_{SA480}	T_{ST360}	T_{ST480}	LSD	FxL
TOTAL INTAKE	1,038.4^{b}	1,184.7^{a}	1,009.4^{d}	1,080.7^{c}	35.9	**
DOMR (g d^{-1})	498.0^{c}	577.0^{a}	487.2^{d}	513.1^{b}	10.05	**
MN (g d^{-1})	15.9^{c}	18.5^{a}	15.6^{d}	16.4^{b}	0.32	**
EMNS (g N kg^{-1} DOMR)	32.0^{a}	32.0^{c}	32.0^{b}	32.0^{b}	0.002	*
P_a (mmol d^{-1})	21.9^{b}	25.4^{a}	21.4^{c}	22.6^{a}	0.44	*
PD_e (mmol d^{-1})	20.4^{c}	23.3^{a}	20.0^{d}	21.0^{b}	0.37	*
A_e (mmol d^{-1})	17.4^{c}	19.8^{a}	17.0^{d}	17.8^{b}	0.32	*
UA_e (mmol d^{-1})	3.1^{c}	3.5^{a}	3.0^{c}	3.2^{b}	0.06	*

*, $P<0.05$; **, $P<0.01$; LSD, Least significant difference. T_{ST}, treatments with *Stylosanthes*; T_{SA}, treatments with *Samanea*.
$^{a, b, c, d}$means within rows with different superscripts differ significantly ($p<0.05$).
DOMR, digestible organic matter fermented in the rumen. MN, microbial N. P_a, purine absorbed. EMNS, efficiency of microbial N supply. PD_e, purine derivative excreted. A_e, allantoin excreted. UA_e, uric acid excreted.

Conclusions *Samanea saman* foliage elicited high voluntary intake and higher microbial N (protein) production than *Stylosanthes hamata* foliage.

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Evaluation of the efficiency of nitrogen utilisation of young Holstein cattle at age of 6 to 22 months

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Introduction The efficiency of utilisation of nitrogen (N) is a nutritional variable used to describe the production efficiency and the effect of N excretion on the environmental footprint (N pollution to ground and surface water and N_2O emissions) of cattle production systems. There is ample information available in the literature on N utilisation of adult cattle. However, there have been few studies involving systematic measurements of N utilisation in young cattle at different stage of growth. The objectives of the present study were to investigate effects of gender of young Holstein cattle on N utilisation at different ages and to use these data to develop relationships between N intake and excretion.

Material and methods Twenty Holstein cattle (10 male steers vs. 10 heifers) at age of 5 months were selected for a 4 periods study (28 d/period) with measurements taken at age of 6, 12, 18 and 22 months, respectively. The animals were blocked into 10 pairs (steers vs. heifers) according to birth date, birth and weaning weights, growth rate and body condition score. Nitrogen intake and output data were measured for 4 days at the end of each period when they were housed in calorimeter chambers for measurements of gaseous exchange and energy utilisation (data reported elsewhere). Prior to the 4-day measurements, they were housed in a cubicle accommodation for 20 days, metabolism units for the next 3 days, and then chambers for 1 day. All cattle were allowed free access to water and offered a single diet for *ad libitum* intake once daily at 0900 h. The diet offered was a typical mixed ration of grass silage and concentrates used on UK commercial farms. A single grass silage was used in all 4 periods with the silage offered during the final 8 d boxed in an evacuated condition at the beginning of each period to maintain its quality. The concentrates were based on barley, maize, sugar beet pulp and soybean meal. All data were analysed using one-way ANOVA and the linear regression technique was also used to develop relationships between ME intake and energy balance. The statistical programme used was Genstat 6.1 (6th edition; Lawes Agricultural Trust, Rothamsted, UK).

Results The period mean data on N intake and outputs and the efficiency of N utilisation are presented in Table 1. Animal gender had no significant effect on N intake or manure N output in any period with an exception of steers having significantly higher N intake than heifers at age of 18 month ($P < 0.05$). The combination of data with heifers and steers demonstrated a linear reduction in N retention/N intake and a linear increase in manure N/N intake with growth of the animals. The regressions using all 4 period data found that there was a significant relationship ($P < 0.001$) between N intake (x, g/d) and N output (y, g/d) in faeces ($y = 0.27 x + 5.46$, $R^2 = 0.69$), urine ($y = 0.59 x – 34.0$, $R^2 = 0.71$) or both faeces and urine ($y = 0.86x – 28.5$, $R^2 = 0.84$, Fig. 1).

Table 1. Mean data on N intake, outputs, and utilisation efficiency

	6 month	12 month	18 month	22 month
N intake and outputs (g/d)				
Nitrogen intake	99.1	170	187	205
Faecal nitrogen	33.4	49.7	50.1	65.4
Urinary nitrogen	23.1	62.4	79.7	92.5
Manure nitrogen	56.5	112	130	158
Nitrogen retention	42.6	58.4	57.0	47.3
N utilisation efficiency				
Faecal N / N intake	0.33	0.29	0.27	0.32
Urinary N / N intake	0.23	0.36	0.43	0.45
Manure N / N intake	0.57	0.66	0.70	0.77
N retention / N intake	0.43	0.34	0.30	0.23

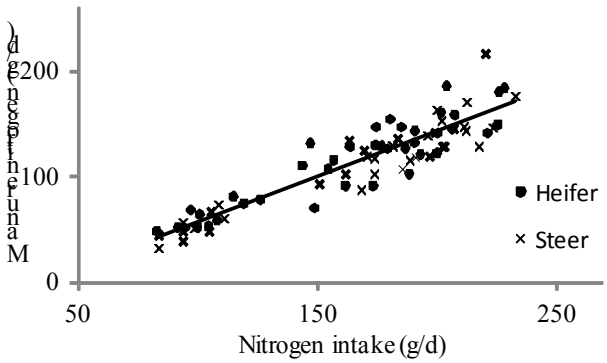

Fig 1. Relationship between N intake and manure N

Conclusions A range of linear relationships have been developed to predict N excretion for young Holstein cattle. These data may enable beef and dairy production industries to develop appropriate mitigation strategies to reduce the environmental footprint of cattle production systems.

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Evaluation of *Moringa oleifera* leaves, twigs and pods as supplemental feed resource for sheep on grass-based diet

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Introduction The dwindling nutritive quality of available pasture grasses for sheep feeding particularly during the dry season is a major factor limiting efficient sheep production. Sheep producers are faced with the challenge of searching for alternatives to the use of expensive conventional concentrate supplements. Browse species have played significant roles as supplements to low quality grasses but few of the browses are being utilized inspite of the vast array of tree species. This could partly be due to deficient information on the nutritional value of these species. The aim of this present study was to evaluate the nutritional potentials of *Moringa oleifera* leaves (ML), twigs (MT) and pods (MP) as supplemental feed for sheep.

Material and methods Samples of ML (n=8), MT (n=8) and MP (n=8) were harvested from different *Moringa oleifer*a tree stands. Separation into the different aerial parts was carefully done by hand picking. Samples (n=8) of *Panicum maximum* (PM) grass was harvested from different pasture areas. Harvesting was done during the dry season. All fresh samples harvested were weighed and oven dried at 60°C to constant weight for dry matter determination. Dried samples were then milled through a 2 mm sieve for proximate (AOAC, 2000) and fibre fraction (Van Soest *et al*., 1999) determination. For *in vitro* digestibility study, samples of PM were mixed with ML, MT and MP in the ratio 70: 30, respectively. Three West African dwarf (WAD) sheep were used as donors of rumen liquor. The sheep were previously fed with 40% concentrate (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal). Rumen fluid was collected from the animals, within 15 min before the morning meal into thermo flasks and strained through four-layered cheesecloth and kept at 39°C soon after collection. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2. Incubation of weighed (200 mg) samples was carried out according to Menke and Steingass (1988). The rumen liquor and a buffer solution were mixed in the ratio 1:2 (v/v), respectively. Incubation was carried out at 39°C and the volume of gas production was measured at 3 h interval from 3 to 48 h. Three blanks containing 30 ml of medium only were included in the run. Average volume of gas produced from the blanks was deducted from the volume of gas produced per sample. Indicators of energy value which included the organic matter digestibility, OMD (Menke and Steingass, 1988) and short chain fatty acids, SCFA (Getachew *et al*., 2000) were estimated at 24 h post gas production. Data were analysed for each observation (n=8) using the analysis of variance (ANOVA) with the *Panicum maximum* and *Moringa oleifera* mixtures as fixed effects.

Results Supplementation with *Moringa oleifera* leaves, twigs and pods resulted in higher crude protein ($P < 0.05$) relative to sole *Panicum maximum*. Gas production, organic matter digestibility and short chain fatty acid production values were higher with supplementation than without supplementation. Supplementation with the leaves showed higher variation than supplementation with twigs and pods.

Table 1 Supplementation effect of *Moringa oleifera* leaves, twigs and pods on proximate and fibre content (g/kg DM), gas production (ml/200 mg), organic matter digestibility (g/kg) and short chain fatty acid production (µ mol/g DM)

	Proximate and fibre composition			Incubation time		Energy value	
	DM	CP	NDF	24h	48h	OMD	SCFA
Sole Panicum	356	81^{c}	611^{a}	14.6^{c}	19.7^{c}	325.0^{d}	0.200^{d}
Panicum/Moringa leaves	323	131^{a}	562^{b}	30.3^{a}	37.7^{a}	465.0^{a}	0.370^{a}
Panicum/Moringa twigs	329	108^{b}	578^{b}	21.7^{b}	29.0^{b}	397.0^{c}	0.260^{c}
Panicum/Moringa pods	337	112^{b}	582^{b}	24.3^{b}	30.3^{b}	419.0^{b}	0.310^{b}
s.e.m	5.1	7.9	8.5	3.02	3.49	26.7	0.033

Conclusions These results show that *Moringa oleifera* leaves, twigs and pods have potentials as supplemental feed resource for sheep. The higher crude protein content, gas production, organic matter digestibility and short chain fatty acids with supplementation indicates better availability of nitrogen in the feed and improved utilization. The eventual acceptability of the different parts of *Moringa oleifera*, particularly the pods merits further investigation.

Acknowledgements The authors gratefully acknowledge the assistance of Dr. O. M. Arigbede of Pasture and Range Management for the establishment of Moringa oleifera.

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Creating a murine neonatal obesity model by adjusting litter size

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Introduction Obesity has in the last few years become very widespread, both among adults and children. Obesity causes a whole range of health problems from the predisposition for type 2 diabetes to cardiovascular and liver diseases. The trend of earlier onset of obesity in childhood points to the possibility of influence of the caloric intake by the mother during pregnancy and lactation on the development of early childhood obesity and on the offspring's adult health (developmental programming). There is debate regarding the relative importance of the intrauterine and neonatal environments (Sun *et al* 2012), hence there is a need for the creation of animal early-obesity models that can differentiate the two phases.

Material and methods NMRI (Harlan) mice were kept in a 12 hours light/dark cycle at a temperature of 21.5ºC and humidity of 35%. Nulliparous females were mated at around 9 weeks of age and at a minimum weight of 30g. After detection of vaginal plugs they were housed in groups of 5 and fed high-caloric diet (cafeteria food: equal proportions by weight of rodent breeding chow, chocolate and mixed nuts). The food consumption was measured of each ingredient separately. At 16th day of pregnancy females were transferred to individual cages where they stayed until the end of the experiment. Litter size was adjusted to 5 (TEST) or 10 (CONTROL) pups within the first 24 hours of birth. A total of 20 pups, of which 11 were females and 9 were males, were involved in this preliminary experiment. At 16th day of lactation pups were euthanized by cervical dislocation and the weight of the pups and of subcutaneous adipose tissue, abdominal adipose tissue, peri-renal adipose tissues, left kidney, right kidney, liver, gastrointestinal tract, spleen and heart was taken. Statistical analyses were performed with Minitab 11 statistical package (Minitab Inc., USA). Data were analyzed by General Linear Model and a two-way ANOVA (treatment × gender). The data are expressed as means ± SD (standard deviation). Results were considered significant when $P<0.05$.

Results The cafeteria diet was consumed in the proportions nuts>chocolate>chow. There was an overall significant effect of the litter size reduction, resulting in increased body weight of the TEST pups (+59%, $P<0.001$, table 1). The values of tissue and organ weight were body weight-corrected and expressed in grams per 10g of body weight. Weighing of the adipose tissue during dissection showed that TEST pups had more subcutaneous adipose tissue (+154%, $P<0.001$) and abdominal adipose tissue (+87%, $P<0.001$), but there was no significant difference in peri-renal adipose tissue. The body-weight adjusted liver weight of TEST pups was also increased (+15%, $P<0.01$), but the body-weight adjusted heart weight was decreased (-15%, $P<0.05$). The reduced litter size did not impact the weight of the kidneys, spleen and gastrointestinal tract. There were no significant differences between the genders, except for some small changes in the distribution of visceral fat.

Table 1 Influence of litter size on body weight (g) and body weight-corrected weight (g/10g) of some tissues/organs
Values are means and standard error, n=10 per group

Variable	Weight	S.C. adipose tissue	Abd. adipose tissue	Liver	Heart
Litter size 5	15.89±0.25	1.27±0.03	0.15±0.02	0.39±0.01	0.05±0.00
Litter size 10	9.99±0.15	0.50±0.02	0.08±0.01	0.34±0.01	0.06±0.00
Significance	P<0.001	P<0.001	P<0.001	P<0.01	P<0.05

Conclusions These results show that the reduction of litter size together with the mother's high-caloric diet affects the body weight and adiposity of the neonatal (sixteen-day old) pups. This represents a simple model for further investigation of the relative impact of prenatal and postnatal nutrition on the early development of adiposity, and consequences for metabolic disturbances in later life. In contrast to the recent report of Sun *et al* (2012) where a maternal high fat diet fed during lactation caused neonatal obesity irrespective of litter size, in our model the effect was only evident in the smaller litter size. This suggests that the effect is a consequence of neonatal caloric intake, rather than a specific maternal dietary component *per se.* The absence of difference in weight between the genders suggests that any sexual dimorphism in size, weight and adiposity occurs later. Enlarged liver in the group with reduced litter size may have been due to fatty liver (which was evident visually during dissection) but may also have involved stimulated growth to cope with the additional metabolic load of the increased caloric intake. Decreased body-weight corrected heart weight suggests that heart growth cannot follow the rapid growth of the obese body and may, therefore, be a predisposition for heart disease. This conclusion remains tentative, since as a proportion of lean body mass the reduction in heart size did not achieve significance.

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060

Characterization of diversity in the sheep MHC Class II Antigen presenting genes

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Introduction The major histocompatibility complex (MHC) is the most diverse region of the mammalian genome. Different alleles and genotypes influence susceptibility to infectious, parasitic and autoimmune disease. Pathogen-mediated selection is largely responsible for the observed diversity but the precise selective mechanisms are unknown. The aim of this project was to characterise MHC diversity as part of a larger project exploring the optimal use of the MHC in selective breeding for disease resistance.

Material and methods Five cohorts of 200 Scottish Blackface lambs were studied in five consecutive years on an upland farm in southwest Strathclyde. DNA was extracted from peripheral blood and typed for genetic variation by direct sequencing of PCR amplicons. Five loci were studied: *DRB1*, *DQA1*, *DQB1*, *DQA2* and *DQB2*. Gene frequencies and linkage disequilibrium were estimated with the allele procedure on SAS 9.3.

Results The number of distinct alleles ranged from 11 in DQA1 to 21 in *DQB1*. There was a null allele at each of the four DQ loci. Gene frequencies varied from less than 0.001 to nearly 0.5. The most common allele was the null allele at the *DQA1* locus. The number of potential haplotypes was calculated by multiplying the number of alleles at each locus. Of the 1,500,000 potential haplotypes only 30 were observed in our sample of Blackface sheep indicating extremely high levels of linkage disequilibrium. The proportion of heterozygotes at each locus ranged from 0.75 to 0.85. Diversity was higher for the B loci than the A loci.

Discussion The ovine MHC was more diverse than comparable human populations as assessed by the number of alleles but less diverse when assessed by the number of haplotypes. High levels of linkage disequilibrium can be maintained by selection but probably also reflect the harem structure of sheep populations. The next stage of this project is to use these results to fine-map the previously reported MHC gene for nematode resistance and explore the way selection acts to maintain MHC diversity.

Acknowledgements We thank the BBSRC for funding.

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MHC class II DQA1 diversity and nematode resistance in Scottish Blackface

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Introduction Detecting some of the genes that influence disease resistance would improve our understanding of the processes that cause disease and also simplify disease control. Genes within the Major Histocompatibility Complex (MHC) are strong candidates for disease resistance and they have been intensely studied for a long period. Recently several groups working independently have reported the existence of alleles within the MHC that are associated with enhanced resistance to nematode infection. The aim of this project is to describe the DQA1 locus and its association with FEC.

Material and methods All sheep were Scottish Blackface from a commercial upland farm in Southwest Strathclyde, central Scotland. All lambs were kept on the same field after weaning at three or four months of age until the grazing season. A total of 881 genomic DNA were extracted. Two specific PCR primers NikDQA1_F (ACTGGCCACAAATGAAGCCCACAA) and NikDQA1_R (AGAAGGCAGAAGATGAGGGTTCAG) were used to amplify the second exon of the ovine DQA1 gene. All PCR products were directly sequenced from genomic DNA. Faecal egg counts were estimated every 28 days from May until October. Sequence alignment, translations and comparisons were carried out using CLC Genomic Workbench version 5.1 (CLC bio, Denmark). The allele procedure on the SAS suite of statistical programs version 9.3 (SAS Institute, Carolina) was carried out to estimate the DQA1 allele frequencies among lambs.

Results Figure 1 shows the DQA1 alleles present in Scottish Blackface sheep in this study. There were 11 alleles with the gene frequency ranging from 0.001 to 0.5. The most common allele was null (01) followed by Z28418 (02) and M33304 (03). In this population, Ovar DQB1 locus exhibited low diversity compared to DRB1. Figure 2 illustrates the effect of DQA1 alleles on the log egg count. The alleles have been compared to the null allele which has been set to zero. It can be seen that all alleles except c and g alleles negatively associated with FEC. This indicated that the null allele was relatively susceptible in this population, however none of the association were statistically significant. Overall the effect of DQA1 was weaker than other loci within MHC.

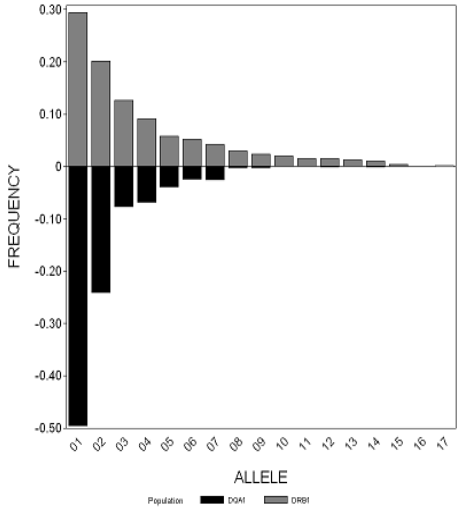

Figure 1 Comparison of alleles frequencies at DRB1 and DQA1 loci

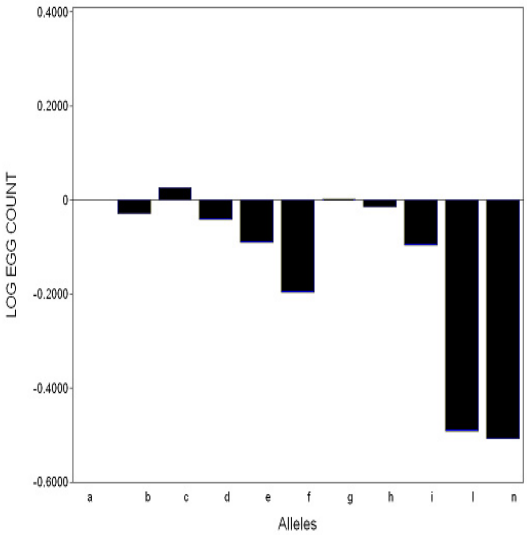

Figure 2 The effect of DQA1 alleles on log egg counts

Conclusion There are fewer alleles at the DQA1 locus than the DRB1 locus indicating that DQA1 is less diverse than DRB1. None of the allele were significantly different from allele a. These results indicate that the mutation or mutations responsible for the association between the MHC class II region and resistance to nematodes is unlikely to lie within DQA1 locus.

Acknowledgement We thank C McComb and N McLaren for their technical assistance. This research was supported by BBSRC, Universiti Putra Malaysia and Ministry of Higher Education Malaysia.

Explaining high levels of MHC diversity

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Introduction Major histocompatibility (MHC) molecules are encoded by highly polymorphic genes, and are known to play an important role in the immune defence of many vertebrates, including commercial species such as sheep and cattle. The exact nature of the biological forces driving this extraordinary diversity is still not fully understood. It has been suggested that pathogen-mediated selection is the main force maintaining MHC polymorphism, with heterozygote advantage being a possible explanation for how balancing selection could operate. Different models for heterozygote advantage have been proposed in recent decades.
Our objectives are to explain whether and to which extent heterozygote advantage is capable of maintaining the polymorphism of the MHC genes, and which of the proposed models fits observed data best.

Material and methods We ran stochastic simulations of an allele-based model over evolutionary time scales to explore the evolution of MHC genes of the bovidae family. This enables us to compare alternative models of heterozygote advantage in terms of allele numbers and other features, and also gives insights important for practical applications. Simulations start with a single allele at the emergence of the bovidae family; over the time span of many million generations allele numbers, amino acid sequences encoded by MHC alleles and other allele properties are tracked.
The simulation assumes discrete generations and adjusts allele frequencies according to their relative marginal fitnesses, taking possible mutations in the current generation into account. Variable initial conditions in terms of allele fitness variation, the amount of heterozygote advantage and population size were explored for different popular models of heterozygote advantage. Parameterisation, where appropriate, was taken from biological data (Stear *et al*, 2005); however, there is hardly any such data on some of the parameters. In these cases a broad range of parameter values had to be explored. The results of these simulations are compared to observed data.

Results The predicted number of coexisting alleles in the divergent allele advantage model of Wakeland *et al.* (1990) is in the same order of magnitude as allele numbers found on MHC loci of sheep, with many hundred alleles being predicted for population sizes of 100 million animals and more. Additionally, the distribution of the age of the final set of alleles, as well as the level of heterozygosity are corresponding well with existing biological data for this model. Other models, however, differ considerably in many important features of MHC genes.
Furthermore, our findings confirm theoretical expectations of a strong dependency of the final set of MHC alleles on population size; other parameters of importance are the amount of heterozygote advantage, and the variation in fitness of newly mutated MHC alleles.
Table 1 presents the dependency of allele numbers on population size for a specific set of parameter values in an exemplary overview.

Table1 Dependency of allele numbers on population size – divergent allele advantage model

Population Size	Heterozygote Advantage	Allele Fitness Variation	Number of Alleles
25,000	~ 50%	+/- 16%	~5
100,000	~ 50%	+/- 16%	~ 10
250,000	~ 50%	+/- 16%	~ 15
1,000,000	~ 50%	+/- 16%	~ 30
2,500,000	~ 50%	+/- 16%	~ 60

Discussion The divergent allele advantage model of Wakeland *et al.* (1990) is closest to our biological observations. We argue that this model is currently the best representation of how heterozygote advantage operates in MHC genes.
Our results have wide-ranging consequences for both livestock breeding and conservation. For example, schemes to breed animals for disease resistance could be adapted to give more emphasis to heterozygosity, and, particularly, allelic dissimilarity. In terms of conservation genetics, the divergent allele advantage model suggests that populations may be more vulnerable to contractions in the size of the gene pool.

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063

Validation of novel RNA transcripts detected in pathogenic leptospires by RNA-Seq

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Introduction Leptospirosis is one of the most geographically distributed zoonotic diseases and occurs following exposure to pathogenic species of *Leptospira*. Leptospires colonise renal tubules of reservoir hosts, which include domestic and wild animal species, from which they are excreted via urine into the environment. Disease transmission occurs through contact with contaminated urine or contact with contaminated water sources. Currently, the genomes of 5 pathogenic strains of leptospires have been sequenced. Whilst recent work has demonstrated that leptospires regulate gene and protein expression in response to environmental signals, relatively little is known about mechanisms of gene regulation in leptospires and to date, no small RNAs have been identified.

Objectives Characterisation of the transcriptome of pathogenic leptospires and validation of expression of putative small RNAs

Methods RNA-Seq data (6 biological replicates) characterising the transcriptome of *Leptospira interrogans* serovar Copenhageni strain FIOCRUZ L1-130 was visualised using IGB Viewer. Analysis revealed non-annotated regions of the genome with high transcriptional activity. These transcripts are predicted to encode a series of novel small RNAs. Preliminary characterisation of these small RNAs was carried out *in silico* and their expression validated using quantitative reverse-transcriptase PCR.

Results and Discussion 12 novel RNA transcripts were identified in non-annotated regions of the genome. The expression of 9 of the 12 non-annotated transcripts identified was validated by quantitative reverse-transcriptase PCR. Four transcripts show sequence homology to known small RNA families including tmRNA, PyrR, RNAseP and Cobalamin. The transcripts are unique in their conservation amongst *Leptospira interrogans* and show homology within *Leptospira biflexa* and *Leptospira borgpetersenii*. This suggests a regulatory mechanism for gene expression which enables *Leptospira* to adapt to environmental cues such as those encountered within a host during infection.

Further characterisation of these novel small RNAs will provide insights into their function and facilitate a greater understanding of pathogenic mechanisms of leptospirosis.

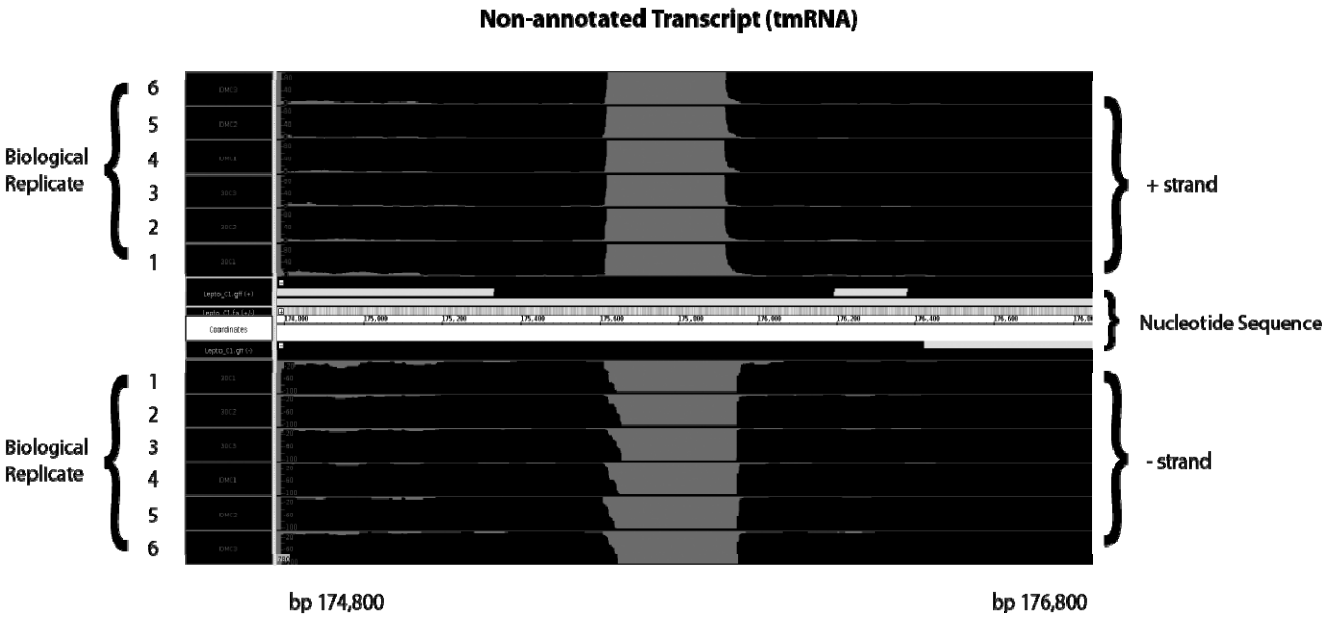

Figure 1 IGB visualisation of RNA-Seq data identifying a novel small tmRNA in the genome of *Leptospira*

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Characterisation of the phylogenetic relationship of monophasic *Salmonella enterica* subsp. enterica (Subspecies I) serotype 4,[5],12:i:- DT193 and other phage types strains by a complete genome sequencing

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Introduction '*Salmonella Typhimurium* – like' ('STM-like') strains also called 'monophasic *Salmonella*` strains with an antigenic formulas 1,4,[5],12:i:- have become a major concern of the EU Community Reference Laboratory for *Salmonella* as since 1990 isolates with this serotype have been responsible for outbreaks in several EU and non-EU countries. These emerging 1,4,[5],12:i:- isolates that are not fully typeable, can not be called *S. Typhimurium* and can not be officially reported as *S. Typhimurium*. In the Europe these isolates are generally of phage type DT120 or DT193 and appear to be replacing DT104 as the dominant phage type associated with multi-drug resistance (MDR).

Material and methods Using next-generation sequencing (NGS) technology we have used whole genome sequence variation to determine the phylogenetic relationship of 127 monophasic Typhimurium isolates from animals in the UK from 1995-2010 and from human clinical cases of disease from 2007-2010. Short Illumina-generated reads were mapped to the finished reference genome of *S. Typhimurium* strain SL1344 using smalt software (for mapping DNA sequencing reads) and single nucleotide polymorphisms (SNPs) identified. Based on the SNPs discovered, phylogenetic relationship of these strains was infered using RAxML and the phylogeny was displayed using Dendroscope or Figtree software.

Results Deletion within the *fljA*, *fljB*, *hin* region is responsible for the monophasic phenotype. Deletion at this locus is variable within the monophasic clade and appears to be under ongoing rearrangement during the clonal expansion (Fig 1). This could indicate an evidence for ongoing selection. Most *S.* 4,5,12:i:- isolates encode a novel genomic island (designated SGI-2) encoded at approximately 4.5Mb that encodes heavy metal resistance genes (Fig 1). Time-dependent phylogenetic analysis (using Bayesian MCMC analysis) applied to date branches on the phylogenetic tree for the DT193 epidemic strains showed that the vast majority of the monophasic isolates collected after 2005 were from a single distinct clade. These were largely DT193. However, earlier monophasic isolates (pre-2005) were from several different clades some of which include well-characterised isolates such as DT104, LT2 and ATCC14028. DT193 isolates from before 2005 were from a distinct clade and were not direct ancestors of the current DT193 epidemic, indicating that DT193 is a polyphyletic phagetype.

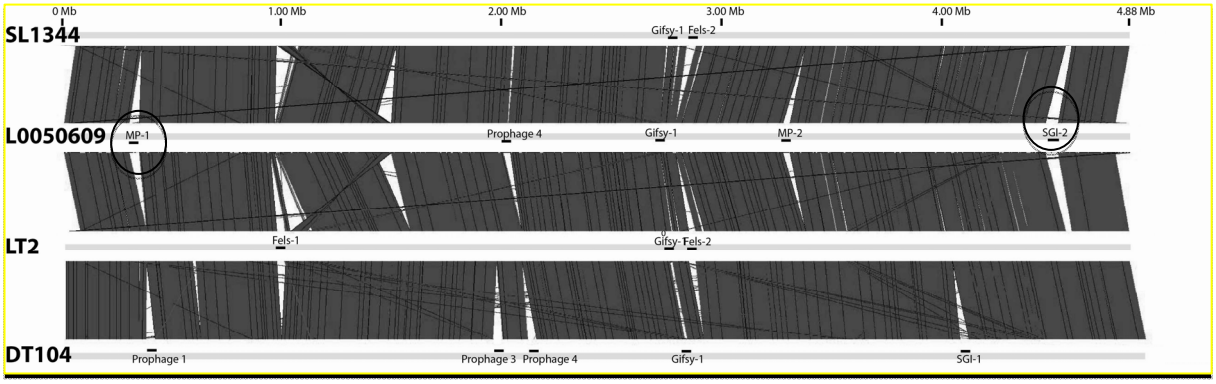

Figure 1 Artemis comparison tool (ACT) view comparing the whole genomes of *S. Typhimurium* SL1344, *S.* 4,5,12:i:- L0050609, LT2 and DT104

Conclusion Bayesian phylogenetic analysis using BEAST indicate a clonal expansion of a clade of *S. Typhimurium* beginning in about the year 2000, that are phylogenetically distinct from the epidemic DT104 clade and monophasic isolates prior to the year 2000. Human isolates were more commonly associated with an older subclade containing many pig isolates and more recently chicken isolates. Phylogenetically younger clades have a more diverse animal and few human origin. Comparative genomics identified two novel genomic islands and a region encoding multiple antibiotic resistance. The monophasic phenotype was due to multiple deletion events that occurred during the epidemic (Figure 1). Novel diagnostic SNP array will be developed based on the discovered SNPs from the whole genome sequences.

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065

The effect of high versus low polyphenol oxidase red clover silage on polyunsaturated fatty acids across the rumen of beef steers

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Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce both proteolysis and lipolysis in silo and the rumen (Van Ranst *et al*. 2011). However all comparisons *in vivo* have compared red clover with other forages with typically lower levels of PPO, which brings in other confounding factors as to the cause for the greater flow of polyunsaturated fatty acids (PUFA) on red clover silage, other than just PPO. This study will compare two red clover silages which when ensiled had contrasting PPO activities against a control of perennial ryegrass silage to ascertain the full role of the enzyme in protecting PUFA across the rumen of beef steers.

Material and methods Six Hereford × Friesian steers (623 ± 33.6 kg), prepared with rumen and duodenal cannulae were allocated at random to one of three big bale silages: a high PPO red clover (RC+); a low PPO red clover (RC-) or perennial ryegrass (PRG). All diets were offered at 14-15 g DM/kg live weight with the experiment consisting of two 3 × 3 Latin Squares. Each period was 21 d consisting of 14d adaptation to the diet, 4 d faecal collection, 2 d duodenal sampling and 1 d for rumen sampling. Digesta flow at the duodenum was estimated using a dual phase marker system with $Yb(CH_3COO)_3$ and Cr EDTA as particulate and liquid phase markers, respectively**.** Lipid extraction and GC analysis was as described by Lee *et al.* (2011). Statistical analysis was undertaken using ANOVA, blocking according to period + animal (Genstat 11.1).

Results All silages were well preserved with mean dry matter (DM) of 33.4, 39.8 and 57.0% for RC+, RC- and PRG, respectively. PPO activity and phenol substrate concentration in RC before wilting was 69.4 and 20.8 ukatal/g DM and 2367 and 4100 µg/g FW for RC+ and RC-, respectively. Protein bound phenol (PBP; mg/g DM) as a measure of the degree of oxidation and therefore PPO protection in the silages was however comparable for the RC silages: 10.2, 9.6 and 3.0 for RC+, RC- and PRG, respectively. DM and fatty acid intake along with fatty acid flow and biohydrogenation are reported in Table 1. Due to different fatty acid compositions of the silages there were differences in intake with subsequent differences in fatty acid flow to the duodenum. Flow of C18:0 and C18:1 trans was comparable between PRG and RC+, with RC- higher for all fatty acids other than C18:0. Biohydrogenation of C18 PUFA was significantly lower on RC silages compared to the grass silage with no difference between RC+ and RC-.

Table 1 Effect of red clover silage with contrasting PPO levels versus grass silage on intake and ruminal fatty acid metabolism

	RC+	RC-	PRG	s.e.d	P value
DM Intake (kg/d)	9.26	9.40	8.76	0.344	NS
Fatty acid intake (g/d)					
C18:0 stearic	4.56^{b}	4.64^{b}	2.84^{a}	0.145	***
C18:2*n-6* linoleic	35.6^{b}	39.3^{c}	22.7^{a}	1.06	***
C18:3*n-3* linolenic	70.8^{b}	84.5^{c}	59.7^{a}	2.27	***
Total fatty acids	165^{b}	189^{c}	129^{a}	5.21	***
Duodenal flow (g/d)					
C18:0 stearic	116^{ab}	131^{b}	111^{a}	6.68	*
C18:1 trans	12.2^{a}	17.0^{b}	11.8^{a}	0.83	***
C18:2 CLA	1.50^{b}	1.69^{c}	0.43^{a}	0.058	***
C18:2*n-6* linoleic	8.58^{b}	9.76^{c}	3.93^{a}	0.487	***
C18:3*n-3* linolenic	16.2^{b}	19.0^{c}	5.34^{a}	0.782	***
Total fatty acids	261^{b}	294^{b}	233^{c}	14.1	**
Biohydrogenation (%)					
C18:2*n-6* linoleic	75.8^{a}	75.1^{a}	82.8^{b}	0.16	**
C18:3*n-3* linolenic	77.0^{a}	77.4^{a}	91.1^{b}	0.13	***

Values with different superscripts (^{abc}) differ significantly (P<0.05).

Conclusions As previously reported RC resulted in a lower biohydrogenation of C18 PUFA than grass silages. Higher levels of C18 fatty acid flow on the RC- diet compared to RC+ was driven by higher substrate intakes. The lack of difference between RC- and RC+ in terms of biohydrogenation would indicate that the RC effect in reducing biohydrogenation is not driven by PPO and may be more a reflection of differences in digestion kinetics between grass and RC silage. However as PBP levels were similar between RC- and RC+ it may reflect similar levels of PPO driven protection in both silages as a result of greater substrate concentration in RC- and the role of non-PPO driven oxidation (Lee *et al*., 2012).

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Development of maintenance energy requirement for young Holstein cattle using calorimeter data measured at age of 6, 12, 18 and 22 months

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Introduction The efficiency of energy utilisation plays an important role in animal nutrition. A considerable volume of research in energy metabolism of dairy cows has been undertaken over the last 3 decades. However, there is little information available in terms of energy utilisation in young stock of Holstein cattle. The lack of such information may impact on the development of appropriate rearing regimes for high genetic merit Holstein cattle. Consequently, the objectives of the present study were to evaluate energy utilisation for young Holstein cattle at different stages of growth and to use these data to quantify their maintenance energy requirement.

Material and methods Twenty Holstein cattle (10 male steers vs. 10 heifers) at age of 5 months were selected for a 4 periods study (28 d/period) with measurements taken at age of 6, 12, 18 and 22 months, respectively. The animals were blocked into 10 pairs (steers vs. heifers) according to birth date, birth and weaning weights, growth rate and body condition score. In each period, they were housed as a single group in a cubicle accommodation for the first 20 d, then transferred to metabolism units where animals were tied individually in stalls for 3 d, before being housed in indirect open-circuit respiration calorimeter chambers for 5 d, with measurements of feed intake for all 5 d, and faeces and urine outputs and gaseous exchange during the final 4 d. All cattle were allowed free access to water and offered a single diet for *ad libitum* intake once daily at 0900 h. The diet offered was a typical mixed ration of grass silage and concentrates used on UK commercial farms. A single grass silage was used in all 4 periods with the silage offered during the final 8 d boxed in evacuated conditions at the beginning of each period to maintain its quality. The concentrates were based on barley, maize, sugar beet pulp and soybean meal. All data were analysed using one-way ANOVA and the linear regression technique was also used to develop relationships between ME intake and energy retention. The statistical programme used was Genstat 14.2 (14th edition; Lawes Agricultural Trust, Rothamsted, UK).

Results The period mean data on production and energy metabolism are presented in Table 1. Animal gender had no significant effect on any energetic efficiency variable in any period in terms of energy digestibility or metabolisability or proportion of ME intake used as heat production or for energy retention. The linear regression between ME intake and energy retention indicates a decreasing trend in net energy requirement for maintenance (NE_m) from periods 1 to 4, although ME requirement for maintenance (ME_m) was marginally higher in period 4 than in period 3. The similar linear regression (Fig. 1) using data in all 4 periods produced a NE_m of 0.41 MJ/kg$^{0.75}$and ME_m of 0.59 MJ/kg$^{0.75}$.

Table 1. Mean data on production and energy metabolism

	6 month	12 month	18 month	22 month
Live weight (kg)	176	320	493	570
Live weight gain (kg/d)	0.71	0.75	0.82	0.78
ME intake (MJ/d)	52.5	89.5	98.5	106
Heat production (MJ/d)	41.5	57.4	69.9	85.3
Energy retention (MJ/d)	11.0	32.1	28.6	20.5
DE/GE	0.79	0.78	0.74	0.74
ME/GE	0.70	0.68	0.65	0.64
Heat production/ME intake	0.79	0.64	0.71	0.81
Energy retention/ME intake	0.21	0.36	0.29	0.19
NE_m (MJ/kg$^{0.75}$)	0.57	0.48	0.46	0.42
ME_m (MJ/kg$^{0.75}$)	0.77	0.62	0.59	0.63

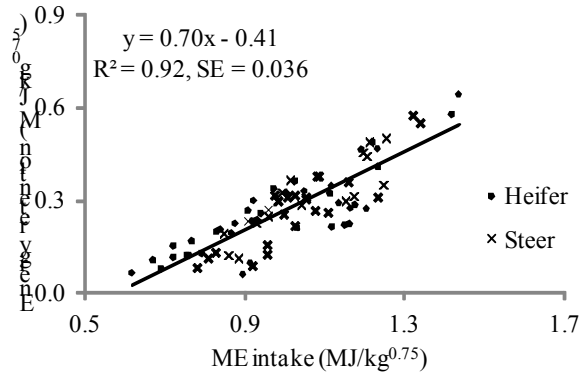

Fig 1. Relationship between ME intake and energy retention

Conclusions The NE_m and ME_m for young Holstein cattle derived from the present study were much higher than those recommended in energy feeding systems used in UK (NE_m = 0.35 MJ/kg$^{0.75}$ and ME_m = 0.47 MJ/kg$^{0.75}$; AFRC, 1993) or elsewhere. The use of these systems to ration young stock may underestimate total feed requirements, thus reducing production efficiency.

Acknowledgements The authors gratefully acknowledge their colleagues at Heifer and Ruminant Nutrition Units for collation of data. This study was funded by DEFRA and the Devolved Administrations (FFG 0914; Project AC0115).

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The effect of high versus low polyphenol oxidase red clover silage on nitrogen and amino acid metabolism across the rumen of beef steers

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Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce both proteolysis and lipolysis in silo and the rumen (Van Ranst *et al*. 2011). However all comparisons *in vivo* have compared red clover with other forages with typically lower levels of PPO, which brings in other confounding factors as to the cause for the greater protection of dietary N on red clover silage, other than just PPO. This study will compare two red clover silages which when ensiled had contrasting PPO activities against a control of perennial ryegrass silage to ascertain the full role of the enzyme in protecting N across the rumen of beef steers.

Material and methods Six Hereford × Friesian steers (623 ± 33.6 kg), prepared with rumen and duodenal cannulae were allocated at random to one of three big bale silages: a high PPO red clover (RC+); a low PPO red clover (RC-) or perennial ryegrass (PRG). All diets were offered at 14-15 g DM/kg live weight with the experiment consisting of two 3 × 3 Latin Squares. Each period was 21 d consisting of 14d adaptation to the diet, 4 d faecal collection, 2 d duodenal sampling and 1 d for rumen sampling. Digesta flow at the duodenum was estimated using a dual phase marker system with $Yb(CH_3COO)_3$ and Cr EDTA as particulate and liquid phase markers, respectively**.** Lipid extraction and GC analysis was as described by Lee *et al.* (2011). Statistical analysis was undertaken using ANOVA, blocking according to period + animal (Genstat 11.1).

Results All silages were well preserved with mean dry matter (DM) of 33.4, 39.8 and 57.0% for RC+, RC- and PRG, respectively. PPO activity and phenol substrate concentration in RC before wilting was 69.4 and 20.8 ukatal/g DM and 2367 and 4100 µg/g FW for RC+ and RC-, respectively. Protein bound phenol (PBP; mg/g DM) as a measure of the degree of oxidation and therefore PPO protection in the silages was however comparable for the RC silages: 10.2, 9.6 and 3.0 for RC+, RC- and PRG, respectively. Rumen ammonia concentration was highest on RC- followed by RC+ and PRG: 173, 134 and 64.5 mg NH_3-N/L, respectively. Intake of N was higher on RC- than RC+ followed by PRG. However flow of N and amino acids followed the same pattern with both RC silages similar and greater than PRG (Table 1). There were no I x DF interactions.

Table 1 Effect of red clover silage with contrasting PPO levels versus grass silage on intake and duodenal flow of amino acids

	RC+		RC-		PRG		s.e.d		P value	
	Intake	Duodenal Flow	Intake	Duodenal Flow	Intake	Duodenal Flow	I	DF	I	DF
Dry Matter (kg/d)	9.26	6.42	9.40	6.55	8.76	6.21	0.344	0.437	NS	NS
Total Nitrogen (g/d)	256^{b}	280^{B}	279^{c}	291^{B}	138^{a}	197^{C}	6.7	14.3	***	***
Ala	63.8^{b}	61.0^{B}	67.7^{b}	67.5^{B}	38.1^{a}	47.0^{A}	1.71	4.57	***	**
Arg	36.4^{b}	47.2^{B}	43.3^{c}	43.8^{B}	20.9^{a}	32.1^{A}	1.54	3.79	***	**
Asp	209^{b}	112^{B}	230^{c}	125^{B}	52.5^{a}	82.5^{A}	6.40	8.17	***	**
Glu	96.1^{b}	122^{B}	107^{c}	135^{B}	39.5^{a}	90.8^{A}	2.32	8.96	***	**
Gly	46.6^{b}	71.3^{B}	50.8^{c}	78.4^{B}	26.3^{a}	46.7^{A}	1.31	5.57	***	**
His	22.9^{b}	23.0^{B}	24.6^{c}	25.8^{B}	9.84^{a}	15.0^{A}	0.602	1.57	***	***
Ile	53.5^{b}	53.8^{B}	57.7^{c}	60.0^{B}	26.7^{a}	39.5^{A}	1.37	3.93	***	**
Leu	86.8^{b}	84.1^{B}	94.9^{c}	94.2^{B}	45.6^{a}	59.3^{A}	2.37	5.82	***	***
Lys	73.3^{b}	72.0^{B}	78.6^{c}	80.7^{B}	35.2^{a}	54.6^{A}	1.77	5.46	***	**
Met	13.0^{b}	20.2^{B}	14.2^{c}	22.4^{B}	8.57^{a}	15.9^{A}	0.404	1.66	***	*
Phe	53.9^{b}	56.0^{B}	58.8^{c}	63.0^{B}	27.6^{a}	39.7^{A}	1.44	3.93	***	**
Pro	92.8^{b}	44.4^{B}	110^{c}	48.9^{B}	40.9^{a}	31.6^{A}	1.82	3.02	***	**
Ser	49.7^{b}	46.4^{B}	54.0^{c}	51.4^{B}	22.8^{a}	32.6^{A}	1.16	3.26	***	**
Thr	48.9^{b}	51.4^{B}	52.7^{c}	57.3^{B}	24.5^{a}	38.7^{A}	1.22	3.70	***	**
Tyr	28.0^{b}	39.7^{B}	31.9^{c}	44.5^{B}	11.1^{a}	25.5^{A}	1.29	3.47	***	**
Val	65.0^{b}	60.5^{B}	70.6^{c}	66.9^{B}	34.0^{a}	43.5^{A}	1.25	4.29	***	**
ΣAA DF / ΣAA I (g/g)	0.93^{a}		0.94^{a}		1.49^{b}		0.103		***	

Values with different superscripts (^{abc} for intake (I) and ^{ABC} for duodenal flow (DF)) differ significantly.

Conclusions Rumen ammonia-N was lower with PRG and RC+ than RC-, which reflects N intake. The lack of difference in N and amino acid duodenal flow between RC+ and RC- despite greater N intake for RC- may reflect a slight protective role of PPO for N. However the effect is small between RC+ and RC- in the study possibly as a consequence of similar PBP levels in the silage (Lee *et al.,* 2013)

Acknowledgement The work was funded by a DEFRA LINK project (LK0686) with project partners Germinal Holdings, AHDB-DairyCo, AHDB-EBLEX, HCC, QMS, LMC(NI), BGS

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Lee, M.R.F., Fychan, R., Barnes, B., Theobald, V., Tweed, J., et al. 2013. Proceedings of the BSAS, 65.
Van Ranst et al. (2011). Animal 5(4), 512-521.

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The effect of high versus low polyphenol oxidase red clover silage on nitrogen balance in dry cows

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Introduction Polyphenol oxidase (PPO) in red clover has been shown to reduce both proteolysis and lipolysis in silo (Lee *et al*. 2008) and the rumen (Lee *et al*. 2007). However all comparisons *in vivo* have compared red clover with other forages with typically lower levels of PPO, which brings in other confounding factors as to the cause for the greater flow of non-microbial non-ammonia nitrogen on red clover silage, other than just PPO. This study will compare two red clover silages which when ensiled had contrasting PPO activities against a control of perennial ryegrass silage to ascertain the full role of the enzyme in protecting dietary N in dry cows.

Material and methods Six barren non-lactating cows (mean liveweight 692 +/- 37.6 kg), were allocated to either: high PPO red clover silage (RC+); low PPO red clover silage (RC-) or perennial ryegrass silage (PR). All silages were fed at a fixed rate of 17 gDM/kg live weight. The experiment consisted of two 3 × 3 Latin-Squares. Each period was 28 d, consisting of 16 d adaptation to the diet, 7 d recovery after gluing equipment and 5 d for N balance. N balance was measured by collecting the total production of urine and faeces from each animal over a 5 d period, using externally applied urine and faeces separators (Aston *et al*., 1998). Statistical analysis was undertaken using ANOVA, blocking according to period + animal (Genstat 11.1).

Results All silages were well preserved with mean dry matter (DM) of 23.1, 26.4 and 37.7% for RC+, RC- and PRG, respectively. PPO activity and phenol substrate concentration in RC before wilting was 48.3 and 10.4 ukatal/g DM and 424 and 2552 µg/g FW for RC+ and RC-, respectively. Protein bound phenol (PBP; mg/g DM) as a measure of the degree of oxidation and therefore PPO protection in the wilted RC prior to ensiling was 11.8 and 8.5 for RC+ and RC- respectively. Figure 1 shows the N balance of the three silages with feed, faecal and urinary N all showing the same pattern of RC->RC+≥PRG. Both RC+ and PRG showed a small retained level of N, whereas RC- showed a small loss. Figure 2 shows the percentage of feed N converted into faecal, urinary and retained/lost N. No difference was found for faecal N, whereas for urinary and retained/lost PRG and RC+ were lower and higher than RC-, respectively.

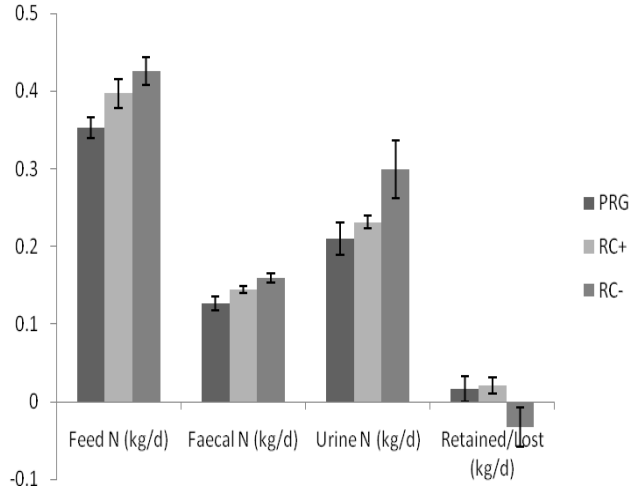

Figure 1 N balance – feed N intake and N outputs (kg/d)

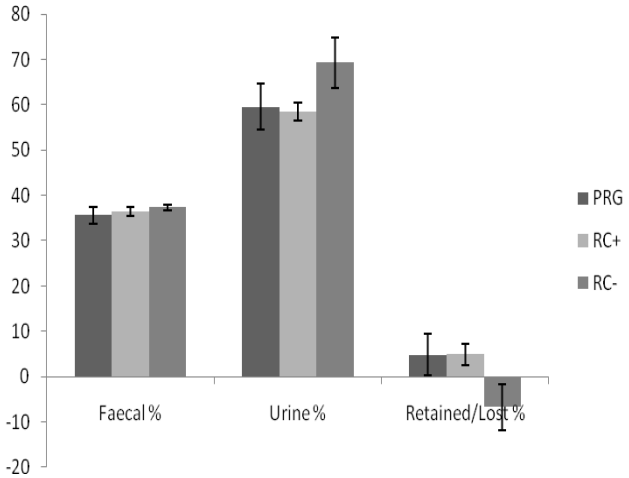

Figure 2 Percentage of dietary N in N outputs

Conclusions As reported previously (Lee *et al.,* 2013) RC+ despite having higher levels of PPO had lower levels of substrate than RC- resulting in smaller differences in the formation of PBP than would be expected as a result of non-PPO oxidation of phenol (Lee *et al.* 2012). Despite the small difference in PBP a significant effect in N balance was found with RC+ converting less N into urinary N than RC- with a greater proportion being retained. Further investigation is required to determine the relationship between PPO activity and substrate concentration to be able to select for high PPO cultivars with high levels of substrate. This may result in a greater protection of dietary N.

Acknowledgement The work was funded by a DEFRA LINK project (LK0686) with project partners Germinal Holdings, AHDB-DairyCo, AHDB-EBLEX, HCC, QMS, LMC(NI), BGS

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An evaluation of two sampling positions for estimating methane emissions from housed cattle using the SF_6 tracer technique

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Introduction The SF_6 technique for estimating methane (CH_4) emissions from ruminant livestock (Johnson *et al*., 1994) was conceived as a way to determine CH_4 emissions from free-ranging animals. However, it can also be applied to housed animals. While considered to be not as accurate as the direct measurement of CH_4 from individual animals in respiration calorimeters, the SF_6 technique has some advantages: it is less costly than calorimetry and it can be applied to large numbers of unconfined, grazing animals simultaneously. After preparation and intra-ruminal dosing with a slow-release source of SF_6, the apparatus required is minimal and consists of a length of flexible plastic tubing that connects an evacuated reservoir to a sampling port located over the nostrils and fixed to a head harness worn by the animal. Samples of exhaled breath mixed with ambient air are continuously drawn into the evacuated reservoir for periods of 20-24 hours and the ratio of CH_4:SF_6 in the composite sample is determined, allowing methane production rates to be calculated. When used with animals in closely confined accommodation (eg. stalls or digestibility crates), the sampling port and plastic tubing can be damaged by normal animal activity or blocked by water or feed particles, necessitating sometimes frequent repairs and adjustment. Both problems might be avoided by relocating the sampling port close to, but not on, the animal's head. The current study compared SF_6-derived CH_4 emissions from cattle housed in digestibility stalls, and using two sampling ports: one above the animal's nostrils ('on-animal') and another fixed to an adjacent surface ('off-animal').

Material and methods Eighteen dairy origin (Holstein-Friesian) bulls with a mean starting live weight of 265 kg (SD 13.5 kg) were intra-ruminally dosed with a brass SF_6 continuous-release permeation tube before being placed in digestibility crates and offered, *ad libitum*, one of three zero-grazed forages (immature upland permanent pasture, mature upland permanent pasture and an immature lowland perennial ryegrass sward; 6 animals per forage type). After adaptation to diet, each animal was fitted with a head harness and sampling apparatus for conventional 'on-animal' sampling (with the sampling port closely juxtapositioned above the nostrils) as devised by Johnson *et al* (1994). A second sampling port for 'off-animal' sampling was attached above the feed bin in each stall, close to, but not on, the animal's head (Fig 2). The two evacuated reservoirs required for each animal were suspended on the stall frame for protection and for ease of access for changing (every 24 hours). The SF_6: methane ratio was determined in each of the reservoirs within one working day after their removal and replacement each day and used to generate methane production values (g/d). 'Off-animal' methane emission values were subsequently regressed against 'on-animal' methane emission values (Fig 1).

Results The 'off-animal' and 'on-animal' mean CH_4 emission rates estimated for each animal were strongly correlated (R^2 = 0.8699; Figure 1) but significantly different (P <0.001) with the overall mean 'off-animal' value 9.6% higher than that derived by 'on-animal' sampling (Table 1).

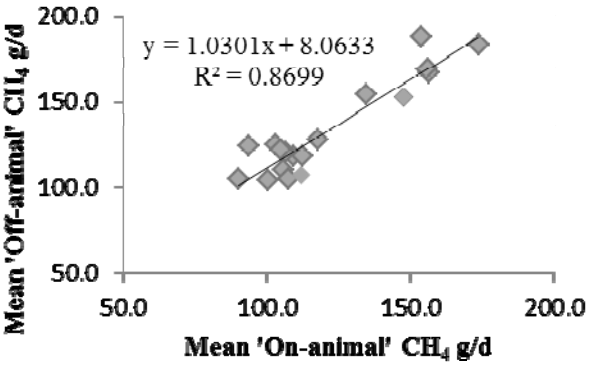

Figure 1 Comparison of off-animal and on-animal sampling protocols on CH_4 emissions estimated by the SF_6 technique

Table 1 Mean of all methane emission values (g/d) obtained by the on-animal and off-animal sampling techniques

	On-animal	Off-animal	S.E.M.	Sig.
Mean methane (g/d)	121.6	133.4	2.39	***

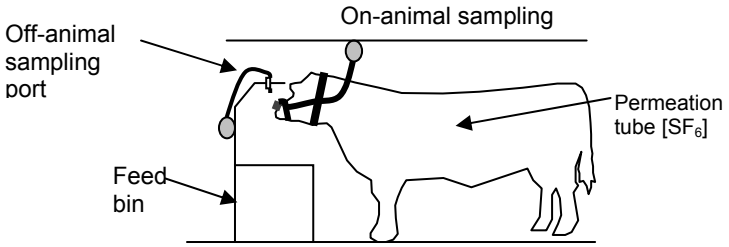

Figure 2 position of the on-animal and off-animal sampling ports

Conclusion The strength of the correlation between methane production estimated using the conventional 'on-animal' and novel 'off-animal' sampling protocols suggests that the technically easier and less intrusive 'off-animal' sampling protocol has potential as an alternative approach to acquiring methane emission data in housed cattle with minimal disturbance to the animal during reservoir changeovers and sampling line maintenance and repair. Further validation of the technique is required.

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Grass pellets acceptability by calves as influenced by age at harvest and feeding pattern

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Introduction Ruminants relish guinea grass but such forages become very scarce in the dry season. If grass of any age is effectively managed, it can strategically be exploited to ameliorate forage scarcity in the off season. Conservation through various means such as pellet production which can be stored for feeding during the dry season is needed to meet the nutritive needs of animals in periods when there is low availability of forage. Since ruminants according are the best assessors of the nutritive value of any feed, the aim of this work therefore to assess the acceptability of grasses at two difference ages when harvested and pelleted for dry season feeding.

Material and methods Guinea grass (*Panicum maximum*) and Elephant grass (*Pennisetum purpureum*) of four and eight weeks regrowth collected in triplicates were staged to provide forage regrowth either 4 or 8 weeks at the time needed for pelleting. The forages for pelleting was harvested and after dehydration the dried forage was milled with a hammer mill fitted with 3 mm sieve and pelleted using a 6mm mesh size to produce pelleted hay of average length of 4 cm. Water was used as the binding agent. A total of 12 pure bred Muturu calves of ages ranging from 9-12 months old were used with an initial adaptation period of 7 days when the animal are fed with *Andropogon gayanus* to accustom the animals to the pen conditions.
One kg of each sample of pelleted forage were measured and served each animal daily. Feed was delivered into feeders in a cafeteria style where confined animal were provided with several choice feed to select from. Feeds were placed in a pen that consisted of four containers. At the end of 15 min, the feeding bowls were withdrawn and the left-over in the plastic feeding bowls weighed and recorded to determine the intake per 15 min. This trial was recorded for 7 days.
In a second feeding experiment, all the animals were released in same holding yard. Where the four pelleted forages of known weights were placed in heaps in an enclosed yard into which animals were released. At the end of 15 min, the amount of grass remaining was weighed and recorded. A forage type was said to be preferred by animals to the others when its intake is more than others. All data were analysis using General Linear Model (GLM) procedures of SAS 2001 and separation of treatment means was done using Duncan Multiple Range Test.

Results Preference was observed using intake from the cafeteria method as recorded in figure 1.*P. purpureum* was more preferred than *P. maximum* either at 4 or 8 weeks old. Age at harvest influenced preference as forages pelleted at 4 weeks old had higher intake forages at 8 weeks old. Group feeding tended to show increased in intake as preference for pelleted samples increased than when served individually. Mustafa *et al* (2009) reported that cafeteria calves show more eating behavior and less idle standing, licking object and drinking behavior which led to increased ruminating and intake in comparison to individually fed calves.

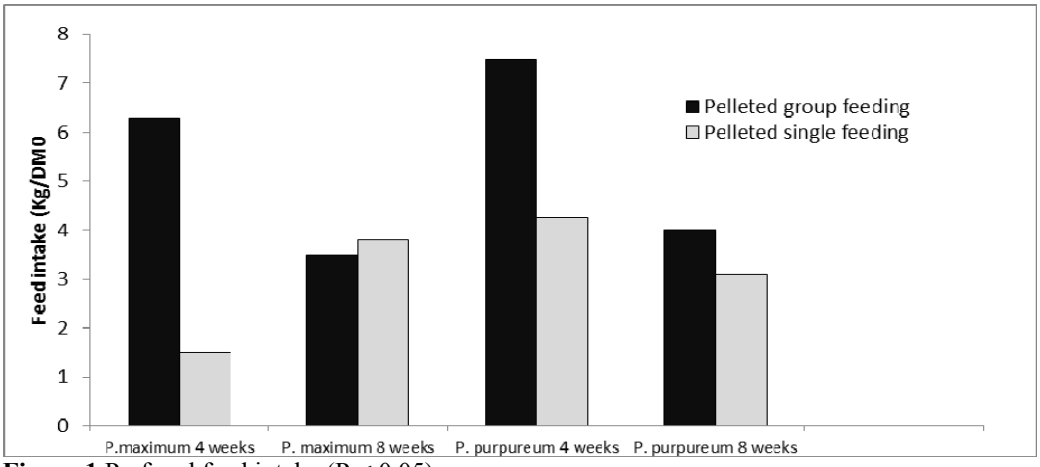

Figure 1 Prefered feed intake ($P < 0.05$)

Conclusion The instinct for completion invariably decreased idle standing which could probably result to increased intake of forages when served in group. Forage acceptability by animals on pasture or under zero grazing conditions is a function of inherent chemical traits which decline with age and reduced acceptability while processing samples into pellet reduced the surface area and digests easily with lesser retention time. Therefore forage harvested at 4 week and also processed into pellets increased digestibility and hence acceptability

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Effect of rate of inclusion of grass and maize silage fed without or with copper antagonists on the performance and indicators of copper status in dairy cows

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Introduction Copper (Cu) is one of the most important trace elements for normal health and performance in dairy cattle and is required by approximately 300 different proteins whose function range from antioxidants to release of hormones (Suttle, 2010). Clinical symptoms of Cu deficiency are often related to interactions with antagonists such as molybdenum (Mo) and sulphur (S) which form thiomolybdates and bind available Cu. It is recognized that the degree of thiomolybdate formation is dependent on the basal forage, although understanding of the mechanisms remains poor. The objectives of the current study were to determine the effect of inclusion rate of grass and maize silage fed without or with added S and Mo on the performance and Cu status of dairy cows.

Material and methods Fifty six Holstein-Friesian dairy cows that were 35 d (SE +/- 2.2) into lactation were blocked according to parity, days in milk, milk yield and live weight and randomly allocated to one of four dietary treatments for 14 wks. Two basal diets were formulated to have a forage to concentrate ratio of 53:47 (DM basis), and a grass to maize silage ratio of 0.75:0.25 (G) or 0.25:0.75 (M: DM basis). The diets were supplemented with $CuSO_4$ to contain 19 mg/kg DM. Background S and Mo concentrations were 1.26 g/kg DM and 1.32 mg/kg DM respectively. Each of the two basal diets were either unsupplemented (-) or supplemented (+) with additional S and Mo, to result in a total dietary concentration of 3.5 g S/kg DM or 7.5 mg Mo/kg DM. The 4 diets were therefore; 0.75 grass silage: 0.25 maize silage with no additional S and Mo (G-) or added S and Mo (G+); 0.25 grass silage: 0.75 maize silage with no additional S and Mo (M-) or added S and Mo (M+). The diets were fed as a total mixed ration which was provided once daily at 1.05 of *ad libitum* intake. Cows were milked twice daily at 06:00 and 16:00 h with milk yield recorded at each milking. Milk samples were taken weekly for subsequent analysis of fat and protein. Cows were weighed and condition scored fortnightly following the evening milking. Liver biopsy samples were taken by inserting a needle through the 11th intercostal space from all cows during week 0 and 14 of the study with blood samples collected via jugular venepuncture during weeks 0, 2, 4, 8 and 14 of the study. Liver and blood plasma samples were analysed for Cu and Mo by ICP-MS. Data was analysed as 2 x 2 factorial design with main effects of forage (F), antagonist (A) and their interaction (Int) using Genstat (v14.1).

Results The dry matter intake of cows offered M was approximately 2.2 kg/d higher ($P < 0.001$) than those offered G (Table 1). Adding S and Mo reduced DM intake by 2.1 kg/d in cows offered the grass but not maize silage based ration ($P < 0.05$). Adding S and Mo also decreased milk yield in cows offered G but increased yield in those offered M ($P < 0.05$). There was no effect of dietary treatment on milk composition but milk protein yield was higher ($P < 0.05$) in cows receiving M compared to G. Cows fed added S and Mo gained less condition over the study period than those that were not supplemented ($P < 0.05$). Final liver Cu concentration was lower ($P < 0.05$) in cows offered G vs. M and when additional S and Mo were fed. There was also a trend ($P = 0.08$) for an interaction between forage source and Cu antagonists on the rate of change in liver Cu concentration, with a net decrease of 0.62 mg/kg DM/d in cows fed G with added S and Mo, but increase of 0.11 mg/kg DM/d in cows fed M with S and Mo. There was no effect ($P > 0.05$) of dietary treatment on plasma Cu concentrations. Ceruloplasmin (CP) concentrations were higher ($P < 0.01$) in cows fed G vs. M and lower when additional S and Mo were fed. In contrast, there was no effect of dietary treatment on CP:Cu ratio, although there was a trend ($P < 0.1$) for a lower ratio in cows fed M compared to G, or in animals receiving added S and Mo.

Table 1 Effect of basal forage (Maize (M) or grass (G) silage based) fed without (-) or with (+) added S and Mo on the performance and Cu status of dairy cows

	Diet					Significance		
	M-	M+	G-	G+	s.e.d.	F	A	Int
Intake, kg DM/d	23.5	24.0	22.6	20.5	0.74	<0.001	0.111	0.012
Milk yield, kg/d	38.1	40.6	38.9	37.9	1.22	0.225	0.373	0.034
Milk protein yield , kg/d	1.23	1.30	1.22	1.21	0.034	0.049	0.242	0.142
Body condition change	0.35	0.13	0.27	0.09	0.114	0.470	0.019	0.801
Final liver Cu, mg/kg DM	587	437	490	357	58.0	0.038	0.002	0.837
Liver Cu change, mg/kg DM/d	0.66	0.11	0.84	-0.62	0.357	0.275	0.001	0.078
Plasma Cu, µmol/L	13.3	13.7	14.3	13.7	0.77	0.340	0.889	0.332
Ceruloplasmin, mg/dL	17.9	15.9	20.3	18.1	1.12	0.006	0.010	0.909
Ceruloplasmin:Cu	1.37	1.22	1.41	1.36	0.081	0.096	0.090	0.377

Conclusions A higher level of maize inclusion increased DM intake and milk protein yield. The addition of S and Mo reduced feed intake and milk yield in cows fed the grass but not maize silage based ration. The addition of S and Mo also reduced liver Cu status by a greater extent in cows fed grass compared to maize silage, but this was not reflected in plasma Cu or ceruloplasmin:Cu ratio.

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Effect of forage type on the efficiency of energy utilization in lactating dairy cows

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Introduction Manipulation of dietary fibre and starch contents can alter the rumen fermentation pattern and consequently influence nutrient degradability and the efficiency of energy utilisation of the host animals. The aim of this experiment was to investigate how different forage types affected energy utilization in lactating dairy cows.

Material and methods The experiment was designed to evaluate energy metabolism of 6 mid lactation Holstein cows offered 3 forages (grass silage, maize silage and whole crop wheat (WCW)) in a complete changeover study with 3 periods of 3 weeks/period. The forages were offered *ad libitum* together with 5 kg/d (fresh weight) of a protein supplement (3.5 kg soybean meal and 1.5 kg rapeseed). This supplement was used to balance the protein supply for maize silage and WCW diets. In addition, each animal was given 140 g/d of a Vit/Min supplement (V/M 208). In each period, the animals were housed in cubicle accommodation for the first 13 days, and then transferred to digestibility units and remained there for 5 days with feed intake and faecal and urine outputs collected for the final 3 days. Afterwards they were housed in calorimeter chambers for 3 days with gaseous exchange measured for the final 2 days. Grass silage, maize silage and WCW contained g/kg DM: ash 110, 35 and 38; CP 163, 84 and 99; ADF 311, 260 and 210; NDF 547, 460 and 379; and starch 0, 274 and 352, respectively. The details on data collection, feed intake and milk production were reported by McCourt *et al*. (2007). Data were analysed as one-way ANOVA with treatment as block using the Genstat statistical package.

Results The effects of the 3 silages on the efficiency of energy utilization are presented in Table 1. Cows offered grass silage had lower GE intake (P<0.001) and lower faecal (P<0.001) and methane (P<0.01) energy outputs, but higher urinary energy output (P<0.05) compared to those given maize silage and WCW silage. The WCW silage and maize silage produced a significantly higher milk energy output than grass silage (P<0.05). However, neither heat production nor energy retention was affected by treatments. Moreover, feeding of grass silage resulted in the highest DE/GE (P<0.05) and ME/GE (although the differences were not significant). There were no significant differences among the 3 diets in terms of heat production/ME intake, milk energy/ME intake or the efficiency of use of ME for milk production (ME requirement for maintenance estimated from Agnew *et al*., 2004).

Table 1 Effects of forage type on energy intake and outputs and the efficiency of energy utilization

		Grass silage	Maize silage	WCW silage	SEM	Sig.
Energy intake and output (MJ/d)	GE intake	293[a]	366[b]	358[b]	8.7	***
	Faecal energy	65[a]	102[b]	101[b]	5.7	***
	Urinary energy	15.2[b]	11.9[a]	11.6[a]	0.86	*
	Methane energy	19.4[a]	25.8[c]	21.3[ab]	0.98	**
	Heat production	123	134	130	3.0	NS
	Milk energy	41.7[a]	53.9[b]	55.9[b]	3.02	*
	Energy retention	29.3	38.8	37.3	6.84	NS
Energy utilization efficiencies	DE/GE	0.78[b]	0.72[a]	0.72[a]	0.014	*
	ME/GE	0.66	0.62	0.63	0.016	NS
	Heat production/ME intake	0.64	0.60	0.58	0.022	NS
	Milk energy/ME intake	0.21	0.24	0.25	0.014	NS
	k_l	0.59	0.62	0.64	0.026	NS

Sig. = significance; *** = P<0.001, ** = P<0.01, * = P<0.05, NS = not significant
[a,b,c] means within rows with same superscripts are not significantly different (P>0.05)

Conclusions Dairy cows offered grass silage diets had lower GE intake and milk energy output than those given maize silage diets or WCW silage diets. However, the forage type had no significant effects on the rate of energy portioning into milk or the efficiency of use of ME for milk production.

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Effect of concentrate allocation strategy on the performance of high-yielding dairy cows

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Introduction Concentrate feed levels on many dairy farms have increased considerably in recent years (from approximately1.1 tonnes/cow/year in 1998 to 2.3 tonnes/cow/year in 2012). During this same period 'milk produced from forage' decreased from approximately 3200 to 2150 litres/cow/year. Concentrate inputs have increased for a number of reasons, including historically low world cereal prices, and the rapid increase in the genetic merit for milk yield of dairy cows during the last two decades. As concentrate feed levels have increased, out-of-parlour feeders and complete diet mixer wagons have increasingly replaced in-parlour feeding systems. However, at high concentrate feed levels, concentrate feeding system and allocation strategy become much more important. In addition, as electronic feeding systems become more advanced, it is possible to add a much greater degree of 'precision' to allocation strategies. Nevertheless, the impact of different feeding systems, and of the benefits of increased 'precision' within allocation strategies, is still subject to debate. To address this issue, a study was undertaken to examine a number of concentrate allocation strategies.

Material and methods From calving onwards, ninety autumn-calving Holstein Friesian cows (mean parity 3.2) were allocated to one of four concentrate allocation strategies. All treatments were targeted to achieve an average concentrate intake / cow / day of 12 kg (fresh), with 1.0 kg of this concentrate being offered daily via 'in-parlour' concentrate feeders. The concentrate allocation of 12.0 kg / cow / day was designed to meet the expected nutrient requirements of the average cow on the experiment. With treatment 'Complete diet, flat rate', all cows were offered a complete diet designed to provide an average concentrate intake for the group of 11.0 kg / cow / day. With treatment 'Out-of-parlour feeding, flat rate', all cows were offered exactly 11.0 kg concentrate per day through an out-of-parlour feeder. With treatment 'Feed-to-yield', cows were allocated to one of three concentrate curves based on milk production in week 2 and 3 of lactation. Concentrates were offered through an out-of-parlour feeder with the aim of achieving an average concentrate intake for the group of 11.0 kg / cow / day. With treatment 'Basal diet + feed-to-yield', all cows were offered a basal ration containing 6.0 kg concentrate / cow / day as well as being allocated to one of three concentrate curves based on milk production in week 2 and 3 of lactation. This treatment was designed to provide an average concentrate intake for the group of 11.0 kg / cow / day. The forage component of the diet contained 70% grass silage and 30% maize silage on a dry matter (DM) basis. Cows remained on these four treatments until day-150 of lactation. Data were analysed using the residual maximum likelihood procedure via Genstat.

Results Total dry matter intake, milk yield and milk composition were unaffected by concentrate allocation strategy (Table 1). Forage intake was significantly higher in the basal diet + feed-to-yield system compared any of the other feeding systems. There was no effect of concentrate allocation strategy on body condition score or live weight.

Table 1 Effect of concentrate allocation strategy on DM intake, milk production and energy balance (day 1-150 of lactation)

	Complete diet, flat rate	Out-of-parlour feeding, flat-rate	Feed-to-yield	Basal diet + Feed-to-yield	SED	P value
Total intake (kg DM / day)	21.7	22.2	21.8	22.8	0.53	0.089
Forage intake (kg DM / day)	12.2	11.9	11.6	12.8	0.28	<0.001
Concentrate intake (kg DM / day)	9.6	10.3	10.2	9.8	0.49	0.450
Milk yield (kg / day)	38.7	40.8	42.1	39.3	1.42	0.100
Milk fat (g / kg)	44.0	43.4	42.2	43.2	1.28	0.439
Milk protein (g / kg)	32.5	32.5	32.6	32.6	0.54	0.971
Milk fat + protein yield (kg / day)	3.1	3.2	3.2	3.1	0.12	0.468
Live weight (kg)	643	654	645	646	18.8	0.908
Body condition score	2.4	2.5	2.5	2.6	0.10	0.732

Conclusions Across the four very different concentrate allocation strategies examined, treatment had very little effect on cow performance. Thus when allocating concentrates to meet the expected nutrient requirements of the average cow in a group of cows with a tight calving pattern, concentrate allocation strategy will have little effect on overall production performance.

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Effect of different lipid supplements on the fatty acid profile of bulk milk from commercial dairy herds

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Introduction Milk and dairy products are a major source of saturated fatty acids (SFA) in UK diets (Hulshof *et al*., 1999). Inclusion of unsaturated lipid supplements in dairy cow diets has been shown to replace medium chain SFA in milk fat with unsaturated fatty acids and 18:0 (e.g. Kliem *et al*., 2011), but there is very little information on the effects of such supplements at the commercial farm level, where variations in management arise. To implement large scale changes in dairy cow nutrition in order to reduce milk SFA concentrations at source, it is essential to assess the efficacy dietary supplements in practice. The objective of this study was to determine whether the inclusion of four different types of lipid supplements exerted similar effects on milk fatty acid composition to those seen in research, across a number of commercial herds.

Material and methods Twenty-two commercial herds (mean ± SEM annual yield 8460 ± 267.7 L, and mean ± SEM cows in milk 162 ± 17.8) in the South West of the UK were recruited from one milk purchaser. Farms were divided into one of four treatment groups depending on supplement fed which were: calcium salts of palm oil fatty acids (CPO; n=5), extruded linseed (EL; n=6) calcium salts of palm/linseed fatty acids (CPL; n=6) and milled rapeseed (MR; n=5). For a four week period over the winter of 2011/2012 each farm supplemented all cows on farm with an inclusion rate that supplied each cow with 350 g lipid/day. Bulk milk was preserved and analysed at every collection for total butterfat, protein, total SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) by Fourier-Transform Infra-Red spectrometry (Foss UK) prediction. Data were averaged per week (0-4), and analysed using the Mixed Model procedure of SAS, with a model that included repeated fixed effect of week and random effect of farm. Baseline concentrations (week 0) and amount of supplement lipid (g/cow/day) fed prior to the four week period were included as covariates.

Results Supplements EL, CPL and MR decreased (P<0.001) milk SFA concentration compared with the baseline value (week 0; Fig. 1), but there was no effect of CPO (P=0.149). When treatments were compared within week, there was no difference (P>0.05) between EL, CPL and MR for week 4, thus they had similar effects on SFA concentrations. The average decrease over the four week period was 3.4 g/100 g fatty acids. Total MUFA increased (P<0.001) over four weeks for EL, CPL and MR (mean increase of 2.4 g/100 g fatty acids; Fig. 2). Again there was no effect (P=0.986) of CPO, and there was no difference (P>0.05) between the oilseed-based treatments at week four. Total PUFA response varied according to treatment; the oilseed (linseed and rapeseed) supplements increased (P<0.05) PUFA but at week four EL was higher (P<0.01) than CPL and MR. CPO had no effect (P>0.05). Milk fat concentration was decreased by EL (P=0.002) but the other supplements had no effect (P>0.05). Milk protein concentration decreased (week effect, P<0.01), but there was no treatment effect (P=0.122).

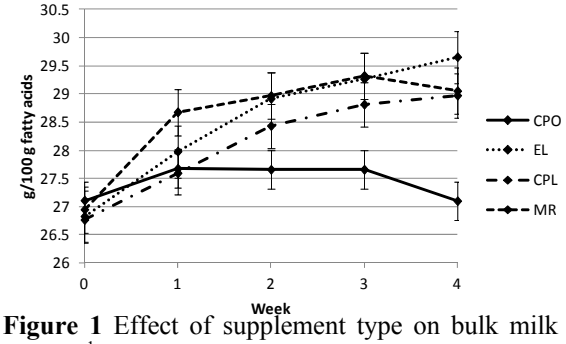
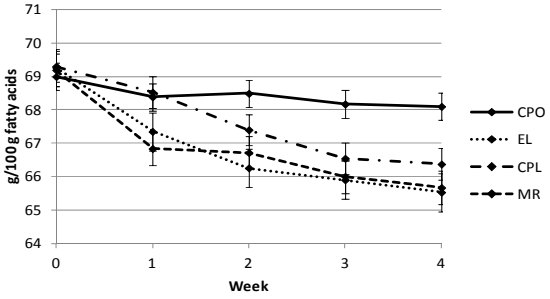


Figure 1 Effect of supplement type on bulk milk fat SFA[1] concentration over four weeks



[1]SFA – saturated fatty acids, MUFA – monounsaturated fatty acids

Figure 2 Effect of supplement type on bulk milk fat MUFA[1] concentration over four weeks

Conclusions The results demonstrate that lipid supplements based on linseed or rapeseed decrease SFA concentration in bulk milk across a pool of milk producers. The SFA were replaced by both MUFA and PUFA. The EL, CPL and MR supplements were equally effective at achieving these changes, although the increase in PUFA was greater for EL.

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Effect of dietary form of copper fed with or without added sulphur and molybdenum on the indicators of copper status and expression of copper transporters and chaperones in dairy cows

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Introduction Dietary copper (Cu) is one of the most important trace elements for normal health and performance in dairy cattle (Suttle, 2010). Interactions between Cu, sulphur (S) and molybdenum (Mo) reduce Cu bioavailability either by the formation of insoluble thiomolybdates in the rumen or, in the absence of adequate Cu, are absorbed and bind to Cu present in biological compounds. Cellular Cu homeostasis is mediated by transporters and chaperones including COX17, CTR1 and ATP7B, although the effect of form of dietary Cu or the presence of Cu antagonists such as S and Mo on gene expression is unclear. The objectives of the current study were to determine the hepatic expression of key members of the copper transport system in response to inorganic or organic forms of dietary Cu in the presence or absence of added S or Mo.

Material and methods Fifty six Holstein-Friesian dairy cows that were approximately 35 d into lactation were blocked according to parity, days in milk, milk yield and body condition and randomly allocated to one of four dietary treatments for 16 wks. Cows were fed a basal ration composed of concentrates, grass and maize silages which was predicted to contain 6.99 mg Cu/kg DM, 1.67 mg Mo/kg DM and 1.68 g S/kg DM. The basal diet was supplemented with 10 mg/kg DM as $CuSO_4$ (I) or organically complexed Cu (Bioplex®, Alltech UK; O), and received either no added S or Mo (-) or added S and Mo to result in a dietary concentration of 3.5 g/kg DM S and 8.5 mg/kg DM Mo. The four dietary treatments were therefore $CuSO_4$ fed without (I-) or with (I+) added S and Mo mix, and organicially complexed Cu fed without (O-) or with (O+) added S and Mo. Cows were milked twice daily at 06:00 and 16:00 h with intake recorded daily. Gene expression in the liver was estimated using mRNA extracted from liver biopsies taken at wk 0 (control) and wk 16 (test). Changes in expression were calculated using the Pfaffl method and were expressed as wk 0:16 ratios. Fold changes were normalised against a reference gene (GAPDH). Associations between gene expression and Cu source, antagonist inclusion and their interaction were assessed using ANOVA in Genstat 14.1.

Results There was an interaction (P = 0.025) between Cu source and antagonist on DM intake, with cows offered I- having an increased DM intake compared to those offered I+ or O-. Milk yield was 5% higher in cows fed I vs O (P < 0.05). Hepatic Cu concentration decreased to a greater extent (P < 0.001) in cows when fed S and Mo, with a mean reduction of approximately 0.9 mg/kg DM/d, compared to an increase of 0.13 mg/kg DM/d for animals not receiving added S and Mo. There was no effect (P > 0.05) of dietary form of Cu on the rate of change or final hepatic Cu concentration. There was a trend (*P* = 0.06) for ATP7B to be up regulated in cows fed S and Mo along with O but not I. There was no effect (P > 0.05) of dietary Cu source or the inclusion of Cu antagonists on hepatic mRNA expression of ATOX1, ceruloplasmin or CTR1. There was no effect of treatment on the expression of the reference gene (P > 0.05).

Table 1 Effect of diet on performance and daily change in hepatic Cu concentration

	Diet					Significance (P)[2]		
	I-	I+	O-	O+	s.e.m.	Cu	Ant	Int
Intake, kg DM/d	22.6	20.8	21.0	21.4	0.54	0.271	0.134	0.025
Milk yield[1], kg/d	33.0	32.9	31.5	33.5	1.10	0.825	0.254	0.511
Hepatic Cu change, mg/kg DM/d	0.33	-0.91	-0.07	-0.87	0.197	0.650	<0.001	0.260

[1]adjusted to 38 g fat/kg; [2]Cu = main effect of Cu source, Ant = main effect of antagonists and Int = interaction between Cu and Ant

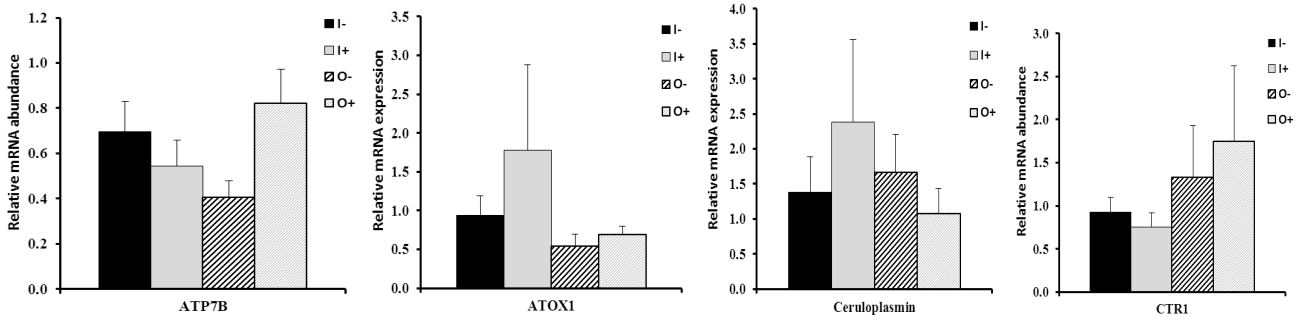

Figure 1 Hepatic mRNA relative expression of a) ATP7B, b) ATOX1, c) ceruloplasmin and d) CTR1. Values are expressed relative to GAPDH and are presented as the ratio of the value obtained at wk 16 to wk 0 of the study. Error bars indicate SE.

Conclusions Form of dietary Cu had no effect on fat adjusted milk yield. Added S and Mo reduced DM intake in cows fed the inorganic but not organic Cu. Added S and Mo also reduced liver Cu levels by approximately 0.9 mg/kg DM/d. There was no effect of dietary treatment on liver copper chaperone or transporter mRNA levels, except ATP7B which tended to be increased in cows when fed the organic, but not inorganic Cu with added S and Mo.

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Milk yield and composition in dairy cows fed sugar cane-based diets with different levels of sunflower oil

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Introduction Investigations into nutraceuticals have stimulated consumers' demands for healthier products and new scientific data relating to their effects on human health. Milk fat from ruminants contains several components that promote beneficial human health effects, and conjugated linoleic acids (CLA) are given special attention due to their known anticarcinogenic properties. Sugarcane is an important tropical gramineous species of plant because of its potential for high dry matter production per unit area, low-cost per dry matter unit produced and its period of maturity coincides with a period of pasture shortage. However, no studies in the literature have reported its potential as a forage in association with vegetable oils as most research on CLA has been performed in temperate areas, compared to the tropics. Therefore, the purpose of this study was to assess diets based on sugarcane with different concentrations of sunflower oil (SFO) with respect to the milk yield and composition of dairy cattle.

Material and methods Four multiparous Holstein x Gir cows with a 107±10 lactation days and average milk production of 15±5 kg/d with cannulas in the rumen received four dietary treatments (levels of SFO inclusion, % diet dry matter, DM) *ad libitum* in a 4 x 4 Latin Square design composed of 19-day experimental periods (10 days for adaptation and the last 9 days for data collection).The treatments were: 1) Control: diet without SFO; 2) SFO1: diet containing 1.5% of SO; 3) SFO2: diet containing 3.0% of SFO and 4) SFO3: diet containing 4.5% of SFO. The diets were isoproteic with 14.5% crude protein (CP) in accordance with the NRC, 2001 and fed once a day as total mixed rations (TMR) composed of whole sugarcane plant and a concentrate mixture (60:40, % of diet DM). Milk samples were collected from morning and afternoon milking (6:00 and 14:00 h, respectively) during the last 6 days of each experimental period and analyzed for total solids, fat, protein, lactose contents using the technique described by the IDF (1996). The chemical analyzes were analysed following the procedures described in AOAC (2000). The neutral detergent fiber content corrected for ash and protein (NDFap) was estimated following Mertens' (2002) recommendations. Humane animal care and handling procedures were followed in accordance with the Federal University of Viçosa guidelines (Viçosa, MG, Brazil). The results were analysed through variance and regression with the *Statistical Analysis System* software (SAS, 2002) at a 5% probability for type I error.

Results The different levels of SFO had no effect (P>0.05) on DM, CP, and NDFap intakes. However, there was linear increase (P<0.05) in EE intake (Table 1). The linear increase in EE intake (P<0.05) for the cows supplemented with SFO may be explained as a function of the increase in EE content from the experimental diets without a significant difference in DM intake. Dietary SFO affected neither (P>0.05) milk yield (corrected or not corrected for 3.5% fat) nor protein and lactose content. However, fat content (P<0.05) decreased linearly with SFO.

Table 1 Daily nutrient intake and milk composition of cows fed different levels of SFO

Items	SFO levels				MSE[1]	Effect (P value)	
	0	1.5	3	4.5		L	Q
DM (kg/day)	14.6	15.5	16.1	14.4	0.703	ns[2]	ns
CP (kg/day)	1.9	2.0	2.0	1.8	0.091	ns	ns
EE (kg/day)	0.3	0.5	0.7	0.9	0.039	<0.001	ns
NDF ap(kg/day)	5.0	5.4	5.8	5.1	0.289	ns	ns
Milk yield (kg/day)	15.1	15.6	16.1	15.1	0.831	ns	ns
Corrected yield (kg/day)	15.5	14.9	13.8	13.0	1.070	ns	ns
Fat (%)	3.7	3.3	2.7	2.6	0.165	<0.01	ns
Protein (%)	3.3	3.2	3.3	3.4	0.053	ns	ns
Lactose (%)	4.3	4.4	4.3	4.3	0.032	ns	ns
Regression equations							r^2
EE intake (kg/cow/day)	$\hat{y} = 0.28 + 0.14xX$						0.96
Fat (%)	$\hat{y} = 3.62 - 0.25xX$						0.88

[1]MSE = Mean standard error; [2]ns = not significant (P>0.05); r^2 = coefficient of determination

Conclusions Inclusion of up to 4.5% SFO in sugarcane-based diets affects neither the dry matter intake nor milk yield in Holstein x Gir, but it reductions in levels of milk solids, as fat can pose financial implications in view of the producer subsidy schemes adopted by Brazilian dairy.

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A survey of dietary levels of selected macro and trace elements fed on UK dairy farms

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Introduction Minerals are a key component in the diet of dairy cows and their effects on performance, health, fertility and welfare are well documented. There is evidence that dairy farms in the UK are feeding minerals in excess of requirements (e.g. Bidewell *et al*., 2000), although other farms may be under supplementing. There is however, a lack of accurate information on mineral intake levels on UK dairy farms, particularly in situations where minerals are provided from several sources. The objectives of the current study were to determine the intake of several important minerals on UK dairy farms from all dietary sources and compare these to recommended levels.

Material and methods A random sample of 50 dairy farms located in the Midlands and North of England were visited once between November 2011 and March 2012. Of the farms visited a maximum of 7 were using one feed company or nutritionist, and a total of 10 farms had organic accreditation. The basal ration fed to the high and low (where appropriate) groups of milking cows were sampled within 10 min of feed delivery, with forage and concentrate samples collected as required. A sample of fresh drinking water from the running water in a trough was also collected. All samples were stored at -20°C prior to analysis. Feed samples were dried, ground and their mineral content determined by ICP-MS. Dry matter intake (kg/d) was estimated as per NRC (2001), with water intake estimated according to Little and Shaw (1978). The quantity of mineral supplied by additional sources (e.g. bolus, mineral lick block, free access minerals, injections) on the day of the visit was calculated using the farmers recorded rate of use and the stated mineral concentration of the product. The sum of the calculated daily supply of minerals from the diet, water and supplementary sources was divided by the daily DM intake to provide a mineral concentration (g or mg/kg DM). Data were analysed using Genstat (version 14.1) and results compared to recommended levels (NRC 2001).

Results The mean milk yield of the farms sampled was 7982 kg, ranging from 4000 to 12,600 kg. Mineral lick blocks were the most popular source of supplement used, being offered on 54% of the farms surveyed, with rumen boluses used on 36% of farms. The calculated mean and median concentration of macro-minerals in the diet of early lactation dairy cows was in excess of recommended guidelines (Table 1). For example, there was on average a 47% excess of Ca and 20% excess of P, with some farms feeding up to 205% excess Ca and 65% excess P. In contrast, some farms were feeding below recommended levels, with a minimum Ca concentration of 5.08 g/kg DM, 22% below the recommend dietary concentration, whilst the lowest P level fed was 27% below requirements. Similarly, compared to recommended values, mean and median trace-mineral concentrations were in excess of requirements. For example, Cu was on average, 120% in excess with the mean dietary concentration of Zn being 58% above requirements, whilst Fe and Mn were also well in excess. When accounting for all sources of Cu, 4 farms were supplying above 40 mg/kg DM with a total of 31 out of 50 feeding above 20 mg/kg DM. Dietary concentrations of Mo were generally low at 1.18 mg/kg DM, although one farm had a high concentration at 5.16 mg/kg DM.

Table 1 Selected mineral concentrations in the diet of in 50 herds in the UK.

	NRC (2001) recommendations	Mean	SD	Median	Max	Min
Macro elements (g/kg DM)						
Sodium (Na)	2.2-2.3	3.15	0.82	3.15	5.04	0.74
Potassium (K)	10-10.7	22.6	4.24	22.1	33.8	14.6
Calcium (Ca)	6.2-6.7	9.46	2.91	8.39	19.7	5.08
Magnesium (Mg)	1.8-2.1	3.11	0.68	2.96	4.97	1.78
Phosphorus (P)	3.2-3.8	4.20	0.71	4.17	5.76	2.56
Trace elements (mg/kg DM)						
Copper (Cu)	11	24.2	8.35	22.9	44.3	12.9
Zinc (Zn)	43-55	77.5	28.5	72.1	169	30.8
Iron (Fe)	12.3-18	315	99.6	308	591	111
Manganese (Mn)	13-14	100	31.1	97.7	193	41.2
Molybdenum (Mo)	--	1.18	0.84	1.02	5.16	0.28

Conclusions When accounting for the mineral supply from all dietary sources there was a considerable range in the quantity of minerals being fed with most farms feeding excess, although some were underfeeding. The particularly high dietary concentration of Cu did not appear to be justified by high dietary Mo levels.

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Metabolic parameters in lactating cows fed sugar cane-based diets with different levels of sunflower oil

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Introduction Fats are supplemented to increase ration energy density, in this context, fat is the most variable component in milk and is markedly affected by diet, in other words, nutrition affects both the quantity and composition of milk fat and a striking example is milk fat depression. Sugarcane is exceptional among tropical gramineous plants because of its potential for dry matter production per unit area and low-cost per dry matter unit produced. Also, sunflower oil (SFO) provides 13% of the worldwide production of edible vegetable oils, and it has excellent nutrition characteristics, including a high ratio of polyunsaturated to saturated fatty acids (65.3% and 11.6%, respectively). The polyunsaturated fatty acid content almost entirely comprises linoleic acid (65%), which is classified as essential because it is not synthesised by the animal and is involved in several physiological functions (Andrade, 1994). However, no studies in the literature have reported its potential use or association with vegetable oils because virtually all research has been performed in temperate areas, and little research has been conducted in the tropics. Thus, the purpose of this study was to assess diets based on sugarcane with different concentrations of sunflower oil (SFO) with respect to the metabolic parameters in dairy cattle.

Material and methods Four multiparous Holstein x Gir cows with a 107±10 lactation days and average milk production of 15±5 kg/d with cannulas in the rumen received four dietary treatments (levels of SFO inclusion, % diet dry matter, DM) in a 4 x 4 Latin Square design composed of 19-day experimental periods (10 days for adaptation and the last 9 days for data collection).The treatments were: 1) Control: diet without SFO; 2) SFO1: diet containing 1.5% of SO; 3) SFO2: diet containing 3.0% of SFO and 4) SFO3: diet containing 4.5% of SFO. The diets were isoproteic (14.5% CP) in accordance with the National Research Council (NRC, 2001) and fed once a day as total mixed rations (TMR) composed of sugarcane and a concentrate mixture (60:40, % of diet DM). Blood samples were collected on day 16 using coccygeal venipuncture and test tubes (Vacutainer, Becton-Dickinson, Rutherford, NJ) with an anticoagulant (ethylenediaminetetraacetic acid – EDTA); they were centrifuged immediately at 3,000 x *g* for 15 minutes, after were collected, placed in capped plastic tubes and stored at –20°C for future analyses of nonesterified fatty acids (NEFA), glucose, cholesterol, triglycerides and high-density lipoprotein (HDL).. A commercial kit was used to measure the serum levels of NEFA (kit FA 115 - Randox®, UK), and the glucose, triglycerides and total cholesterol serum levels were measured using the enzymatic colorimetric method described by Trinder (1969). Humane animal care and handling procedures were followed in accordance with the Federal University of Viçosa guidelines (Viçosa, MG, Brazil). The results were analysed through regression with the *Statistical Analysis System* software (SAS, 2002) at a 5% probability.

Results The serum concentration of cholesterol and HDL had quadratic effect (P<0.05) in the cows fed increasing levels of SFO (Table 1). This increase in the serum lipid concentration may be explained as a function of the higher intake of fatty acids associated with the experimental diets, which increased the corresponding fractions of fatty acids related to lipid metabolism in the blood.

Table 1 - Mean blood parameters in lactating cows fed different levels of SFO

Variable	SFO levels				MSE[1]	Effect (P value)	
	0.0	1.5	3.0	4.5		L	Q
Glucose (mg/dL)	52.52	52.91	58.46	56.26	1.63	ns[2]	ns
NEFA (mmol/L)	0.17	0.22	0.24	0.16	0.04	ns	ns
Cholesterol (mg/dL)	88.73	137.3	186.66	159.28	12.75	<0.01	0.03
HDL (mg/dL)	47.76	80.13	74.21	60.53	10.14	ns	0.02
Triglycerides (mg/dL)	3.27	3.43	3.86	4.44	0.97	ns	ns
Regression equations							r^2
Cholesterol (mg/dL)	$\hat{y} = 84.855 + 55.376*X - 8.439*X^2$						0.73
HDL (mg/dL)	$\hat{y} = 49.289 + 25.184*X - 5.117*X^2$						0.85

[1]MSE = Mean standard error; [2]ns = not significant (P>0.05); r^2 = coefficient of determination

Conclusions Inclusion of up to 4.5% SFO in sugarcane-based diets has no effect on blood-lipid metabolism, however had quadratic effect in the serum concentration of cholesterol and HDL in Holstein x Gir.

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Efficiency of the use of nitrogen parameters in dairy cows fed sugar cane-based with different levels of sunflower oil

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Introduction Lipid supplementation the diet of dairy cows aims to increase the energy density of the diet, however, considerable amounts can affect the performance of the animal. Milk composition can be changed when providing lipids in ruminant diets. A major deleterious effect of the inclusion of high concentrations of lipids in the diet of ruminants is the reduction in ruminal fiber digestion. Thus, the amounts and proportions of volatile fatty acids produced in the rumen can be negatively altered, especially the acetate:propionate ratio, promoting the reduction of milk fat. In addition milk protein is reduced due to reduction of microbial synthesis, since lipids are not energy sources for microbial growth or reduction in the availability of amino acids in the mammary gland.The purpose of this study was to assess diets based on sugarcane with different concentrations of sunflower oil (SFO) with respect to nitrogen use efficiency parameters in dairy cattle.

Material and methods Four multiparous Holstein x Gir cows in lactation for 107±10 days with average milk production of 15±5 kg/d) fitted with rumen cannulas received four dietary treatments (levels of SFO inclusion as % of diet DM) in a 4 x 4 Latin Square design composed of 19-day experimental periods (10 days for adaptation and the last 9 days for data collection). The treatments were: 1) Control: diet containing no SFO; 2) SFO1: diet containing 1.5% SFO; 3) SFO2: diet containing 3.0% SFO and 4) SFO3: diet containing 4.5% SFO. Diets were isoproteic (14.5% CP) and fed *ad libitum* once a day as total mixed rations (TMR) composed of whole sugarcane plant and a concentrate mixture (60:40, % of diet DM). The production of faecal DM was estimated from samples collected over six consecutive days using indigestible NDF as an internal indicator. Milk samples were collected at the morning and afternoon milking (6:00 and 14:00 h, respectively) during the last 9 days of each experimental period and analyzed for urea and alantoin content. Spot urine samples were acquired on day 13 of each experimental period four hours after the morning feeding during spontaneous urination .The allantoin, cratinine and acid uric in urine and milk was measured by the colorimetric method in accordance with Fujihara *et al*. (1987), whereas the urea content was measured using an enzymatic colorimetric method with an equivalence point reaction (Bergmeyer, 1985). The total levels of purine derivatives (PD) excreted were calculated by Verbic *et al*. (1990) and ruminal N compound synthesis was calculated based on Chen & Gomes (1992). The results were analysed by regression with the *Statistical Analysis System* software (SAS, 2002) at a 5% probability.

Results A linear decreasing effect of different levels of SFO on the excretion of purine derivatives was found (Table 1). This decrease reflected in lower intestinal absorption of purines, lower production of microbial protein in the rumen and lower microbial efficiency.

Table 1 Means of N use efficiency parameters in lactating cows fed different levels of SFO

Items	SFO levels				MSE	Effect (P value)	
	0.0	1.5	3.0	4.5		L	Q
N intake (g/day)	296.4	312.9	318.7	287.3	14.623	ns[2]	ns
Faecal N (g/day)	90.3	100.6	97.3	86.3	7.834	ns	ns
Urinary N (g/day)	118.9	120.8	116.9	113.4	2.833	ns	ns
Milk N (g/day)	79.3	80.6	85.1	84.4	3.872	ns	ns
N balance (g/day)	8.0	10.9	19.4	3.2	9.029	ns	ns
N balance (% of N intake)	1.7	2.5	6.0	0.18	2.971	ns	ns
Urine urea N (mg/dL)	134.8	106.6	97.4	109.9	2.583	ns	ns
Milk urea N (mg/dL)	12.2	13.1	10.8	11.1	1.154	ns	ns
Serum urea N (mg/dL)	10.8	14.1	11.8	10.4	1.039	ns	ns
Total purines (mmol)	280.6	248.9	244.2	230.5	3.561	<0,001	ns
Absorbed purines (mmol)	283.6	246.2	240.6	225.2	4.143	<0,001	ns
Ruminal microbial N (g/day)	206.2	179.0	174.9	163.7	3.014	<0,001	ns
Microbial efficiency (g BW/kg TDN)	123.1	97.9	91.5	83.0	4.332	<0,001	ns
Regression equations							r^2
Total purines (mmol)	$\hat{y} = 274.267 – 10.319xX$						0.63
Absorbed purines (mmol)	$\hat{y} = 276.044 – 12.057xX$						0.77
Ruminal microbial N (g)	$\hat{y} = 200.696 – 8.765xX$						0.96

[1]MSE = Mean standard error; [2]ns = not significant (P>0.05); r^2 = coefficient of determination

Conclusions Inclusion of up to 4.5% SFO in sugarcane-based diets had no effect on nitrogen metabolism, however there was a in decrease production of microbial protein in the rumen resulting in the lower microbial efficiency in Holstein x Gir.

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Selecting for improved host tolerance: an alternative strategy for disease control?

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Introduction A host can adopt different potential strategies when coping with infectious pathogens. Two such mechanisms are resistance and tolerance. Host resistance, which has been the focus of most disease control strategies to date, refers to a host's ability to inhibit or limit pathogen replication. A measure of resistance used in the literature is the inverse of pathogen burden (Schneider & Ayres, 2008). Tolerance is the ability of a host to maintain fitness or high performance levels while infected with a pathogen (Schneider & Ayres, 2008). In contrast to resistance, tolerance has no impact on the pathogen; Instead, tolerance mechanisms reduce damage to the host caused by pathogen or self as a result of infection, thus reducing the negative impact of infection (Medzhitov *et al*, 2012; Roy & Kirschner, 2000). When considering breeding for improved host resistance and tolerance, there must first be evidence for genetic variation in both traits, and how these are related. There is abundant evidence for genetic variation in host resistance, but little is known whether there is also genetic variation in tolerance, and about the relationship between these traits. The objective of this study was to determine, using *listeria* infections in mice as a model system, whether genetic variation exists in both resistance and tolerance, and whether these two mechanisms are indeed antagonistically related as suggested by the evolutionary theory.

Material and methods Data were provided by Bergmann *et al* (2012). The mice derived from four genetically diverse lines (A-D), chosen for their differing susceptibility to infections. They were orally administered with a mouse adapted *Listeria* strain (n=8 per lines). The lines were selected based on their differing response to pathogen challenge. A measurement of body weight was taken for each mouse prior to infection, and recorded over time. Similarly, colony forming units (CFU) were measured in different organs, at 1, 3, 5 and 7 days post infection, to analyse the systemic dissemination of bacteria. Using ANCOVA, differences in resistance and tolerance to *Listeria* infection were identified. As outlined by Simms (2000), the method of quantifying tolerance used was general linear models (GLMs), where comparisons of slopes (tolerance) of regression lines of Body Weight as a measure of performance (dependent variable), and log-transformed CFU as a measure of pathogen burden (predictor) were used. Additionally, to identify a trade-off between resistance and tolerance, the Pearson's correlation coefficient between the two traits was calculated.

Results

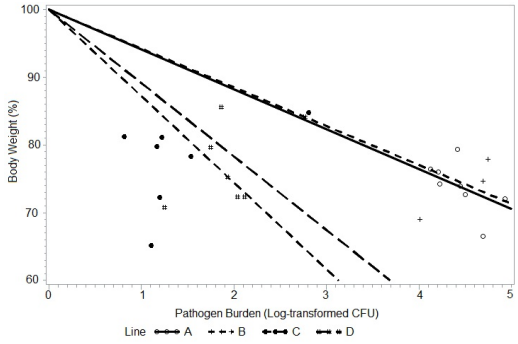

Figure 1 Plot of regression slopes for mouse lines A-D in the intestine at day 5 post infection.

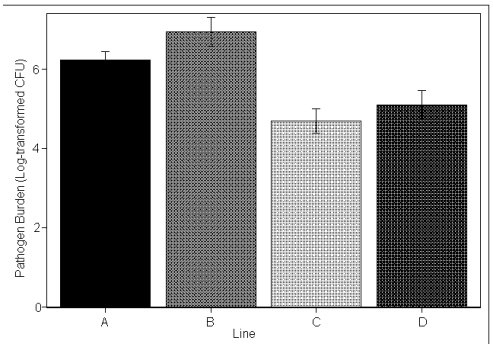

Figure 2 Means of pathogen burden with standard errors for mouse lines A-D in the intestine at day 5 post infection.

As demonstrated in Figure 1, there was variation in slopes between the four mouse lines across all days and all tissues ($p<0.05$). Furthermore, Figures 1 and 2 demonstrate the differences between mouse lines in both resistance and tolerance. These differences were found to be statistically significant ($p<0.05$). Moreover, the most resistant line frequently turned out to be the least tolerant line. There was a consistently strong negative correlation between resistance and tolerance over all analysed tissues at different days (significant r-values between -0.973 and -0.989). The correlation was strongest at day 5 ($r=-0.987$, $p<.0001$) and for intestine measurements ($r=-0.987$, $p<.0001$).

Conclusions The results show evidence for genetic variation between mouse lines for both tolerance and resistance. Furthermore, the results suggest that there is a trade-off between resistance and tolerance. This provides evidence to suggest that genetic selection for/against host tolerance, in addition to resistance, may be a potential disease management strategy, when harmful pathogens which are difficult to eradicate from a population could be controlled. The results have implications on how to deal with infectious disease in livestock in the future.

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Genetics of host response to infection with porcine reproductive and respiratory syndrome virus (PRRSv)

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Introduction Porcine Reproductive and Respiratory Syndrome (PRRS) is an important viral disease of pigs. Mummification of piglets and other reproductive losses are a direct outcome of PRRS alongside other clinical signs such as respiratory disease, increases in co-infections and occasionally blue colouration on the ears, vulva or hind (Zimmerman *et al.* 2003). Compromised production alongside increased monitoring and treatment costs contribute to a recent cost estimate of €126 per sow per PRRS epidemic in Europe (Nieuwenhuis *et al.* 2012). One option for mitigation of PRRS effects is the exploitation of host genetics, as part of a multifaceted solution incorporating management strategies. Heritable variation in response to the virus has been demonstrated both *in-vivo* and *in-vitro*, reviewed in Lewis *et al.* (2007), indicating the potential for the genetic improvement of host resistance to PRRS. This study aims to investigate the heritability of PRRS resilience in a commercial sow herd using both pedigree and genetic marker information.

Material and methods Data were available from a Chinese commercial multiplication unit over 83 months for 1,821 sows covering 7,907 farrowing events and 87,445 total piglet births. The herd experienced repeated PRRSv outbreaks and data from the farm were partitioned into two groups; epidemic and non-epidemic. Partitioning was done on the basis of trend analysis investigating the rolling 30 day average reproductive traits recorded on farm, as per the threshold-threshold method used by Lewis *et al*. (2009). The available reproductive traits were explored for their ability to identify epidemics; only traits which identified the three, ELISA confirmed outbreaks, were used to partition the data. An initial baseline phase was identified within time periods not exhibiting signs of PRRS for any of the traits; the 95th percentile of this initial baseline was used to identify time windows breaching this threshold. The data within epidemic phase and the remaining non-epidemic phase were used to estimate variance components and heritabilities for the different reproductive outcomes. Pedigree information was available for the sows over 11 generations including 4,249 animals. 60k SNP chip data (Illumina) were available for 637 animals. Traits analysed include mummified piglets per litter, total dead per litter, dead as a fraction of total litter size and gestation length. Restricted Maximum Likelihood (REML) heritability estimates for each trait were generated using ASReml 3.0 for Linux, analysing epidemic and non-epidemic phase farrowing events separately. The significance of fixed effects (Line n=9/Parity n=10) was assessed using the conditional Wald F-Statistic. Random genetic effects were assessed fitting an A matrix (pedigree) in the first instance and a G Matrix (genomic kinship). A Genome Wide Association (GWA) analysis was conducted using the R based GenABEL package to fit the GRAMMAR method.

Results Parity was consistently significant in the models used (P<0.001) with differences up to 3.49 piglets between parities for total born dead. Line, whilst not significant, was retained in the model to account for breed differences, with differences up to 3.59 dead piglets. The heritability estimates are shown in Table 1. Total dead piglets showed a 9-fold increase in phenotypic variation from the non-epidemic to epidemic phase, with the increase in genetic variance being even larger, leading to an increased heritability. Similar changes were seen in the other reproductive failure traits, which were all markedly more heritable during epidemic phase. Conversely the heritability of gestation length decreased during the epidemic phase. Using the G matrix altered the heritabilities, however the dataset size was reduced. GWA analysis found chromosome-wide significance on chromosome 4 (SSC4) for the trait of total piglets born dead.

Table 1 Heritability estimates for reproductive traits affected by PRRS

Trait	Non-epidemic phase:	Epidemic phase:	
	A Matrix	A Matrix	G Matrix
Mummified Piglets	0.02 ± 0.01	0.20 ± 0.06	0.09 ± 0.05
Total Dead Piglets	0.05 ± 0.01	0.23 ± 0.06	0.16 ± 0.06
Dead / Total Litter Size	0.03 ± 0.01	0.32 ± 0.06	0.21 ± 0.06
Gestation Length	0.48 ± 0.02	0.23 ± 0.06	0.42 ± 0.05

Conclusions We observed that lowly heritable traits, associated with PRRS outcome, increase in heritability under PRRS challenge. Supported by the increases in genetic variance, this indicates different host control of these outcomes, in the presence of PRRS as opposed to non-epidemic situations. Conversely, gestation length becomes less heritable during PRRS epidemics due to increased phenotypic variance. The significant effect of parity corroborates an earlier finding by Lewis *et al*. (2009), however it is not yet understood whether parity is a proxy for true age effects or risk of previous exposure. The presence of a significant association on SSC4 supports previous findings by Boddicker *et al*. (2012) who also identified a region on SSC4 conferring resistance/susceptibility to PRRSv in growing pigs. These findings indicate that such genetic effects may be translated into potential reproductive benefits, i.e. reductions in mummified and stillborn outcomes. Further work, such as principle component analysis, is required to fully disentangle and quantify the line effects. Current analyses are single breed populations to verify the results from this study.

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Genetic analyses of Johne's testing data from UK dairy herds

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Introduction Johne's disease (or paratuberculosis) is found worldwide and causes significant efficiency losses to both the farm business and the agricultural industry through reduced production, and it also compromises animal welfare. As yet, there is no cure and control strategies are based upon timely detection and culling of infected animals together with good hygiene practices to reduce transmission. Breeding for resistance may be an additional tool to control the disease. Testing milk or blood for Johnes disease (by detection of antibodies using an ELISA test) is a service offered by National Milk Records (NMR). The objective of this study was to estimate genetic parameters for antibody response to Johne's disease using field data milk ELISA test results.

Material and methods Test results of milk ELISA from a five year period, 2007 to 2012, from UK herds were obtained from NMR. Milk ELISA test results were natural log transformed resulting in a normal distribution. The dataset edited for the purposes of genetic parameter estimation consisted of 91,830 Holstein Friesian cows, born from 1998 to 2010, from 1,660 herds with a total of 270,039 records (edits included daughter and contemporary group size, ages at calving, pedigree available etc.). The number of tests per animal ranged from 1 to 30 (average 2.9 tests). The animal pedigree file was generated by tracing the pedigrees of cows back 5 generations resulting in a file containing the relationship of 285,695 animals. Genetic parameters were estimated in ASReml (Gilmour *et al.,* 2006), using both sire and animal models. The fixed effects included were parity of animal at test, birth year of animal, weight of milk closest to test date (within 10 days), age at test in months (linear and quadratic), days in milk at test (linear and quadratic), age of dam at birth (months), heterosis, recombination and herd-year-season at calving. The full animal model included the following random effects: direct additive genetic effect of the animal (A), maternal additive genetic effect (M), the covariance between animal and maternal additive genetic effects (AM), permanent environmental effect of the animal ((PE) due to repeated records) and the residual error. Four models were run to select the most suitable random effects.

Results Trends for transformed milk ELISA tests differed according to lactation stage; initially high after parturition, followed by a steep fall, and then gradually increased towards the end of lactation. Also, transformed milk ELISA tests tended to increase with increasing parity number from parities 1 to 3. According to AIC and BIC tests, the full model (4) was the preferred choice (with lowest value). Essential components in the model were A and PE effects. PE effects would be expected due to animals with repeated tests, but omitting the effect caused an over-estimate of heritability. The estimates for heritability and repeatability using an animal model were 0.07 and 0.38 respectively (Table 1). Corresponding estimates from a sire maternal grandsire model gave similar estimates of 0.06 and 0.37 respectively.

Table 1 Variance components, heritabilities and repeatabilities (with s.e.) for four animal linear models with differing random effects

	Model 1	Model 2	Model 3	Model 4
σ_a^2	79.8 (0.69)	10.1 (0.78)	10.27 (0.78)	10.60 (0.88)
σ_{pe}^2		48.6 (0.73)	52.55 (0.73)	48.55 (0.74)
σ_m^2			0.42 (0.22)	0.34 (0.22)
σ_{am}^2				-0.72 (0.38)
σ_e^2	99.2 (0.32)	98.2 (0.32)	85.99 (0.28)	98.2 (0.32)
σ_p^2	179.0 (0.68)	156.9 (0.55)	149.2 (0.54)	156.9 (0.55)
h^2	0.45 (0.003)	0.06 (0.005)	0.07 (0.005)	0.07 (0.006)
R		0.37 (0.002)	0.42 (0.003)	0.38 (0.003)
AIC	-14320.98	3814.64	-9456.34	-16188.8
BIC	-14310.43	3810.64	-9462.34	-16196.8

σ_a^2 direct additive genetic variance; σ_{pe}^2 permanent environmental variance; σ_m^2 maternal additive genetic variance; σ_{am}^2 animal-maternal covariance; σ_e^2 error variance; σ_p^2 phenotypic variance; h^2 heritability; R repeatability; AIC Akaike Information Criterion; BIC Bayesian Information Criterion.

Conclusions The heritability was in line with similar studies (Attalla *et al.,* 2010; Van Hulzen *et al.,* 2011) and for other health traits (Pritchard *et al.,* 2013). Sire and animal models produced similar heritability estimates but were slightly higher using the animal model. Despite the low heritability (<10%) of health/fertility traits genetic progress can still be made. For instance, there has been a turnaround in the genetic trend of fertility almost immediately after the introduction of the fertility index which includes traits with heritabilities of 1-4%. Testing of cattle for Johne's is important for its use in control programs and the results of this study show that the same field data could be used to develop breeding tools as part of an overall disease control strategy.

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Genetic parameters and profile of dairy cattle locomotion score and lameness before and after calving in primiparous Holstein cows

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Introduction Lameness is of great concern in dairy cattle because of economic and welfare considerations and its high prevalence. Lameness creates direct and indirect economic losses; the former are due to treatment and the involuntary culling of cows. Lameness is the third most common reason for involuntary culling after infertility and mastitis (Whitaker *et al.* 2004). Indirect costs 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). Heritability estimates of locomotion scores for diagnosing lameness (Van Dorp *et al.*, 2004) or as a linear trait (Onyiro and Brotherstone, 2008) range from 0.05 to 0.14. Still, no discrimination is made between before and after first calving or between different stages of lactation. Calving and lactation onset are stressful events and environmental effects are expected to be greater around and, cumulatively, after lactation peak. The objective of this study was to investigate the profile and estimate genetic parameters of locomotion score and lameness before the first calving and throughout the first lactation.

Material and methods The study was carried out in a large commercial farm located in Northern Greece and included 237 first 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. Cows with a score greater than or equal to two were considered as lame. Body condition was scored starting six weeks before calving and then weekly for the first 13 weeks and monthly until the end of lactation, using a five-point scale in increments of 0.25. Body weight was also recorded at the time of body condition scoring. These records were matched to corresponding weekly milk yield records, where the latter were seven-day averages on the week of veterinary inspection. Total number of repeated records amounted to 9,643. Each trait was analysed with a univariate random regression model, including year-season of calving, country of origin, calendar month, barn, age at calving, fixed regression on week from calving, and random regressions on week from calving associated with the additive genetic effect and the permanent environment effect of cow. Estimates of (co)variance components from this model were used to calculate weekly heritabilities for each trait.

Results Fixed curves for locomotion score and lameness are presented in Figure 1, illustrating the relative frequency of the traits throughout lactation. Weekly estimates of the genetic variance are shown in Figure 2; all of which were statistically greater than zero ($P < 0.05$), demonstrating the presence of genetic factors that control the animal's capacity for uninhibited movement and resistance to lameness. Consistent with Figure 2, heritability estimates were highest before 1st calving and decreased as lactation progressed. Average estimates for the entire period were 0.39±0.06 and 0.26±0.06 for locomotion score and lameness, respectively.

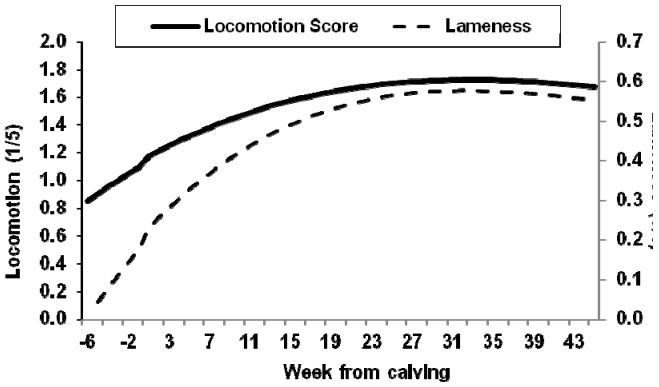

Figure 1 Locomotion and lameness profile across lactation.

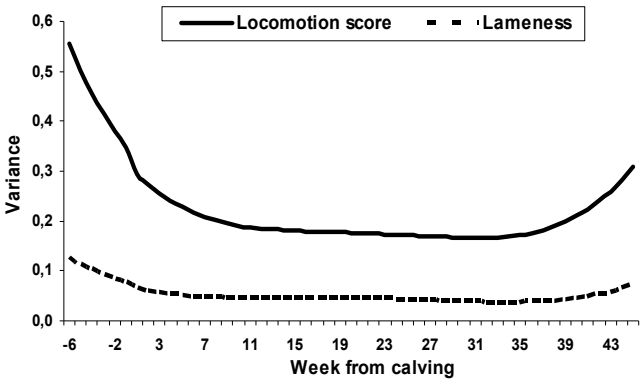

Figure 2 Estimates of the genetic variance across lactation.

Conclusions Locomotion score and lameness are amenable to improvement with genetic selection. Highest gains are expected before first calving and in the beginning of lactation.

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Genomic prediction for tuberculosis resistance in dairy cattle

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Introduction Cattle are susceptible to *Mycobacterium bovis*, the causative bacterium of bovine Tuberculosis (bTB). BTB is a zoonotic disease with the potential to impact on animal performance and welfare causing significant financial losses to the dairy cattle industry worldwide (Allen *et al*. 2010). Eradication strategies based on available diagnostic methods including the single intradermal comparative tuberculin test (SICTT) and post-mortem examination at slaughter have proven ineffective in eliminating the disease in a number of countries and particularly those with a wildlife reservoir. Genetic selection for disease resistance may offer a complementary strategy, by contributing to a reduction in the herd-level incidence and its severity. The presence of exploitable genetic variation in *M. bovis* susceptibility in dairy cattle and in the responsiveness to the diagnostic tests has been confirmed by previous studies (Bermingham *et al*. 2009; Brotherstone *et al*. 2010), in which phenotypes describing whether or not a cow developed bTB were shown to be heritable. The aim of this study was to investigate the possibilities of genomic prediction for bTB resistance using a dense single nucleotide polymorphism (SNP) array, which would enable selection in the absence of disease phenotypes.

Material and methods Phenotypic data were available for 1,151 Holstein-Friesian dairy cows (592 cases and 559 controls) from 165 herds in Northern Ireland and all individuals were genotyped for 617,885 SNP loci (Illumina Bead Chip). Cases were defined as animals positive to SICTT and with confirmed lesions, while controls were animals with multiple negative test results coming from high disease prevalence herds to maximise the probability of exposure to the pathogen (Bishop and Woolliams, 2010). A genomic Best Linear Unbiased Prediction (BLUP) model was used to obtain genomic estimated breeding values (GEBVs) for bTB resistance for all the individuals in a two-stage process: (i) adjusted phenotypes corrected for all non-genetic factors were obtained from an initial fixed effects model and (ii) GEBVS were estimated from the adjusted phenotypes fitting a marker-based genomic kinship relationship matrix, using ASReml. The ASReml analysis also gave an estimated heritability for bTB resistance. Five-fold Cross Validation (CV) was conducted to assess the predictive ability of the genomic EBVs, where the individuals were partitioned in five random groups of equal size, each time omitting one as the test-set and using the remaining four as training-sets to predict GEBVs for the test-set (Luan *et al*. 2009). The procedure was performed with a balanced proportion of cases and controls across the five groups and repeated with the 164 cows designated as Friesians being excluded. The correlation between adjusted phenotypes and the predicted GEBVs was calculated and the accuracy was derived as the correlation divided by the square root of the heritability, i.e. $r(g,\hat{g}) = r(\hat{y},y)/h$. The estimate of the accuracy reported here is the average across six runs of different randomisation. To further assess the performance of the GBLUP, ROC curves and their corresponding "area under curve" (AUC) values were calculated.

Results The heritability was estimated to be 0.23 ± 0.06 and the estimated accuracy ($r(g,\hat{g})$) for the analysis was 0.33, in line with theoretical expectations given the size of the dataset. When the analysis was repeated with Friesian breed cows removed from the dataset the accuracy was 0.34. When the dataset was randomly reduced to give the same number of cases and controls within each herd (hence herds with zero control animals were deleted), the estimated heritability was 0.24±0.10. The ROC curves for the full data set were calculated and their corresponding AUC values were 0.56, 0.59, 0.58, 0.57, 0.57 and 0.59 for the six different randomizations applied (Figure 1). Hence, there was a probability close to 0.58 of correctly classifying cows on the basis of genotype alone using these data.

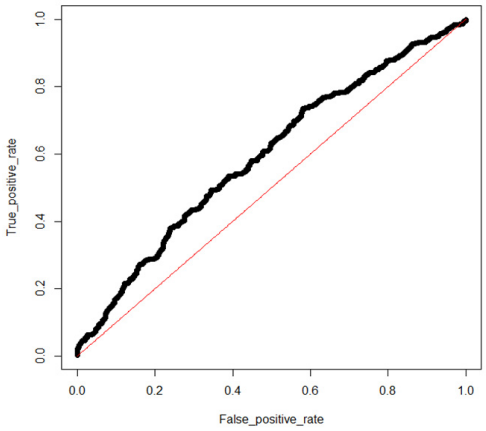

Figure 1 The ROC curve obtained from the GEBVs and the phenotypes, AUC = 0.59. The diagonal is the line of no-discrimination.

Conclusion Genomic selection is potentially feasible for bTB resistance, even in populations with no pedigree data available. Further, it can be applied to animals lacking bTB phenotypes. The accuracies are in line with expectations given the population size (Luan *et al*. 2009), but before implementing genomic selection a greater number of animals will be required to increase the prediction accuracies.

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Can low birth weight pigs exhibit catch up growth post weaning if fed according to their size?

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Introduction Variation in body weight (BW) of pigs is a major concern for the industry with both financial and welfare implications (Patience *et al*., 2004). The low birth weight piglet (LBW) is considered to contribute to this weight variability, exhibiting poor postnatal growth rates (Gondret *et al*., 2005). Therefore, improving the growth of these pigs is considered the most viable remedial option. The aim of this study was to determine if LBW pigs will respond to enhanced nutrition post weaning (9-13 weeks of age) and compensate for low BW, reaching the same BW as normal birth weight pigs. The hypothesis addressed here was that the ability of LBW pigs to compensate will depend on the nutritional environment they are given access to post weaning, and in particular the amino acid: energy ratio in the feed.

Material and methods The experiment was a 3 x 2 factorial with 6 replicate groups using a total of 180 pigs. Treatments comprised 3 BW categories (N= normal birth weight (1.7 to 2.0kg), NR= normal birth weight but feed restricted from day 49-63 and L= low birth weight (≤ 1.2 kg)) and two food specifications given from day 63-91 (High or Standard lysine). In period 1, from birth to day 49, piglets were selected within 24 hours of birth to create one cross-fostered litter for each BW category (N, NR and L) in each of 6 consecutive farrowing batches. In period 2, from day 49 to 63, each litter was split to form two treatment groups of 5 pigs (balanced for sex and litter origin) for each BW category. The NR pigs received restricted amounts of feed (600g/day per pig) with the remaining N and L groups fed *ad libitum* the same commercial weaner diet (20.55% crude protein, 1.28% digestible lysine, 14.46MJ/kg digestible energy). The aim was for NR and L pigs to have the same BW by day 63. For the third period, from day 63-91, groups within litter were randomly allocated a high (16 g/kg) or a normal lysine (13 g/kg) grower diet for their age, offered *ad libitum* for four weeks. Pigs were individually weighed twice a week from day 49 to 91 and feed intake per group was recorded. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion efficiency (FCE) were calculated. Data were analysed on a group mean basis by ANOVA using SAS version 9.2, with BW category (N, NR and L) and feed offered in period 3 (High or Standard lysine) as factors.

Results BW on day 63 was 25.41, 21.00, 21.26 kg (0.38 s.e.d) for N, NR and L pigs respectively. There was a significant effect of BW category on performance from day 63-91, with L pigs exhibiting the lowest ADG and FCE; this was the case for all sub-periods examined. NR pigs exhibited the highest ADG for all periods (post restriction) however there was no significant difference in the FCE when compared to N pigs for all periods examined (P>0.05). There was no difference in the performance of pigs fed a high or normal lysine diet (P>0.05), nor was there a significant interaction between BW category and lysine content of the feed (P>0.05).

Table 1 Effect of BW category (normal, N, normal restricted NR, or lightweight L) on growth and feed intake of pigs from day 49 to 91[1]

	Average daily gain (g/day)			Feed conversion efficiency (g gain per g feed)		
	Time period (days)					
BW category	63-77	77-91	63-91	63-77	77-91	63-91
NR	767^{A}	869^{A}	818^{A}	0.661^{A}	0.609^{A}	0.631^{A}
N	688^{B}	799^{B}	743^{B}	0.619^{A}	0.607^{A}	0.611^{A}
L	622^{C}	732^{C}	677^{C}	0.557^{B}	0.532^{B}	0.540^{B}
sed	35.2	37.7	27.8	0.300	0.028	0.021
Significance[2]	*	*	**	*	*	**

1. Within a period, means with a different superscript differ (P<0.05)
2. *P<0.05; **P<0.01

Conclusions L pigs did not exhibit catch up growth, even on a higher specification diet, and consistently exhibited the lowest ADG and FCE. However they had a similar FI to other treatment groups. NR pigs exhibited higher ADG post restriction than both N and L pigs, indicating that these pigs can compensate for low BW due to a reduction in feed intake. This suggests that feeding LBW pigs a higher specification diet, i.e. feeding them for their BW rather than age, has no effect on overall performance and that it might not be possible to enhance the growth of LBW pigs post weaning.

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Are compensatory live weight gains consistently observed in pigs following lysine restriction during the weaner phase?

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Introduction Providing cost effective growth and improving feed efficiency is vital for long term profitability of the pig industry. Compensatory growth, defined as a period of accelerated growth after a period of growth restriction (Hornick *et al.*, 2000), has been associated with an increase in feed efficiency. However despite a large amount of work on compensatory growth it continues to be poorly understood with many conflicting reports. The aim was to determine whether or not compensatory gains were observed after feeding a reduced lysine diet for three weeks post weaning followed by a high lysine diet up until slaughter and to determine whether compensatory gains could be consistently observed over a number of experiments.

Material and methods Four separate experiments were conducted. Experiment 1 was conducted from March to October 2008, experiment 2 from December 2008 to July 2009, experiment 3 from December 2009 to July 2010 and experiment 4 from May to December 2011. For each experiment pigs were weighed at weaning (28 days of age) and allocated to pens on the basis of live weight and litter origin. Throughout each experiment pigs were fed *ad libitum* receiving either a high lysine (Control; 17.5 g/kg) or a low lysine (Weaner restrict (WR); 8.0 g/kg) diet during the first three weeks post weaning creating two dietary treatments. Eight replicates were used for each experiment. In each experiment, both weaner diets were followed by a high lysine diet up until slaughter, 15.5 and 12.0 g/kg of lysine for the grower (7 to 12 weeks of age) and finisher (12 weeks of age to slaughter) diets respectively. All pigs were individually weighed at the start of the experiment (weaning) and then weighed at 7 and 12 weeks of age and prior to slaughter. Average daily intake (ADI), average daily gain (ADG), feed conversion ratio (FCR) and average daily lysine intake (ADLI) were determined for each experiment. When the pen average reached 95.0-105.0 kg pigs were sent to a commercial abattoir and slaughtered under commercial conditions. All pigs were given daily health scores (recording any ill health). A General Linear Model (Version 15.0; Minitab Inc.) was used to analyse each experiment independently as a two treatment design. The pen mean was the experimental unit for performance data.

Table 1 Effect of weaner dietary lysine level on rate of gain throughout the weaner, grower and finisher stage.

	ADG, kg Weaner phase				ADG, kg Grower phase				ADG, kg Finisher phase			
	Control	WR	SE	P-Value	Control	WR	SE	P-Value	Control	WR	SE	P-Value
Exp 1	0.389	0.254	0.0051	<0.001	0.772	0.818	0.0113	0.013	0.855	0.858	0.0220	0.945
Exp 2	0.316	0.175	0.0080	<0.001	0.725	0.718	0.0101	0.633	0.855	0.915	0.0104	0.001
Exp 3	0.344	0.157	0.0131	<0.001	0.755	0.723	0.0092	0.029	0.885	0.881	0.0172	0.871
Exp 4	0.288	0.179	0.0105	<0.001	0.730	0.713	0.0219	0.589	0.849	0.844	0.0236	0.889

Results Growth performance was successfully reduced during the weaner phase in WR pigs. These pigs grew more slowly ($P<0.001$) and less efficiently ($P<0.001$) than Control pigs in all experiments. Feed intake during this period was lower for WR pigs in experiments 1, 2, and 3 ($P<0.05$), whereas feed intake did not differ significantly between WR and Control pigs in experiment 4. Although lysine intake was lower for WR pigs in all experiments compared to Control pigs ($P<0.001$), WR pigs utilised the lysine in the diet more efficiently for growth than Control pigs ($P<0.001$). In the subsequent growth period, WR pigs in experiments 1 and 2 demonstrated improved rate of gain ($P<0.05$, $P<0.05$) and feed efficiency ($P<0.1$, $P<0.1$; experiments1 to 2 respectively) and thus compensatory live weight gains were evident. However, previous lysine restriction did not result in compensatory gains in pigs from experiments 3 and 4 as these pigs had a lower feed intake throughout the grower stage ($P<0.001$, $P=0.056$). No difference in health score was observed between treatments in any experiment.

Conclusions The above results demonstrate that pigs are capable of compensating for a reduction in performance when dietary lysine levels as low as 8.0 g/kg lysine are fed for a period of three weeks post weaning, but that compensatory growth is not consistently observed. In experiments 3 and 4 feed intake was lower during realimentation for WR pigs compared to Control pigs which may have prevented them from compensating, however the reasons for this lower intake are difficult to explain. The difference in weight created at the end of the weaner stage was greatest for pigs in experiment 3 which may account for the greater difference in feed intake during realimentation between the treatments. However this was not the case for pigs in experiment 4. Many environmental factors can affect feed intake, however the housing conditions, temperature, genotype and feeder type were kept constant for each experiment. Although health were similar for each trial, more robust health measurements are needed to rule out any effects caused by poor health which may have resulted in a reduced feed intake. These results confirm that the occurrence of compensatory growth cannot be reliably predicted.

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Finishing pig performance and eating behaviour when using up to 90 g/kg rapeseed meal in the diet

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Introduction Magowan *et al*., (2013) found a reduction in pig performance when rapeseed meal (RSM), with a low glucosinolate level (4.8 µmol/g), was included in finishing pig feed at levels between 70 and 210 g/kg. However, there remains a strong desire within the pig industry to utilise RSM in pig feed since its successful inclusion should reduce reliance on imported soya bean meal and reduce diet costs and the carbon footprint of pig production. The aim of the current experiment was to investigate finishing pig performance and eating behaviour when diets containing up to 90 g/kg of RSM were offered.

Material and methods A total of 80 pigs (PIC337 x (Landrace x Large White)) were offered one of four isoenergetic (13.8 MJ/kg digestible energy), isonitrogenous (170 g/kg crude protein; 11 g/kg total lysine) diets containing either 0, 30, 60 or 90 g/kg RSM. Pigs were housed in groups of 10 which were balanced for gender and weight. Dietary treatments were offered from 84 to 145 days of age. The feed intake and eating behaviour of individual pigs was recorded continuously using ACEMO electronic feed stations. Each pig represented one replicate. Pigs were weighed at 12, 15 and 18 weeks of age (Wks) and at slaughter (145 days of age). The composition of the control diet (0 g/kg RSM) was (g/kg) maize 393; wheat 283; soya 246; soya hulls 47; vegetable oil 6.0; limestone 4.7; dicalcium phosphate 7.0; minerals and vitamins 5.0; salt 4.6; lysine 3.3; methionine 1.1 and L-threonine 0.8. As the inclusion of RSM increased the inclusion of maize decreased and the inclusion of wheat increased to 313 and 366 g/kg respectively for the RSM diet containing 90 g/kg. Soya hulls inclusion was reduced to 40, 33 and 27 g/kg for the 30, 60 and 90 g/kg RSM diets. Vegetable oil inclusion was 8.3, 10.7 and 13.1 g/kg for the 30, 60 and 90 g/kg RSM diets. Inclusion of all other dietary ingredients remained similar to that of the control diet. The raw RSM was analysed for total glucosinolate content using an HPLC method conforming to BS4325 Part 12 (NIAB Labs, England). Pig performance (average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)) and eating behaviour (average number of visits per day (ANV/D), average feed intake per visit (AFI/V) and the average time spent per feeding visit (AT/FV)) was calculated on a per pig basis and data were statistically analysed using analysis of variance with the 12-week weight of pigs being used as a covariate (using Genstat Version 10).

Results The total glucosinolate level of the RSM was 2.5 µmol/g. The 12-week weight of pigs averaged 43.5 kg. There was a quadratic effect of RSM inclusion on the finish weight ($P<0.01$) and ADG ($P<0.05$) of pigs between 12 weeks of age and finish (Table 1). Pig performance and finish weight increased when pigs were offered diets containing 30 and 60 g/kg RSM but decreased again when the diet containing 90 g/kg RSM was offered to a level comparable with the control diet (Table 1). There was no significant effect of RSM inclusion on the ADFI or FCR of pigs (Table 1). There was no significant effect on the ANV/D but there tended to be ($P <0.1$) a quadratic effect on the AFI/V and AT/FV (Table 1).

Table 1 Effect of RSM inclusion on pig performance and eating behaviour between 12 weeks of age and finish

	RSM inclusion (g/kg)					P value		
	0	30	60	90	SEM	Treatment	Linear	Quadratic
Finish weight (kg)	101.6[a]	103.8[b]	104.5[c]	102.1[ab]	0.72	<0.05	NS	<0.01
ADG (g/kg)	944[a]	976[ab]	1005[b]	962[ab]	16.0	0.052	NS	<0.05
ADFI (g/kg)	2169	2196	2220	2167	33.2	NS	NS	NS
FCR	2.31	2.26	2.22	2.26	0.035	NS	NS	NS
ANV/D	8.7	8.7	8.3	7.9	0.52	NS	NS	NS
AFI/V (g)	330	305	324	353	16.4	NS	NS	<0.1
AT/FV (mins)	8.4	7.7	8.0	8.9	0.45	NS	NS	<0.1

Conclusions In contrast to Magowan *et al*. (2013), the inclusion of RSM at a rate of 90 g/kg did not significantly reduce pig performance compared with the control diet. Furthermore, the inclusion of 30 and 60 g/kg of RSM improved pig growth rate between 12 weeks of age and finish. The level of glucosinolates were considered low in this study and the response of pigs is in line with that of other workers, for example Keady & O'Doherty (2000) and McDonnell *et al*. (2010) who included up to 300 and 210 g/kg of RSM respectively and found no reduction in pig feed intake or growth rate. In both this study and that of Magowan *et al.* (2013), diets were cereal/soya based and were similar with regard to crude protein, lysine and energy composition. However, the terminal sire used in this study (PIC) is likely to have been a contributing factor to the lower ADFI and improved FCR reported here compared with Magowan *et al*. (2013) (2169 vs 2464g/kg and 2.26 vs 2.51 respectively using the control diet). The slightly lower glucosinolate level combined with a lower ADFI may have contributed to the lack of negative response to RSM inclusion found in the current study.

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088

Can we reduce current levels of phosphorus in dry sow diets without affecting sow and progeny performance and health?

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Introduction It has been suggested (Kyriazakis, 2008) that the UK pig Industry uses phosphorus (P) levels in diets for dry sows at levels that are 20-35% above the recommended Nutrient Requirement Standards (BSAS, 2003). Reasons for this oversupply are the need for safety margins, a degree of uncertainty over the digestible P (dig-P) contents of feedstuffs, advice offered by veterinarians and the provision of one diet that needs to meet P requirements at all stages of gestation. Excess of P in pig diets raises serious environmental concerns, as excess P is excreted in the environment and contributes to soil and water eutrophication (White and Hammond, 2006). This study aimed to show if P levels in dry sow diets can be reduced in line with recommended requirements, without adverse effects on performance and health.

Material and methods Two dietary treatments were used in the study. One contained the industry standard level of dig-P (2.50g/kg) and the second contained a lower level of dig-P (1.95g/kg), which is approximately 22% below the BSAS (2003) standard. One farm, with 520 dry sows distributed over 4 batches, was used. The sows were fed the diets from weaning until two weeks before farrowing. During lactation, all sows were fed the same, industry standard, diet. Reproductive performance parameters were measured as well as bone strength using samples obtained when the animals were slaughtered at the end of lactation. The environmental impact of the diets was measured by soil samples taken at the entrance and exit from the service and gestation paddocks as well as faecal samples taken at exit from both paddocks. Both soil and faecal samples were analysed for labile P levels using a partial fractionation procedure (Abioye *et al*. 2010). Differences between treatments were examined using ANOVA with appropriate General Linear Models. In the analysis of soil and faecal P, a number of weather indices were developed to summarise the daily weather patterns in the period before sampling, as samples were collected between January and June under very different weather conditions and used as a covariate in the analysis.

Results There was no effect of dietary treatment on the reproductive performance parameters measured ($P > 0.05$) (Figure 1). There was an effect of parity on the number of piglets born alive ($P = 0.040$) and the number of piglets weaned ($P = 0.028$) due to these being higher for parities 2-4. There was no parity effect on the number of stillbirths ($P > 0.05$). The average weaning weight of the piglets did not differ between the treatments (overall mean 7.80 kg) and there was no difference in the return rates between treatments. There was no effect of dietary treatment on bone strength, but there was an effect of parity, with older parities (6 and above) having stronger bones when compared to gilts ($P < 0.001$).

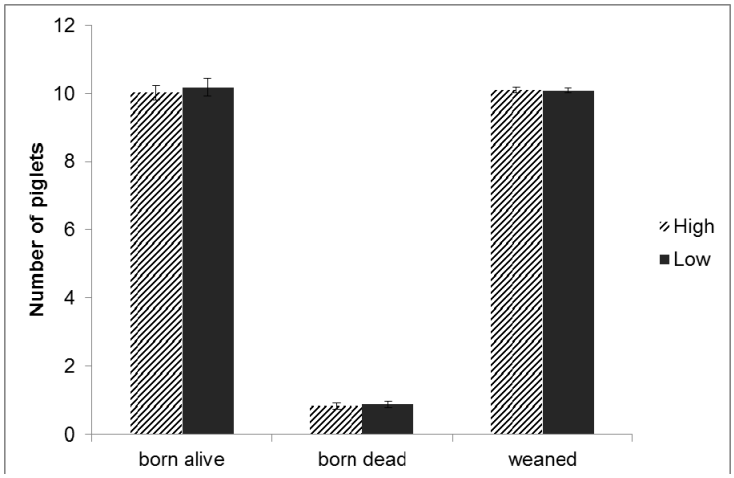

Figure 1 Reproductive performance for sows fed either a High (2.50g/kg) or a (1.90g/kg) digestible phosphorus diet during gestation.

On-farm monitoring of sows found no significant measurable difference in labile P in faeces or surface soil as a result of changes in diet. There was an effect of pig occupation, irrespective of diet, on the soil P levels with increases of 46 mg kg-1 and 26 mg kg-1 in plant available P (bicarbonate extractable) on average in the service and gestation paddocks respectively. Statistical analysis of the levels of labile P pools in soil showed that weather patterns explained a higher proportion of the variation than the differences in diet.

Conclusion As there was no effect on reproductive performance or bone strength caused by the different levels of digP in the diet there might be no need to feed sows above the recommended levels of digP as a safety margin. However the experiment took place over one parity and long term effects of the dietary treatments need to be addressed in future experiments. There was no difference between diets in environmental load which was largely caused by a high variation and seasonal effects. Modelling nutrient balance is being currently considered at paddock scale. This will be compiled with additional biophysical data (e.g. topography, land use) to assess the overall environmental impact of the changes in diet in pig production systems.

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Large-scale demonstration of using peas and faba beans as home grown alternatives to soya bean meal in grower and finisher pig diets

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Introduction The UK pig industry reliance on imported soya bean meal (SBM), which increases concerns about food security, sustainability and environmental impact could be reduced through greater use of home grown peas and faba beans. We observed that the latter could completely replace SBM without impacting on growth performance and carcass measures in small scale experimental trials (Smith *et al.*, 2012a,b; White *et al.*, 2012a,b). Here, we investigate the same under large-scale commercial conditions.

Material and methods Faba beans (var Fuego) or peas (var Prophet) were included at 300 g/kg in commercially formulated test diets without any SBM. Control diets contained SBM at 98.0 and 47.6 g/kg for grower (35-60 kg) and finisher (60-110 kg) pig, respectively. The pelleted feeds were formulated to be iso-energetic (9.75 and 9.30 MJ NE/kg for growers and finishers respectively), have the same standardized ileal digestible lysine content (9.5 and 8.8 g/kg), and meet the minimum requirements of other amino acids (BSAS, 2003) by modifying the inclusion of pure amino acids. Wheat, barley, biscuit meal, wheat feed, fat and macro minerals were allowed to float, whilst rapeseed meal, DDGS and other ingredients were kept constant. Diet were fed to a total of 1230 mixed sex American Hampshire × Landrace/Large White pigs of ~35 kg, housed on slats (10 pens per diet; 11 pigs per pen), or on straw (10 pens per diet, of which there were 8 with 25 pigs and 2 with 50 pigs). Pen live weight was recorded at trial start, and pen live weight and feed refusals recorded at change over to finisher formulation, and when pigs were sent to slaughter, to obtain cold carcass weight, pH at 45 min post-mortem (pH_{45}), P2 value and calculated % lean. Mean pig body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR as FI/BWG) were calculated and analysed separately for the grower, finisher, and combined grower-finisher period using ANOVA to test for diet, housing type and their interaction. To account for unbalanced treatment groups per slaughter day, carcass data were analysed using REML to test for treatment effects, which included slaughter day as random effect.

Results Treatment effects on pig performance did not differ between the grower and finisher period, so are reported for the combined grower-finisher period only (Table 1). Diet did not significantly affect performance and carcass measures (Table 1). However, pigs on slats had lower BWG, lower FI, better FCR, less P2 and consequently higher % lean than pigs on straw. Diet and housing type tended to interact for pH_{45}, although all pH_{45} values were greater than 6, indicating that none of the treatments promoted PSE characteristics. Farm manager comments were favourable, stating normal pig cleanliness, performance and feed handling across diets.

Table 1 Effect of feeding treatment and floor type on growth performance and carcass characteristics

	Treatments							P-values		
	Slats			Straw				Diet	Housing	DxH
	SBM	Peas	Beans	SBM	Peas	Beans	SEM			
Growth performance										
BWG (g/d)	881	889	888	930	959	941	18.35	0.362	0.001	0.697
FI (g/d)	2158	2118	2149	2704	2798	2750	47.4	0.414	0.001	0.580
FCR	2.46	2.39	2.43	2.9	2.92	2.92	0.06	0.805	0.001	0.471
Carcass characteristics										
pH_{45}	6.64	6.59	6.66	6.57	6.66	6.6	0.04	0.520	0.372	0.056
Cold weight (kg)	84.9	83.7	84	84.5	84.9	85.4	1.63	0.805	0.190	0.295
P2 (mm)	10.19	10.17	9.74	11.23	11.59	11.38	0.32	0.304	0.001	0.329
Lean (%)	62.6	62.92	62.53	61.6	61.44	61.27	0.24	0.367	0.001	0.470

Conclusions This large-scale commercial demonstration trial verified that feeding pea- or faba bean-based diets is unlikely to affect pig performance, indicating peas and faba beans are viable home-grown alternatives to SBM for grower and finisher pigs.

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090

Can we reduce current levels of phosphorus in grower/finisher pig diets without affecting performance and health?

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Introduction It has been suggested (Kyriazakis, 2008) that the UK pig industry uses phosphorus (P) levels in the diets of all pig classes that are 20-35% above the recommended Nutrient Requirement Standards (BSAS, 2003). Reasons for this oversupply are the inclusions of safety margins, a degree of uncertainty over the digestible P (dig-P) contents of feedstuffs and advice offered by veterinarians. Excess of P in pig diets raises serious environmental concerns. It is estimated that pig production contributes around 14 per cent of the total diffuse P load from livestock to UK waters (White and Hammond, 2006). This study aimed to demonstrate that P levels in grower and finisher pig diets can be reduced in line with recommended requirements without adverse effects on pig performance and health.

Material and methods There were three dietary treatments each for the grower (Gr: 30-60kg) and finisher (Fr: 60-90kg) pigs. The High diet contained industry levels of dig-P (2.70g/kg Gr, 2.49g/kg Fr). The Medium diet contained dig-P levels just below the BSAS (2003) standard (2.44g/kg Gr, 2.26g/kg Fr). The Low diet had dig-P levels of around 20% below the industry standard (2.19g/kg Gr, 2.00g/kg Fr). A total of 5453 animals were kept on two farms (F1 and F2), with all treatments present on each farm, and replicated over two batches. Performance was recorded as average daily weight gain and food intake consumed and, by calculation, feed conversion ratio (FCR), and lean meat percentage at slaughter. These results were analysed using GLM (Minitab 16, Minitab Ltd.). The potential environmental impact of the pigs and the diets was assessed from faecal and muck samples taken at 2 week intervals and analysed for levels of labile P.
Results There was no effect of dietary treatment on any of the performance parameters measured (P>0.1) (Table 1). There was also no effect of diet on the recorded occurrence of illness or death. There were, however, differences between farms in average daily weight gain ($P < 0.001$) and feed conversion ration (FCR; $P = 0.003$), and an effect of location on lean meat percentage ($P = 0.016$). There was also an effect of batch on the lean meat percentage ($P = 0.027$), as well as on the average daily weight gain ($P = 0.001$). There were no interactions between any of the variables involved.

Table 1 Average weight gain (kg per day), food intake, feed conversion ratio (FCR, kg gain/kg feed) and lean meat percentage (%) for the three dietary treatments (Low, Medium and High) offer to grower/finisher pigs in each of the farms used (F1 and F2); P value for the treatment effect is also given.

	Low			Medium			High			SEM	P-value
	F1	F2	Mean	F1	F2	Mean	F1	F2	Mean		
Weight gain (kg/d)	0.922	0.980	0.951	0.915	0.989	0.952	0.937	0.974	0.955	0.0119	0.989
FCR (kg/kg)	2.53	2.36	2.44	2.63	2.44	2.54	2.62	2.44	2.53	0.103	0.508
Lean meat %	60.7	61.1	60.9	60.6	61.2	60.9	60.8	61.1	60.9	0.0337	0.978

Conclusion There was no measurable effect on pig performance when the levels of dig-P were reduced by up to 20% below the current industry standard. The performance results showed that there is no need to feed above the recommended levels of dig-P as a safety precaution. It must be emphasised that the bone strength of the pigs in the current study was not measured and a reduction of P levels in the diet might therefore not be suitable for pigs destined for breeding stock. Results from the on-going faecal and muck sample P analysis will be analysed to determine the impact of dietary change on P loading in excreta. Furthermore, environmental risk modelling will be used to estimate the potential impact of dietary change on diffuse P load following the application of excreta on to land.

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091

Effect of dietary inclusion of seaweed extracts on ileum morphology and gene expression profile of nutrient transporters in weaned pigs

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Introduction The increased pressure on pig production has resulted in an increase in the use of antimicrobial growth promoters (AGP) to improve pig performance and (or) health. Recent EU legislation prohibits AGP incorporation in animal diets. There is an urgent need to identify reliable alternatives to reduce stress-associated problems in newly weaned pigs. Recently, seaweed extracts such as laminarin (LAM) and fucoidan (FUC) have been investigated as potential feed additives for pigs due to their antimicrobial and immunomodulatory properties(1). The aim of the current study was to investigate the interactions between LAM and FUC levels on intestinal morphology and on the gene expression profile of the nutrient transporters: Glucose transporter 1 (*GLUT1*), *GLUT2*, *GLUT5*, *GLUT7*, *GLUT8*, Peptide transporter 1 (*PEPT1*), Sodium-glucose linked transporter (*SGLT1*), Fatty acid binding protein 2 (*FABP2*) and Cluster of Differentiation 36 (*CD36*) in the weaned pig.

Material and methods Twenty-eight 24d-weaned pigs (6.9 kg, SD 0.50) were blocked by litter of origin and live weight (n=7). The dietary treatments were as follows: (T1) basal diet (BD); (T2) BD + 300 ppm LAM; (T3) BD + 240 ppm FUC; (T4) BD + 300 ppm LAM and 240 ppm FUC. The pigs were individually housed with *ad libitum* access to water. The diets were formulated to have similar digestible energy (14.5 MJ/Kg) and standardised ileal digestible lysine (12.5 g/kg) contents. The diets were offered *ad libitium* for 8 d; after which time the pigs were humanely sacrificed. Ileal tissue (15 cm from caecum) was excised post-mortem: part was fixed in 10 % phosphate-buffered formalin for morphological analysis and part was stored in RNAlater for gene expression analysis. Villus height (VH) and crypt depth (CD) were measured on haematoxolin and eosin stained sections. Total RNA was extracted from the ileal tissue samples using a GenElute Mammalian Total RNA Miniprep Kit. All primers for nutrient transporters were designed using Primer Express™ Software and synthesised by MWG Biotech. Normalised relative quantities were obtained using qbase PLUS software for two stable housekeeping genes: B2M and PPIA. The experimental data were analysed as a 2 × 2 factorial using the GLM procedure of SAS. The statistical model used included the main effects of LAM and FUC supplementation and the associated interaction between LAM and FUC.

Results There was no effect of treatment on VH, CD and VH:CD ratio (P>0.05) (data not shown). There was an interaction between LAM and FUC on *GLUT1* and *GLUT2* expression (P<0.05). Pigs offered the LAM diet had increased *GLUT1*, *GLUT2* and *SGLT1*expression compared with pigs offered the basal diet. However, there was no effect of LAM on *GLUT1* and *GLUT2* expression when combined with FUC. No effect was observed in the expression of *PEPT1*, *GLUT5*, *GLUT7*, *GLUT8*, *FABP2* and *CD36* (*P*>0.05).

Table 1 Effect of dietary LAM and FUC on ileum morphology and on the gene expression of nutrient transporters

Treatment	T1	T2	T3	T4	SEM	Significance		
						LAM	FUC	LAM x FUC
Villous height – VH (µm)	276 5	262·8	245·6	278·2	15·40	ns	ns	ns
Crypt depth – CD (µm)	268·6	258·8	252·6	289·2	15·75	ns	ns	ns
VH:CD ratio	1·1	1·1	1·0	0·08	0·08	ns	ns	ns
PEPT1	0·932	1·530	1·088	1·164	0·204	ns	ns	ns
SGLT1	0·896	1·687	1·153	1·000	0·186	*	ns	ns
GLUT1	1·017	1·472	1·153	0·887	0·101	ns	ns	*
GLUT2	1·034	2·174	1·283	1·043	0·208	ns	ns	*
GLUT5	0·843	1·254	1·328	1·208	0·170	ns	ns	ns
GLUT7	0·983	1·788	1·380	1·930	0·350	ns	ns	ns
GLUT8	1·057	0·980	0·948	0·997	0·053	ns	ns	ns
FABP2	1·207	1·518	0·784	0·758	0·201	ns	ns	ns
CD36	0·821	1·230	1·115	1·254	0·160	ns	ns	ns

* P<0.05, ns P>0.05.

Conclusion The up-regulation of *GLUT1*, *GLUT2* and *SGLT1* suggest that dietary LAM may improve growth performance by improving the absorption of glucose.

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Development of a model to predict phosphorus digestion, retention and excretion in growing and finishing pigs

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Introduction In pig diets, phosphorus (**P**) is the third most expensive nutrient, after carbohydrates (energy) and protein. P is an important mineral for the metabolism and skeletal development; a deficiency in P results in skeletal defects and may reduce lean tissue growth rate. On the other hand, excess P in pig diets contributes to environmental pollution, namely eutrophication. Accurate knowledge of digestible P requirements will allow the supply of digestible P in a manner that more closely matches pig requirements and lead to maximization of the dietary P retained whilst minimizing the P excreted. The objective of this work was to develop a deterministic, dynamic model able to represent P digestion, retention and ultimately excretion in growing and finishing pigs of different genotypes, offered access to feeds of different P contents.

Material and methods P in plant feedstuffs includes some readily digested non-phytate P (NPP), but is mostly in the form of phytate P which is indigestible, unless it is dephosphorylated by phytase enzymes into NPP. Inorganic P is classified as NPP but with different digestibilities to plant NPP. Phytate dephosphorylation in the stomach from exogenous microbial and plant phytase enzymes was expressed by exponential relationships. The model also represented the linear relationship of pig endogenous phytase activity to dephosphorylate phytate, as a function of dietary calcium (**Ca**). The absorption of NPP from the lumen of the small intestine into the blood stream was set at 0.8 and the dephosphorylated phytate from the large intestine was considered undigested. The net efficiency of digested P was set at 0.9 and assumed to be constant across body weight, genotype and sex. P requirements for both maintenance and growth were made functions of body protein mass. Undigested P was assumed to be excreted in the feaces in both soluble and insoluble forms. If digestible P exceeded the requirements for P then the excess digestible P was excreted through the urinary flow; thus the model represented both forms of P excretion (soluble and insoluble) into the environment. Using a UK industry standard diet, (containing 5.19 g/kg total Ca, 4.29 g/kg total P separated into 2.48 phytate and 1.48 g/kg NPP) model behavior was investigated for its predictions of P digestibility, retention and excretion under different levels of inclusion of dietary Ca, exogenous phytase and inorganic P (without the standard 750FTU *E.coli* phytase supplementation), for a pig of an intermediate genotype (BSAS 2003). The evaluation of the model is done in a companion presentation (Symeou *et al.* 2003).

Results Supplementing a P deficient standard UK industry with inorganic P, resulted in a linear increase in P digested (Figure 1). The P retained increased linearly, until P requirements were met and any excess digestible P was excreted as soluble P, though the urinary tract. Beyond the maximum P retention, there was a significant increase in the total P excreted. P digested was inversely proportional to increased levels of dietary Ca (Figure 2). Ca formed insoluble and indigestible Ca-phytate complexes in the small intestine, thus increasing the insoluble P excreted and overall the total P excreted as less dietary P was available for retention and decreasing the soluble P excreted. The supplementation with phytase increased P retention up to 900FTU (data not shown).

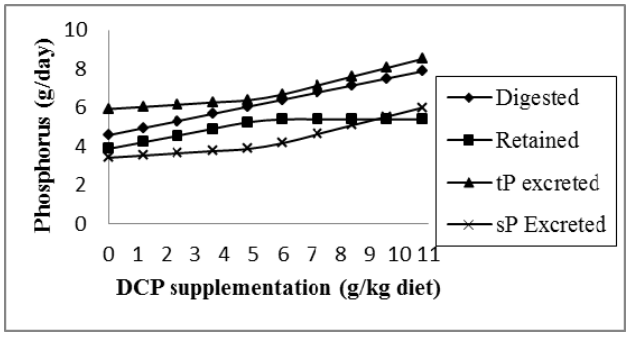

Figure 1 The effect of di-calcium phosphate (DCP) supplementation on P digested, retained, total and soluble P excreted, of a UK standard industry diet (without microbial phytase).

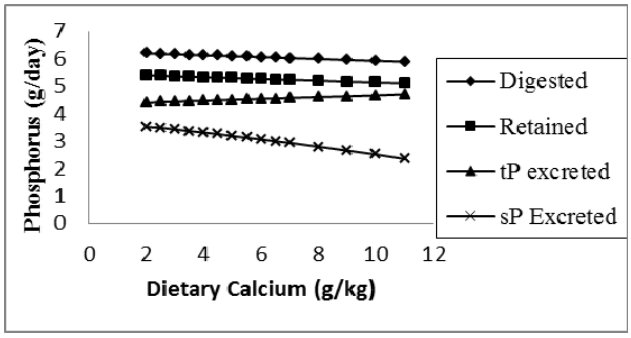

Figure 2 The effect dietary calcium on P digested, retained, total and soluble P excreted, of a UK standard industry diet (with 750FTU *E.coli* phytase).

Conclusion Model behavior was consistent with our understanding of P digestion, metabolism and excretion. The 750FTU *E.coli* phytase supplementation is equivalent to 5.5g/kg diet DCP supplementation. Keeping the dietary Ca content to the minimum before Ca becomes first limiting for bone development, resulted in maximised P digestibility in diet. Maximum P retained was achieved at 900FTU on this particular diet used.

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The use of laminarin as a possible replacement for zinc oxide in weaned pigs: effects on growth performance, nutrient digestibility and faecal microbiology

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Introduction The pressure to expand animal production has resulted in the use of antimicrobial growth promoters (AGP) and zinc oxide (ZnO) to improve performance in pigs and poultry. Recent EU legislation prohibits the use of AGP in animal diets and the use of zinc also may soon be prohibited due to environmental concerns. There is an urgent need to identify alternatives to reduce stress-associated problems in newly weaned pigs. Recently, seaweed extracts such as laminarin (LAM) have been investigated as a potential feed additive in pig diets due to their antimicrobial and immunomodulatory properties(1). The hypothesis of the current study is that LAM could be used to promote growth and could be a possible replacement for ZnO in weaned pig diets.

Material and methods Forty-eight 24d-weaned pigs (6.9kg, SD 0.70) were blocked by live weight. Dietary treatments were as follows: (T1) basal diet (BD); (T2) BD + 300ppm LAM; (T3) BD + 3100ppm ZnO. The pigs were housed in pairs for 32d and had *ad libitum* access to water and feed. The diets were formulated to have similar digestible energy (14.5 MJ/Kg) and standardised ileal digestible lysine (12.5 g/kg) contents. The pigs were weighed at weaning (d0) and d7, 14, 21 and 32. Fresh faeces samples were taken from each pen on d10 and were analysed for *Lactobacillus spp*., *Bifidobacterium spp*. *and Escherichia coli*, and from d10-14 for the determination of nutrient digestibility analysis. The data in the tables are presented as least square means ± SEM. The experimental data were analysed as a completely randomised design using the GLM procedure of SAS. The statistical model used included the main effects of LAM and ZnO supplementation.

Results Neither LAM nor ZnO had any effect on average daily gain (ADG), feed intake (FI) or gain to feed ratio (G:F) during d 0-7 (P>0.05; data not shown - dns). Pigs fed ZnO had higher ADG and G:F than pigs fed LAM (P<0.05) during d 7-14. However, pigs fed LAM had higher ADG than BD and ZnO fed pigs during d 14-21 and 21-32 (P<0.05), and higher ADG and G:F than the BD from d 0-32 (P<0.01) (Table 1). There was no effect of LAM or ZnO on FI (P>0.05; dns). Pigs fed LAM had higher nitrogen and gross energy digestibility than BD and ZnO (P<0.01) (Table 1). Pigs fed ZnO had decreased faecal *E. coli* (8.40 v 9.41 v 9.26 Log_{10} CFU/g, respectively, sem 0.26, P<0.05) and *Bifidobacterium spp.* (7.45 v 8.72 v 8.86 Log_{10} CFU/g, respectively, sem 0.29, P<0·01) than BD and LAM. Pigs fed LAM had higher populations of *Lactobacillus spp*. than BD and ZnO (11.15 v 10.92 v 10.85 Log_{10} CFU/g, respectively, sem 0.08, P<0.05).

Table 1 Effect of laminarin and ZnO on average daily gain (ADG), gain to feed ratio (kg/kg) and apparent faecal nutrient digestibility (g/kg) (L.S.M +/- S.E.M)

	T1	T2	T3	SEM	Significance
ADG 7-14d	204^{ab}	195^{b}	255^{a}	20	*
ADG 14-21d	289^{b}	420^{a}	331^{b}	27	*
ADG 21-32d	483^{b}	576^{a}	484^{b}	24	*
ADG 0-32d	280^{b}	353^{a}	308^{ab}	15.9	*
G:F 7-14d	0·568^{ab}	0·534^{b}	0·638^{a}	0·030	*
G:F 14-21d	0·552	0·634	0·584	0·030	ns
G:F 21-32d	0·568	0·661	0·581	0·038	ns
G:F 0-32d	0·514^{b}	0·608^{a}	0·580^{ab}	0·024	**
Nitrogen digestibility	723 4^{c}	710·8^{a}	620·8^{db}	18·2	***
Gross energy digestibility	677.0^{bc}	725.0^{ae}	608.0^{df}	16.0	***

^{a,b,c,d,e,f} within a row is significantly different. * P<0.05; ** P<0.01, *** P<0.001, ns P>0.05.

Conclusion Dietary supplementation with laminarin post-weaning is a reliable alternative to ZnO as a means to enhance growth performance in newly weaned pigs.

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Does paternal genotype affect the adaptation of piglets to solid feed immediately post-weaning?

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Introduction Feeding behaviour and resulting voluntary feed intake varies between different breeds of grower and finisher pigs. Evidence of genetic variation in feeding behaviour was reported by Hyun and Ellis (1996) who found a significant difference in feeding behaviour between Meishan and Large White breeds. Fernandez *et al.* (2011) reported that Pietrain and Large White pigs had markedly different feeding compared Landrace and Duroc breeds. However, these studies all report on grower or finisher pigs and there have been very few studies comparing the feeding behaviour of newly weaned piglets from different genotypes. This experiment investigated the initiation of feeding immediately post-weaning, the development of feeding throughout the immediate post-weaning week and performance of three genotypes in the immediate post-weaning period.

Material and methods 108 piglets were selected at weaning from Hampshire (H), Large White (LW) and Pietrain (P) crossed piglets and studied for two weeks post-weaning. The experiment was set up as a randomised block design, with three genotype treatments and nine blocks. Piglets were weaned at 27.1 (± 0.21) days of age and 7.8, 8.2 and 7.9 (± 0.31) kg for H, LW and P piglets respectively. Piglets were housed in mixed sex groups of four piglets/ pen. Feed (16.2 MJ DE, 1.6 g lysine /kg) and water were provided ad-libitum. Each piglet's feeding behaviour was recorded by LUFBS (Leeds University's Feeding Behaviour System). Piglets were weighed at weaning (d0), d7 and d14. The experiment lasted for 14 days. Initiation of piglet feeding was recorded firstly as latency to first approach (i.e. when the piglet was first identified at the feeder) and secondly as latency to first feeding visit > 30 seconds. Individual feed intake data was calculated using pen feed intake data apportioned according to feeding time/piglet. All data were analysed using GLM procedures in Minitab15 with pen as the experimental unit for performance data and piglet as the experimental unit, nested within pen, for individual feeding behaviour data.

Table 1 Average pen performance data for Hampshire (H), Large White (LW) and Pietrain (P) piglets in the first two weeks post-weaning

	Period	H	LW	P	(± sem)	p
ADG	Week 1	0.185	0.139	0.184	(0.016)	0.113
(kg/day)	Week 2	0.352	0.340	0.378	(0.023)	0.489
FCR	Week 1	1.296	1.654	1.215	(0.254)	0.457
(ratio)	Week 2	1.307	1.203	1.226	(0.089)	0.691

Table 2 Back transformed means (95 % CI) of latency times immediately post-weaning for individual Hampshire (H), Large White (LW) and Pietrain (P) piglets in the first two weeks post-weaning

Breed	Latency to first approach (min)	Latency to first feed >30 seconds (min)
H	44.17 (25.16 – 72.60)a	89.93 (59.78 – 129.83)c
LW	92.30 (53.04 – 124.95)b	195.59 (113.55 – 288.62)d
P	37.79 (32.11 – 60.94)a	74.44 (53.37 – 126.38)c
p	<0.05	<0.005

Table 3 Apportioned feed intake and eating rate for individual Hampshire (H), Large White (LW) and Pietrain (P) piglets in the first two weeks post-weaning.

Parameter	units	H	LW	P	p
Animals (n)		32	32	32	
FI Day 1	(kg)	0.033a	0.015 b	0.029 a	<0.05
	(± sem)	(0.004)	(0.004)	(0.004)	
FI Week 1	(kg)	1.729	1.433	1.691	0.096
	(± sem)	(0.083)	(0.098)	(0.088)	
FI Week 2	(kg)	3.470	3.162	3.319	0.548
	(± sem)	(0.120)	(0.142)	(0.128)	
Rate of Eating	(g/min)	4.4	4.3	4.4	0.869
	(± sem)	(0.05)	(0.06)	(0.05)	

Results There was no difference in growth performance, feed intake or FCR between the three genotypes (Table 1). Feed intake over the first 24h was also not different between the three genotypes. Latencies to trough approach and first feed are shown in Table 2. The LW piglets were much slower to find solid feed and to initiate feeding activity than the other two genotypes immediately post weaning.

Feed intake/piglet is presented in Table 3 where the total feed intake of H and P piglets was higher than LW piglets over the first 24 h. Subsequent to this period the feeding behaviour differences were less clear over the rest of the first week, though a trend of higher feed intake was observed for Hampshire compared to Large White piglets.

Conclusion Immediately post weaning, Large White piglets were at a distinct disadvantage to the Hampshire and Pietrain pigs that were much faster to find feed and establish eating after weaning. Hampshire and Pietrain piglets were also more active at the feeder in the first 24h. This period immediately after weaning is critical for the weaner piglet (Reynolds et al., 2010) and this experiment suggests that the Hampshire and Pietrain piglets coped better with the stress of weaning and adapted more quickly to the post-weaning environment. This pattern agrees with other studies observing the higher feed intake of Hampshire crossbreeds compared to other genotypes throughout the lifetime of the pigs (Taylor et al., 2009).

Whether the improved performance of Hampshire and Pietrain piglets can be attributed to the better adaptation to weaning requires further investigation.

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The lifetime performance of pigs in relation to their birth and weaning weight

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Introduction It is known that the birth and weaning weight of piglets is a strong predictor of lifetime performance (Douglas *et al.*, 2012). However, Allen *et al.* (2010) found that 28% of piglets with a birth weight under 1 kg achieved a weaning weight of over 8 kg. The subsequent performance of these pigs was unknown. The aim of the current study was to assess the interaction between birth and weaning weight on lifetime growth performance and carcass quality.

Material and methods A total of 200 pigs (Tempo x (Landrace x Large White)) were monitored from birth to slaughter (110 kg) across four time periods. Cross fostering between litters was minimised and at weaning pigs were selected based on their birth and weaning weight to represent four treatments: 1) born light and weaned light (LL), 2) born light and weaned heavy (LH), 3) born heavy but weaned light (HL) and 4) born heavy and weaned heavy (HH) with a fifth treatment 'runts' (Rs) which were reared artificially from birth to weaning. At birth ten runt pigs (birth weight of approximately 1 kg) per time period (40 in total) were allowed to suckle their mother for 24 hrs after which they were transferred to 'Rescue Decks' and offered artificial milk *ad libitum* until weaning (28 days of age). No specific targets of weight were set but pigs were selected at weaning to represent light and heavy birth and weaning weight within the weight profile of all pigs being weaned from a normal batch of pigs (from 18 sows per time period). Pigs were housed in groups of ten according to their weight 'treatment'. Pigs were offered 3 kg/pig of a commercial starter diet (Flatdeck 2000, A One) followed by 6 kg/pig of a second commercial starter diet (Flatdeck 2, A One) after which they were offered a grower diet (14 MJ/kg digestible energy, 186 g/kg crude protein, 12 g/kg lysine) until 12 weeks of age and then a finisher diet (13.5 MJ/kg digestible energy, 170 g/kg crude protein, 9.5 g/kg lysine) to a target slaughter weight of 110 kg. Pigs were weighed at 7, 10, 15 and 20 weeks of age and at slaughter. After slaughter the backfat depth at P_2 (65mm for the top line at the level of the last rib) was measured using the Ulster probe and carcass weight was recorded. The average daily gain (ADG) and kill out percentage (KO%) of pigs was calculated. Data were analysed on an individual pig basis using analysis of variance in Genstat version 10.

Results Table 1 reports the weight and growth rate of pigs throughout their lifetime. The growth rate of runts was poorest at all stages of growth. Furthermore, the backfat depth of runts was significantly greater than that of other pigs (Table 1). HH pigs had the highest growth rate between weaning and 10 weeks of age with the result that they were the heaviest at 10 weeks of age. Although the ADG of these pigs was similar to that of LL, LH and HL pigs between 10 weeks of age and finish, their weight advantage at 10 weeks of age equated to these pigs being heaviest at 20 weeks of age. LL pigs were the lightest at 10 weeks of age and numerically the lightest at 20 weeks of age. However, pigs which were born light but weaned heavy (LH) had a similar growth rate to those pigs born heavy and weaned light (HL). They also had the highest growth rate between 10 and 15 weeks of age with the overall result that LH pigs had a significantly greater 20-week weight LL or HL pigs. The finish weight of LL, LH, HL and HH pigs was similar and averaged 109 kg. The finish weight of runt pigs was significantly lower ($P<0.001$) and averaged 90.7 kg. There was no effect ($P>0.05$) of birth or wean weight on KO% which averaged 74.8% but pigs with a low birth weight had a significantly greater backfat depth at P_2 than pigs with a high birth weight (Table 1).

Table 1 Effect of pig birth and wean weight on lifetime performance (Standard deviation in brackets for birth and weaning weights)

		LL	LH	HL	HH	Runts	SED	P Value
Live wt (kg)	Birth	1.2 (0.19)	1.3 (0.13)	1.7 (0.18)	1.9 (0.15)	1.0 (0.27)	0.04	<0.001
	Weaning	7.0 (0.93)	9.6 (0.74)	7.5 (0.98)	11.0 (0.74)	4.3 (1.19)	0.21	<0.001
	7 wks	15.1^{b}	18.0^{d}	16.7^{c}	20.7^{e}	11.8^{a}	0.50	<0.001
	10 wks	27.6^{b}	31.2^{c}	30.4^{c}	39.2^{d}	21.6^{a}	1.00	<0.001
	20 wks	87.6^{b}	93.8^{c}	90.0^{b}	98.6^{d}	68.1^{a}	3.10	<0.001
ADG (g/day)	Wean - 10wks	419^{b}	441^{c}	467^{d}	577^{e}	354^{a}	18.6	<0.001
	10 - 15wks	713^{b}	760^{c}	704^{b}	713^{b}	514^{a}	45.9	<0.001
	15 - 20wks	1175^{c}	1030^{b}	1205^{d}	1156^{cd}	786^{a}	67.3	<0.001
	10 – Finish	884^{b}	906^{bc}	888b	857^{b}	698^{a}	34.4	<0.001
	P_2 (mm)	12.4^{bc}	12.8^{c}	11.8^{ab}	11.4^{a}	14.9^{d}	0.79	<0.01

Conclusions Whilst pigs born heavy and weaned heavy had the best lifetime performance and those born light and weaned light had the poorest, pigs which were born light but achieved a good weaning weight continued to perform at a high level with the result that their 20-week weight was superior to pigs born heavy but weaned light. These results support the conclusion of Douglas *et al.* (2012) in that interventions to increase weaning weight will maximise lifetime performance. Light birth weight pigs, regardless of their weaning weight were fatter than heavy birth weight piglets. This has important implications for the current pig population in the UK since increasing litter size is resulting in a greater proportion of light birth weight piglets.

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The effects of providing a plant extract or a high intensity sweetener in the drinking water on the welfare and performance of weanling pigs

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Introduction The separation of piglets from the dam at weaning is associated with stress related behaviours including low levels of voluntary feed and water intake resulting in weight loss and growth check. (Lalles *et al*., 2007). As feed intake is partly dependent on water intake it has been suggested that the reduced liquid intake may be responsible for the growth check occurring at weaning (Dybkjaer, 2006). Therefore, encouraging young pigs to drink more may help improve digestibility and stimulate feed consumption. Plant extracts (PE) and high intensity sweeteners (HIS) have been shown to stimulate appetite and to improve welfare and performance parameters in weanling pigs (Manzanilla *et al*., 2004; Sterk *et al*., 2008). Limited data is available on the relationship between flavoured water intake and feed intake. Therefore the aim of the present study was to measure the effects of providing a HIS or a PE in the drinking water during the first week post-weaning on piglet weight gain, faecal consistency and salivary cortisol.

Material and methods 106 piglets of mixed breeds from 10 litters were weaned at 28 days ± 0.686 (± s.e.d.). A second replicate was conducted with 88 piglets from 10 litters weaned at 28 days ± 0.64 (± s.e.d.) in order to increase confidence in the results. At weaning litters were mixed and piglets were divided into 12 groups balanced for weight and sex and breed. Groups were randomly allocated to 1 of 3 dietary treatments (n=4). Treatments were control (plain water), Sucram™ (water + HIS) and Xtract™ (water + bioactive PE) (Pancosma, Geneva, Switzerland). Each group was provided with a cube drinker containing the treatments, water from nipple drinkers and a piglet starter diet (Vito Start 225™, BOCM Pauls, Wherstead, Ipswich, United Kingdom) which were offered *ad libitum*. The water in the cube drinkers was changed twice a day (AM/PM) and refusals were measured. Feed refusals were measured 3 days post-weaning and 7 days post-weaning. Piglets were weighed 3 days post- weaning, 7 days post-weaning (n=194) and then weekly until they left the grower house at an average weight of 45 kg (n=106). Saliva samples were collected from a random selection of individuals (n=30) on the day prior to and post weaning by oral insertion of cotton buds. Samples were frozen and salivary cortisol concentrations were analysed using a commercial kit (Parameter™ Cortisol, R&D Systems, Abingdon, UK). Faecal scores were recorded twice daily (AM/PM) using a 4-point scale. Piglets were housed indoors on slatted floors under monitored temperatures. Statistical analyses were carried using Genstat (Version 14.1, Lawes Agricultural Trust). Feed intakes, water intakes weight gain and differences in cortisol levels the day before and after weaning were analysed using an analysis of variance (ANOVA). A paired t-test was used to analyse overall cortisol levels of individual treatments. A Kruskall-Wallis non-parametric ANOVA was used to analyse faecal scores. Correlation analysis was performed to determine the relationship between water and feed intake.

Results There was a high correlation between water and feed intake over all treatments ($P < 0.01$). Although no statistical differences between treatments in terms of water and feed intake were detected ($P > 0.05$), Table 1 shows intakes were similar in piglets offered a PE or a HIS compared to control. Piglets offered PE gained more weight from day 4-7 than piglets in the control groups (Table 1). With regards to long-term performance there was an overall tendency for differences between treatments ($P = 0.09$). Piglets offered HIS were heavier compared to piglets in the control group in week 7 ($P < 0.05$) and week 9 ($P < 0.05$). Piglets offered the PE did not show higher salivary cortisol levels the day after weaning than the day before weaning ($P > 0.05$), compared to the other groups and presented lower faecal scores on day 2, 3, 4 ($P < 0.01$) and 7 ($P < 0.05$).

Table 1 Total feed and water intake (n=4) and daily live weight gain (n=194) during the first week post-weaning

		Control	PE	HIS	s.e.d.	P
Total feed intake/kg	(n=4)	13.5	15.6	16.6	3.34	n.s.
Total water intake/L	(n=4)	59.5	71.9	74.2	12.31	n.s.
Daily weight gain/kg, day 0-3	(n=194)	-0.0031	0.0132	-0.0042	0.052	n.s.
Daily weight gain/kg, day 4-7	(n=194)	-0.0009^{a}	0.0584^{b}	0.0279^{ab}	0.022	< 0.05

Conclusion The correlation between water and feed intake supports the assumption that feed intake at weaning could be increased by encouraging water intake. A positive impact on weight gain, indicates that the observed differences in feed and water intake may be of biological significance. Therefore offering these supplements may be an effective way of improving welfare and performance at weaning.

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Evaluating a model that predicts phosphorus digestion, retention and excretion in growing and finishing pigs

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Introduction We have developed a model that enables prediction of phosphorus (**P**) retained and excreted for different pig genotypes and under different dietary conditions. Before confidence can be placed on the predictions of the P model, evaluation is necessary. The objective of this work was to conduct a sensitivity analysis and qualitatively and quantitatively compare the model predictions with observations from the literature that were not used for model construction. Experiments covering a broad space of potential diet compositions (varying in inorganic P, dietary calcium and exogenous phytase content) were compared to model outcomes, so more confidence could be placed upon the appropriateness of the concepts and the accuracy of parameters upon which the model was based.

Material and methods A sensitivity analysis of model predictions to ±20% changes in model parameters with an inherent uncertainty was undertaken using a basal UK industry standard diet. The default pig genotype used for the sensitivity analysis was characterized by BSAS (2003) as being of 'intermediate growth'. A sensitivity output of less than 5% compared to the default values was considered as non-significant. Independent data sets of published experiments were used to evaluate model performance based on graphical comparisons and statistical analysis. To assess the goodness-of-fit of simulated predictions against experiments, a series of statistical tests were used. The literature studies were selected based on the following criteria: (1) the studies used growing-finishing pigs (20-120 kg body weight); (2) Pigs grew in thermo-neutral environment; and (3) the studies provided information on P intake, retention and excretion in g/day. Comparison with the studies of Ekpe *et al.* (2002) and Stein *et al.* (2011) are reported here.

Results From the sensitivity analysis, the model parameters for the relationship between microbial phytase and total P excreted and for the relationship between endogenous large intestine phytase and soluble P excreted were proven the most influential. According to Ekpe *et al.* (2002), increasing the di-calcium phosphate (**DCP**) supplementation increased the P retained in a curvilinear manner, while the total P excreted increased at an increasing rate (Figure 1). The simulated values followed the same patterns between observed and predicted, with *r* being 0.88 and 1.00 for P retained and total P excreted, respectively. The predicted results were within the standard error of the mean range of the observed results, an indicative of the low RMSE. Increasing the dietary Ca caused a decrease in the P retained as more Ca-phytate complexes were formed and as a result there was less digested P. The predicted values of P retained were close to those observed by Stein *et al.* (2011). The correlation coefficient and model efficiency were relatively low, 0.54 and 0.16, respectively. The failure of the model to predict the same trend was the result of overestimation of the P digested at higher dietary Ca contents.

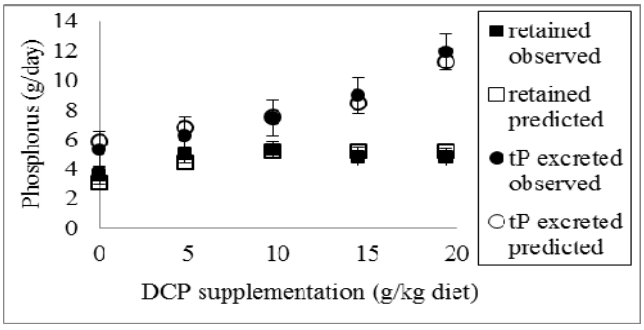

Figure 1 Comparison of experimental observations with simulated predictions for retained phosphorus and total phosphorus (tP) excreted for the study of Ekpe *et al.* (2002), which evaluated the effect of increasing dietary di-calcium phosphate.

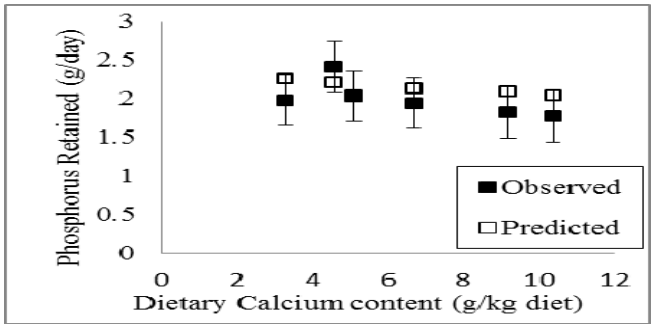

Figure 2 Comparison of experimental observations with simulated predictions for retained P for the study of Stein *et al.* (2010), which investigated the effect of graded levels of calcium levels in the diet.

Conclusion In general, the model satisfactorily predicts the quantitative pig responses in terms of P digested, retained and excreted to variation in dietary inorganic P supply and Ca. The overestimation of retained P at higher dietary Ca contents could be attributed to the assumption that no Ca-phytate complexes could be formed in the stomach. The model can be used to develop feeding strategies to optimise P retention and minimize P excretion excretion, therefore, decreasing the feed costs and the environmental impacts in growing and finishing pigs.

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Activity monitoring in equids at pasture using global positioning systems (GPS) and inertial measurement units (IMUs)

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Introduction Signs of pain are reportedly less obvious in donkeys and different from horses. This makes early detection of diseases such as laminitis more challenging. Equids spend large amounts of their time at pasture grazing and resting, interspersed with locomotor activity (walking, trotting, cantering). Small and lightweight sensors are now available allowing monitoring of location (global positioning system, GPS) in horses (Hampson *et al,* 2010) and even in smaller animals and their handlers (Pfau *et al*, 2011). More detailed quantification of whole body movement can be achieved with inertial measurement units (IMUs) (Pfau et al, 2005). Similar systems have for example been used to evaluate the horse's response to a new feeding system (Hampson et al, in Press). At pasture, continuous monitoring of location and activity might aid early detection of orthopaedic problems by detecting animals showing reduced locomotor activity.
In this study we present data from a first validation study using a sensor based system for continuous monitoring of location and activity in equids during their normal day at pasture. We hypothesised that a combination of a GPS unit and IMU attached to an equid will allow differentiation into different activities, in particular locomotion, resting and grazing.

Material and methods A GPS and a 6 degree of freedom IMU were attached to a head collar of one horse and one donkey. Gold standard activity monitoring was achieved through human observation. A simple threshold based activity detection algorithm was implemented extracting locomotor activities using a running median of the autocorrelation product of sensor acceleration or a 3km/h GPS speed threshold. Quiet standing was identified using a threshold for standard deviation of sensor acceleration and grazing was defined as all other times not engaged in the previous two activities. Validity of testing methods were established via test accuracy, precision, sensitivity and specificity. Three full days of data were compiled i.e. GPS, IMU activity log.

Results The activity extraction methods resulted in accuracy, precision, sensitivity and specificity for locomotor activity of 92%, 68%, 62% and 96% for a 3km/h GPS threshold and 92%, 73%, 56% and 97% for autocorrelation. Accuracy, precision, sensitivity and specificity of 98%, 94%, 72% and 99.7% were achieved for quiet standing using the standard deviation of sensor acceleration.

Conclusions The extraction techniques performed on GPS and x-IMU data can successfully differentiate locomotor and standing activities. Food-intake was inferred as the remaining time. The ancillary GPS data provides-data such as distribution of location and total distance travelled. Further refinement of the classification techniques investigating more sophisticated statistical classifiers and features extracted from longer and shorter time-windows are likely to further improve classification accuracy. This requires the collection of larger 'supervised' data sets for the training of a multidimensional stochastic classifier incorporating several features into the decision. We are currently in the process of collecting larger data sets from an increased number of animals with different grazing regimes. The preliminary results presented here are encouraging and further improvements in sensor technology in terms of sensor size and uninterrupted data collection time (battery life) will further improve the ease-of-use of these systems.

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The efficacy of electrical therapies on increasing the stride length of riding school horses

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Introduction Electrical therapy modalities such as massage pads and heat lamps are becoming more popular in the equine industry. These are not only used in injury recovery, but in prevention as well. To date, there is limited research in to the extent these therapies can help the ridden horse. A recent study by Hill & Crook (2010) identified that massage may play a role in improving equine locomotion and facilitated a subsequent increase in stride length. Thermotherapy is used for both its analgesic and increased circulatory effects, as well as increasing tissue metabolism and extensibility (Kaneps, 2000). As heat enhances muscle elasticity it can aid prevention of muscle injury (Buchner and Schilboeck, 2006). Back injuries can be a major cause of alteration in the equine's gait and strained muscles are common injuries experienced by the horse and can often lead to stiffness and restriction of gait. Increased stride length can give a competitive advantage to performance horses (Deuel & Park, 1990) but can also be an indication of a healthy back in the non-competitive horse (Peham *et al.,* 2001). The aim of this study were to assess the effect of a twenty minute session of several intensities of an electronic massage pad, the Equissage™, and twenty minute solarium (heat lamp) sessions on the natural SL of the horse.

Material and methods Phase one- Massage Pad: The stride lengths (SL) were measured of six riding school horses aged 11 to 15 years, a mixture of mares and geldings and all in a similar level of work. Baseline measurements were recorded for all horses and used to measure the change in stride length for each massage pad setting. Horses were allocated to two groups and followed a crossover design of treatment and control with a week-long senescence period. Three treatments were administered (low, medium and high intensity of the pad) three times per week with SL measurements taken on the final treatment day. Treatments were repeated in 'warm' (>7°C) and 'cold' (<7°C) ambient conditions.
Phase Two- Solarium: The SL were measured of eight riding school hoses aged six to 14 years, a mixture of mares and geldings and all in a similar level of work. Baseline measurements were recorded for all horses and used to measure the change in stride length following solarium treatment. Horses were allocated to two groups and followed a crossover design of treatment and control, SL was recorded immediately after treatment/control.
SL were measured using 2D motion analysis software. Horses were trotted in hand at a controlled speed, the mean SL was calculated from three repeats. Statistical differences were investigated using general linear analysis and Kruskal Wallace

Results Mean SL measurements indicate that both the massage pad and the solarium can increase the SL of riding school horses (tables 1 and 3). Intensity of massage pad settings does not significantly influence SL (table 1). Ambient temperature had an over riding effect on SL, colder temperatures result in decreased SL with and without massage pad treatment (table 2). Ambient temperature did not differ during solarium investigations so this could not be measured.

Table 1 Mean Stride Length following treatment with massage pad. Groups with different letters denote differences with significance of P<0.05

Treatment	Mean Change in SL (m) ± SD
Control	-0.01± 0.08^{A}
Low	0.07±0.12^{B}
Medium	0.15±0.13^{B}
High	0.13±0.16^{B}

Table 2 Mean Stride Length following treatment with massage pad in different temperatures. Groups with different letters denote differences with significance of P<0.05

Treatment	Mean Change in SL (m) ± SD	Temperature
Control	-0.01± 0.08^{A}	Warm
Control	-0.06± 0.13^{B}	Cold
Medium	0.15±0.13^{C}	Warm
Medium	-0.06±0.27^{B}	Cold

Table 3 Mean Stride Length following treatment with solarium.
Groups with different letters denote differences with significance of P<0.05

Treatment	Mean SL (m) ± SD
Control	234.7± 15.1^{A}
Solarium	250.7± 12.2^{B}

Conclusions These results show that both electrical therapy modalities can be used to increase the stride length of riding school horses, indicating that they may aid in relaxation of back muscles, this suggests that there may be positive implications for the horses' welfare. Ambient temperature has an over-riding effect on massage pad efficacy as use on days with colder temperatures has shown a deleterious effect on stride length regards of treatment. This warrants future investigation of combining the two modalities.

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Is there a correlation between the shape of the central and third tarsal bones in horses and the occurrence of OA in the associated joints?

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Introduction Osteoarthritis (OA) of the tarsus is common in horses and it has been suggested that wedging of the central (CTB) and third (3TB) tarsal bones may be a risk factor for developing this disorder (Dutton et al 1998): wedging is defined as decreased height at the dorsal or plantar aspect. The aim of this study was to investigate the relationship between tarsal bone conformation and osteoarthritis of the tarsus.

Material and methods Computed tomographic (CT) images were acquired from 45 cadaver tarsi from a random sample of skeletally mature, thoroughbred-type horses euthanased for reasons unrelated to this study. The height of the CTB and 3TB was measured at the most dorsal aspect and a more plantar aspect in three sagittal planes: lateral sagittal (LS) plane, mid-sagittal plane and medial sagittal (MS) plane. A "wedging index" was calculated as the ratio between the dorsal and plantar measurements. All CTs were graded for signs of OA (absent (0), mild (1), moderate (2) or severe (3)) in PIT, DIT and TMT joints (Byam-Cook & Singer 2009). Wedging indices for varying grades of OA in each joint were not normally distributed in grade 3 cases, they were compared using Kruskal Wallis test and the correlation between the wedging index and degree of OA was assessed by calculating the Kendall's tau.

Results There was a moderate negative significant correlation between wedging index of the CTB in the mid-sagittal plane and degree of OA in the TMT joint (τ = -0.24, P=0.04). (Fig. 1). There was a moderate negative significant correlation between wedging index of the CTB in the LS plane and OA in the DIT joint (τ = -0.27, P=0.02) and mid-sagittal plane (τ = -0.27, P=0.02) (Fig.1). The mean (±SD) wedging index of the CTB in the mid-sagittal plane for PIT joint with no OA was 1.03 (±0.08) this was significantly greater than the mean wedging index for grade 1 OA which was 0.95 (±0.11) (P=0.01). There was a significant difference between the wedging index of the 3TB in the mid-sagittal plane (P= 0.04) and MS plane (P= 0.01), when tarsi were grouped according to grade of OA in the TMT joint (Fig 1.) The wedging index decreased with increasing OA grades 0-2, however grade 3 cases showed increased wedging index. The same trend was seen in the DIT joint, however the differences between groups were not significant either in the MS (P=0.08) or mid-sagittal (P=0.15) planes. No relationship was seen for the wedging index of the third tarsal bone related to grade of OA in the PIT joint.

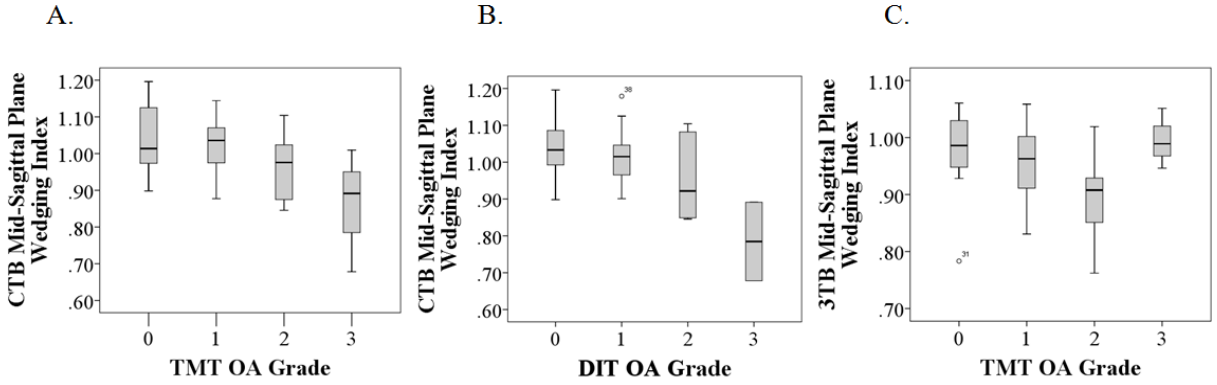

Figure 1 CTB and 3TB conformation in horses of different grades of OA. The wedging index value of below 1 indicates dorsal wedging, a value above 1 plantar wedging. OA: 0= absent, 1= mild, 2= moderate, 3= severe. A- Mid-sagittal plane CTB conformation and TMT OA grade. B- Mid-sagittal plane CTB conformation and DIT OA grade. C- Mid-sagittal plane 3TB conformation and TMT OA grade.

Conclusions Our study suggests that wedging of the small tarsal bones is associated with OA in the associated joints. Care should be taken in foals to prevent the development of this wedging and later corrective farriery should aim to reduce uneven loading of the tarsus.

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Linseed oil in a compound feedstuff offered to horses: effects on haematology and on plasma metabolites over a four months period

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Introduction Sport horses are offered diets high in concentrates in order to meet their energy requirements. Cereals are the main ingredients of such concentrates. Fat and oils, although not usual compounds in horse feeding, have been introduced in the horse regime over the last 10 years. They are usually offered over rather short periods of time during the racing season. The aim of the present study was to assess the effects of linseed oil included in a compound feedstuff on blood metabolites over a 4 months period in trained horses.

Material and methods Eight adult horses – 3 mares and 5 geldings – were divided in 2 groups. The horses were trained four days a week over one and half hour. They were, whatever the weather; taken daily on a pasture in which the grass quickly disappeared. They were offered a diet made of 50% grass hay and 50% concentrate. The concentrate was composed of 48% of whole spelt, 48% of rolled barley, 3% of molasses and 1% of a mineral mixture in the control concentrate. In the oil supplemented concentrate, 8% of barley was substituted by 8% of first pressure linseed oil. The control diet was characterised by a protein content of 108g/kg, an ADF content of 110g/kg and a NDF content of 274g/kg. The ether extract was low at 15g/kg. In the oil supplemented concentrate, the ether extract was increased at 94g/kg and the other chemical compounds reduced – 101g/kg for protein, 104g/kg for ADF and 255g/kg for NDF –. The Ca and P contents were close to 8 and 3g/kg respectively. A transition period of 4 weeks was managed on the beginning of the trial. The amounts of hay and compound feedstuff were adjusted in order to maintain constant the live weight of the horses. Blood samples were taken the last day of each period by a catheter fixed in the jugular vein every 20 minutes over 440 minutes. The samples were analysed according to standard methods. In the morning, hay was offered to the horses before the first sample was obtained while the concentrate was given one hour later, just after the third sample was taken. The sampling session was repeated 4 times on the end of months 1, 2, 3 and 4 of the experiment.

Results The hay and the compound feedstuffs were completely eaten within one hour after being offered. The total feed intake was 6.2kg/d in the control group and 6.3kg/d in the linseed oil group. The added linseed oil was 0.278kg/d corresponding to 4.5% of the feed intake. The inclusion of linseed oil did not affect the plasma concentrations of glucose and of insulin (Table 1). By contrast, there were reductions in plasma concentrations of urea (4.68 vs 5.54 mmol/l, $P<0.001$) and triacylglycerols (0.20 vs 0.26 mmol/l, $P<0.001$). An increase in total cholesterol concentration (2.69 vs 2.41, $P<0.01$) was also observed. There were period effects on concentrations of plasma glucose ($P<0.001$), total cholesterol ($P<0.01$) and insulin ($P<0.01$) with a large increase in plasma insulin when period 4 was compared with period 1 (39.7 vs 65.6/µUI/ml). In term of haematology, the linseed oil inclusion significantly ($P<0.05$) reduced erythrocyte counts (6.8 vs 7.6 10^{12} cells/l), the haemoglobin content (11.6 vs 13.0 g/dl) and the hematocrit (0.32 vs 0.36 l/l) but there were no effects on haematology. Furthermore there were no period effects on haematology.

Table 1 Metabolites and insulin contents in plasma and haematology (averages calculated for each diet on the individual data on the 7 sampling hours of the 4 months and for each month on the individual data of the 2 diets)

	Diets			Periods				
Metabolites	Control	Linseed oil	P>F	Month 1	Month 2	Month 3	Month 4	P>F
Glucose (mmol/l)	5.57	5.55	NS	5.25	5.74	5.33	5.93	***
Triacylglycerols (mmol/l)	0.26	0.20	***	0.27	0.22	0.18	0.25	+
Urea (mmol/l)	5.54	4.68	***	5.03	5.61	4.96	4.83	NS
Total cholesterol (mmol/l)	2.41	2.69	**	2.52	2.82	2.49	2.38	**
Insulin (µUI/ml)	50.54	54.10	NS	39.74	54.79	49.14	65.61	**
Haematology								
Erythrocyte (10^{12} cells/l)	7.58	6.84	*	7.65	7.26	7.39	6.53	+
Haemoglobin (g/dl)	12.99	11.57	*	13.06	12.40	12.60	11.06	+
Hematocrit (l/l)	0.36	0.32	*	0.36	0.34	0.35	0.31	+
Leukocytes (10^9 cells/l)	7.86	7.21	NS	7.30	7.25	7.58	8.01	NS
Platelets (10^9 cells/l)	205.56	210.06	NS	199.38	203.75	203.63	224.50	NS
Lymphocytes (10^9 cells/l)	3.03	2.53	NS	2.61	2.87	2.83	2.81	NS
Monocytes (10^9 cells/l)	0.42	0.41	NS	0.46	0.31	0.43	0.47	+
Neutrophil (10^9 cells/l)	4.25	4.26	NS	4.07	3.97	4.13	4.85	NS

Conclusion The lack of significant differences between control and linseed oil diets in the plasma content of glucose and insulin indicated a well balanced homeostasis in the glucose metabolism pathways. The reduced plasma urea concentration gave further support to such a mechanism owing to a reduced amount of amino-acids being used as energy supply. By contrast, the significant reduction in triacylglycerols content was unexpected. So, since linseed oil supplementation did not affect intakes but improved metabolic pathways, linseed oil supplementation could be of interest for racing horses.

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Hay for Horses: The nutrient content of hay before and after steam treatment in a commercial hay steamer

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Introduction In an attempt to reduce the negative effects of dust on the respiratory health of the horse, many owners soak the hay prior to feeding. However, previous work has shown that soaking can reduce the nutrient content of hay (Moore-Colyer, 1996; Warr and Petch, 1992), whereas steaming 5 kg hay nets did not cause nutrient loss (Blackman and Moore-Colyer, 1998). This study sought to determine the extent of nutrient loss from a wide range of UK conserved hay when complete strung bales were steamed for 50 minutes in the Haygain 1000 hay steamer (HG 1000).

Method Thirty bales of hay from all over the UK were used in this study. Samples from 5 areas of each bale were taken prior to steaming. The bale was then steamed for 50 minutes in an HG 1000 whereupon another composite sample was taken from 5 areas of the bale. Samples were immediately stored in a freezer before being transported to the laboratory where they were dried in a force-draught oven at 60°C. Post drying the 60 samples (30 dry and 30 steamed) were analysed for total nitrogen by use of the Leco FP428 nitrogen determinator; sodium, potassium, calcium and magnesium by ICP-AES; phosphorous by colorimetry, water soluble carbohydrates by an automated anthrone method and trace elements by ICP-AES. A paired t-test was then performed to test for significant differences in nutrient content between the dry and steamed hay.

Results

Table 1 Nutrient content of 30 different samples of hay before and after steaming for 50 minutes in the HG 1000

Nutrient (units)	Dry (mean)	Steamed (mean)	Standard error of mean	Significance (P)
N (%)	1.12	1.19	0.025	0.014
Ca (%)	0.39	0.41	0.027	0.428
K (%)	1.36	1.50	0.068	0.041
Mg (%)	0.12	0.12	0.007	0.407
Na (%)	0.13	0.15	0.025	0.465
P (%)	0.15	0.16	0.008	0.276
WSC (%)	12.6	10.3	0.827	0.009
Cu (mg/kg)	46.5	61.3	15.32	0.341
Mn (mg/kg)	108	124	18.03	0.390
Fe (mg/kg)	288	121	120.3	0.174
Zn (mg/kg)	17.5	23.5	1.54	0.001

Conclusions Steaming for 50 minutes in the HG 1000 had no effect on Ca, Mg, Na, P, Cu, Mn or Fe. The only nutrient to be lost as a result of steaming was WSC which showed a 2.3% loss. This is probably due to partial heat-induced break down of the cellular structure of the hay, allowing nutrient leaching. The loss of WSC would account for the small proportional increases noted in N, K and Zn. The small but significant reduction in WSC may also make this hay a useful fodder when fed to ponies pre-disposed to laminitis.

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The effect of steam treatment on the bacteria, yeast and mould concentrations in haylage for horses

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Introduction Poor weather conditions and increasing knowledge on how to produce haylage for horses has led to many owners now preferring to feed horses haylage. However, aerobic microorganisms known to contribute to serious equine health issues including RAO, botulism and mycotoxicosis rapidly proliferate in haylage after the bales have been opened. Previous work has shown that steaming reduces microbial contamination in hay (Moore-Colyer and Fillery, 2012), however no information is available on the effects of steaming haylage. Using 5 replicate haylage bales divided into 4 this experiment measured total viable count (TVC) and yeast and mould counts (YM) in haylage immediately after opening, 4 days post opening, immediately after steaming, and 4 days post steaming.

Methodology Five commercially produced bales of good quality rye grass haylage were randomly selected from a farm in Gloucestershire. Bales were divided into four sections of equal weight. Sections were tested for microbial contamination: 1 immediately after opening, 2 following four days in an open, clean plastic bag, 3 immediately post steaming and 4 post steaming and four days storage in an open, clean plastic bag. Haylage was steamed in the HG 600 steamer for approximately 50 minutes. . Microbiological testing was conducted as described by James and Moore-Colyer (2010) whereby the 20 haylage samples (5 fresh, 5 steamed, 5 left open for 4 days, 5 steamed and left for 4 days) were prepared as follows: A one gram sub-sample was weighed into a sterile stomacher bag and 79 ml of peptone saline diluent was added. Stomaching was carried out for 2 minutes. Sequential dilutions were prepared down to 10^{-6}. Two x 1 ml from each were placed onto 2 x 3M ^{TM} petrifilms, (3M Microbiology, St Paul, MN 55144-1000), and incubated for 3-5 days at 20^{o}C (mould films) and 32^{0}C (bacteria). Colonies were counted using a standard colony counter. Differences between treatments were determined using analysis of variance and lsd test = $t_{(error\ df)}$ x s.e.d on log transformed data.(Genstat, 13).

Results

Table 1 Bacteria (TVC) and fungi in fresh haylage, haylage opened for 4 days, freshly steamed haylage and 4-day open steamed haylage

CFU	Fresh	Fresh + 4 days	Steamed	Steamed + 4 days	s.e.d
Fungi /g	420	2786	12	128	
Log fungi	2.48^{c}	3.38d	0.45^{a}	1.58^{b}	0.304
pTVC/g	41,600	114,000	10	304	
Log TVC	4.556^{c}	5.048^{c}	0.823^{a}	2.092^{b}	0.2701

^{abc} Values in the same row not sharing letters differ significantly (P<0.001)

Results show that four days post opening, yeast and mould contamination of haylage increased significantly (P<0.001). Steaming significantly reduced TVC and YM of freshly opened haylage (P<0.001) and crucially, 4 days post steaming, microbial contamination of haylage remained significantly lower (P<0.001) than that of a freshly opened bale.

Conclusion These results clearly indicate that steaming haylage in the HG 600 reduced microbial contamination and left 4-day old steamed haylage with a lower microbial concentration than freshly opened haylage.

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An investigation to determine the palatability of steamed hay, dry hay and haylage

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Introduction Horses have evolved to consume large amounts of conserved forage, as a replacement for grass. In recent years, hay has been replaced by silage and haylage (Holmquist *et al.,* 2002). Hay has the potential to contain the highest dust content; consequently soaking hay has become common practise for horse owners to manage respiratory disorders (Clements *et al.,* 2007). The most recent revolution in feeding hay as conserved forage to horses, is the development of the Haygain steamer. Propress Equine (2011) undertook extensive laboratory research over three years to develop their steamer. Unlike soaking hay, steaming at an excess of 100 degrees Celsuis kills, 100% of fungi and yeast spores, and 98.84% of bacteria within hay, if steamed for fifty minutes, thus improving health, performance and well being (James *et al.,* 2009). The main objective of this research was to determine the relative palatability of steamed hay compared with dry hay and haylage.

Method Three methods of feeding forage were used, steamed hay, dry hay and haylage. The hay was steamed for fifty minutes using the Haygain HG-600 steamer. Seven horses were used in the nine-day trial, all owned and stabled by Writtle College Lordships Stud. Prior to the first feed of the day each horse received all three forages in haynets, for a one hour period allowing free choice consumption preference. Consumption rate was determined by the weight difference in forage, before and after the one hour period. During the one hour period observations were undertaken for the first five minutes of each horse receiving all three forages to identify initial preferences. The first choice, second choice and third choice of forage consumption were recorded or if a preference was shown for consumption of one or two forages. The position of the three forage types was determined using a 3x7 Latin square design. Analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test were used to analyse consumption. To determine significance within the forage pattern observations, a Chi-squared goodness of fit test was undertaken.

Results The overall trial results demonstrated a significantly greater consumption of steamed hay (6.72kg ± 1.17 s.e) compared to haylage (2.04 kg ± 0.36 s.e.), ($F = 8.29$, $df = 2$, $P = 0.003$, Bonferroni, $P = 0.002$). However, there was no significant difference between steamed hay and dry hay or between haylage and dry hay (Figure 1). During the first five minutes of each horse receiving all three forages, observations were recorded to demonstrate which forage was consumed first and if the horse moved onto another forage. The Chi-squared goodness of fit test showed steamed hay to be chosen most often as the first choice forage to be consumed, followed by haylage and then dry hay (Chi-squared = 11.81, $df = 2$, $P = 0.0027$).

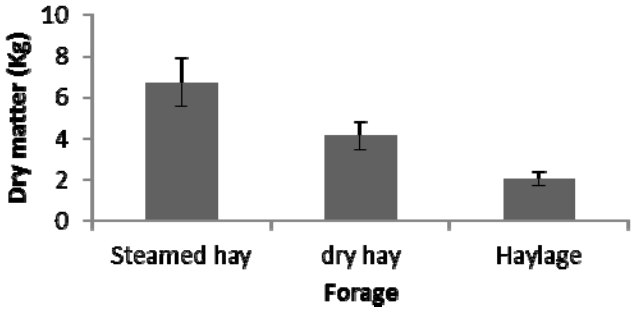

Figure 1 The total mean consumption of the three forages.

Conclusions The results of this experiment demonstrate a highly significant preference towards the consumption of steamed hay compared to haylage when calculated as a mean consumption. However, there was no significant difference between the consumption of steamed hay and dry hay. Steamed hay was chosen as the first choice forage consumed each day over the nine-day trial, demonstrated during the observation period. There was no rejection of any forage by any horse. Each horse showed individual preferences and thus varied consumption rates. This demonstrates that palatability is different within individuals and unless large numbers of horses are used for forage trials, a significant difference in palatability for one forage is difficult to achieve.

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Association between periodontal disease and open or closed diastemata in cheek teeth of horses

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Introduction Equine diastemata are defined as a pathological dental condition that presents as abnormal spaces between 2 adjacent teeth within the same dental arcade (Carmalt, 2003). In open diastemata (oD), feed material can enter and leave, in closed diastemata (cD) feed is trapped and cannot leave. Due to the pathological nature of equine diastemata, feed material can become deeply impacted into the gingiva and periodontal tissues (Dixon *et al.*, 2008), resulting in periodontal food pockets development where food impaction becomes deeper contributing to progressive stretching, inflammation and obliteration of the periodontal ligament (Dixon, 2006). The impacted feed stasis and its decomposition trigger the events of periodontal disease (PD) (Klugh, 2005).

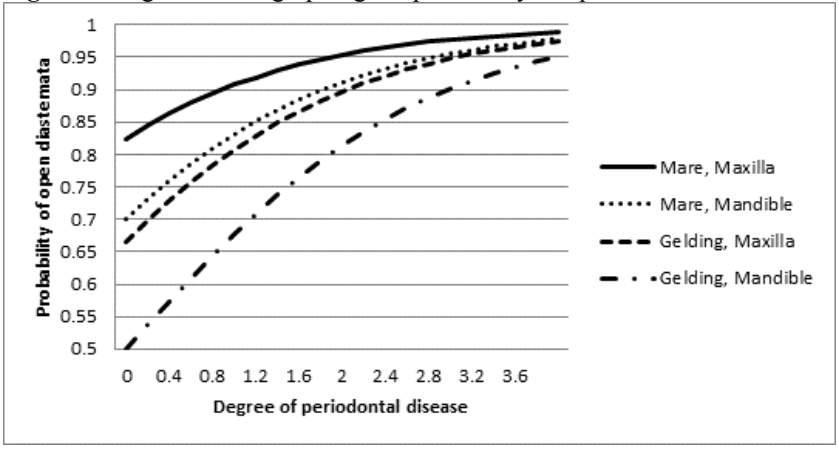
Material and methods 50 cadaver heads were used in this study. These were collected from Potters abattoir, in Taunton, Somerset, UK. All the horses used for collection of data were the ones available for slaughtering during a single day at the abattoir. The severity of the PD present was graded from 0-4 by matching the clinical signs with those proposed by Klugh (2005). The "diastemata prevalence" was visually identified using a torch and dental mirror and the classification as "open" or "closed" was established using the definitions proposed by Carmalt (2003). A logistic regression was used to model "diastemata prevalence" function of the 3 factors ("gender", "jaw" and "side") and the covariate "degree of PD". A backwards stepwise procedure for selection of significant variables and covariate was implemented, and those found to be significant were left in the model.

Results The factors "jaw" ($P<0.05$) and "gender" ($P<0.01$) were found to be significant, as well as the covariate "degree of PD" ($P<0.001$). For the factor "jaw", "maxilla" led to a 135% increase in the odds ratio of "open" vs. "closed" diastema, therefore, diastemata have a higher probability of being open if located in the maxilla. Relatively to the factor "gender", mare" leads to an increase in 99% in the odds ratio of "open" vs. "closed" diastema, therefore, diastemata have a higher probability of being open in mares rather than geldings. Finally, the covariate "degree of PD" was found to be positively related with open diastemata and therefore, the higher the degree of PD the higher the probability of finding open diastemata.

Table 1 Parameters of the fitted logistic regression.

Variables in equation		β	SE (β)	P-value	95% CI (β)		OR (e^{β})	95% CI OR (e^{β})	
Gender	Mare	0.855	0.283	< 0.01	0.301	1.409	2.351	1.798	2.905
	Gelding	0							
Jaw	Maxilla	0.690	0.286	< 0.05	0.129	1.251	1.994	1.440	2.548
	Mandible	0							
Periodontal disease		0.738	0.103	< 0.001	0.536	0.940	2.092	1.538	2.646

Figure 1 Logistic curve graphing the probability of open Diastemata function of gender, jaw and degree of PD.



Conclusions There is limited research relating PD with the prevalence of oD and cD and this study brings some light into this gap, showing an higher probability of development of PD in oD. Mares and maxilla have higher odds for development of oD than gelding and mandible. PD degree is higher in mares, oD and mandible when each variable is considered separately.

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Cholesterol improves the cryo-stability of stallion spermatozoa

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Introduction Semen from approximately one third of all stallions is excessively damaged by the cryopreservation process and the mechanisms behind this variation remain to be elucidated. During the cooling and cryopreservation processes the sperm cell membrane undergoes a transition from a liquid to a gel phase, likely due to the removal of bound water molecules from phospholipids and a tighter packing of the lipid bilayer, while the reversal during thawing has been associated with the leakage of solutes to the extracellular environment. The hypothesis of this study was that the inclusion of cholesterol in a stallion semen extender would stabilise the sperm membrane, increase its fluidity, thereby, improving post-thaw semen quality.

Material and methods Semen was collected from three stallions during the breeding season at a commercial stud using an artificial vagina. Two ejaculates were collected with an interval of 3 days and each ejaculate was processed separately. Following collection the gel fraction was removed and the volume and progressive linear motion (PLM) were assessed. Sperm concentration was assessed using a haemocytometer and each ejaculate was diluted to 120 x 10^6 sperm/mL in one of the four extenders, namely; 1. Tyrosine Albumin Lactate Pyruvate (TALP) extender 2. Methyl-β-cyclodextrin (MβCD) - cholesterol (0.75 mg/mL) 3. MβCD - cholesterol (1.5 mg/mL) and 4. Equipro extender. Samples were incubated for 15 min at room temperature to allow for incorporation of the MβCD-cholesterol before centrifugation at 500 *g* for 10 min. The supernatant was removed, sperm concentration re-assessed and diluted to 100 x 10^6 sperm/mL in Gent freezing extender (Minitüb). The samples were filled into 0.5 mL colour coded straws (Minitüb), cooled to 5˚C over 50 min and frozen using a programmable freezer, followed by immersion and storage in liquid nitrogen. Upon thawing, sperm from each treatment was assessed for (i) PLM (ii) viability (iii) membrane integrity and (iv) membrane fluidity. PLM, viability (ingrain-eosin stain) and membrane integrity (hypo-osmotic swelling test; HOST) were assessed on 18 straws per treatment using standard procedures on a phase contrast microscope at 400X. Sperm membrane fluidity was assessed on 12 straws per treatment using the fluorescent probe merocyanine 540 (M540) with Yo-Pro1 as the viability stain. Sperm were then analysed using flow cytometry (BD-LSR 1; BD Biosciences) with membrane fluidity reported as the percentage of viable cells positive for M540. Data were examined for normality, transformed where appropriate and analysed using univariate procedures in SPSS (version 20.0; IBM). The HOST and membrane fluidity data were transformed using a square root transformation and power transformation, respectively. The model included the main effects of treatment, stallion and treatment × stallion and results are presented as mean ± s.e.m.

Results There was no effect of treatment or stallion on PLM, but both treatment and stallion had a significant effect on viability ($P < 0.05$). There were significantly more viable sperm in the 0.75 mg/mL treatment compared to the Equipro treatment (68.0 ± 3.35 % and 52.6 ± 4.50 %, respectively; $P < 0.05$), and stallion A had a lower proportion of viable sperm than both stallions B and C ($P < 0.01$). There was an effect of stallion on membrane integrity, as assessed by the HOST ($P < 0.05$) with stallion A having a significantly lower proportion of sperm with intact membranes than stallions B and C. There was an effect of treatment on membrane fluidity ($P < 0.05$) with the 1.5 mg/mL treatment having the highest membrane fluidity post-thaw (90.7 ± 2.50 %) and was significantly higher than TALP (Figure 1). The difference between the 0.75 mg/mL and TALP treatments approached significance ($P = 0.065$).

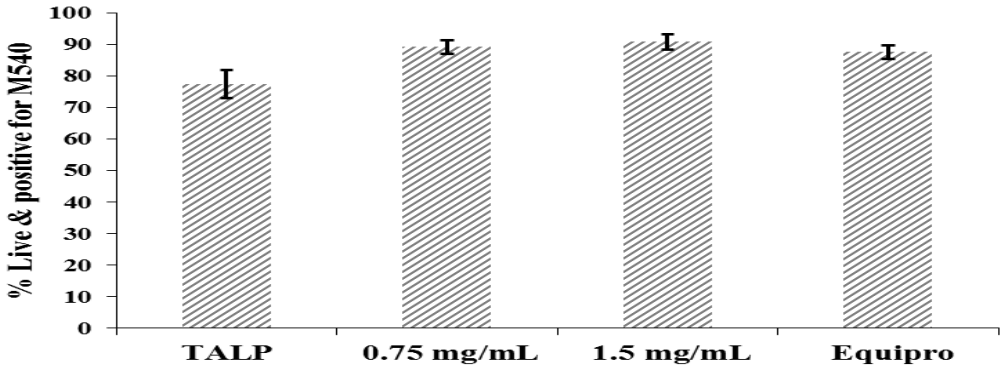

Figure 1 The effect of methyl-β-cyclodextrin cholesterol on the membrane fluidity of viable frozen thawed stallion sperm

Conclusions The results of this study demonstrate that the addition of MβCD cholesterol to stallion sperm prior to cryopreservation increases membrane fluidity and stability when compared to a non-cholesterol control (TALP). Further work is required to establish the effect of the addition of MβCD cholesterol to stallion sperm on subsequent capacitation and fertilising ability.

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The effect of four commercially available extenders on motility of chilled stallion semen over time

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Introduction Semen extenders are routinely added to semen intended for AI once the gel fraction has been removed in order to preserve the ejaculate for a prolonged period of time. A number of commercially produced extenders are available; these can be egg yolk or milk based and can contain antimicrobial agents depending on the manufacturer. The composition of semen extenders is known to have effects on sperm integrity and survival (Pagl et al, 2006). The aim of this study was to differentiate the effects of four commercially available semen extenders- INRA, ARS, Kenney and Minitube- on the progressive motility of chilled stallion semen without accounting for other parameters.

Material and methods This study used three stallions of varying age and breed standing at a commercial semen collection facility in Canada. Each stallion's semen was collected and extended with each of the extenders - INRA, ARS, Kenney and Minitube- on four occasions following standard processing for chilled semen, therefore 48 samples were analysed. Progressive motility was observed by eye using a video microscope immediately after collection and processing by the same observer. Further readings were taken at 8, 16, 24, 32, 40, 48 and 56 hours after collection in chilled conditions until it reached <40% PM, which means the semen is then considered of insufficient quality to be used in AI (Blanchard, 1998). The statistical analysis involved survival analysis techniques. Data were used until the last motility registered show a value above 40%; therefore when the exact time where 40% of motility was achieved is unknown. A Kaplan Meyer analysis was performed and comparisons between extenders were analysed using a Log Rank (Mantel-Cox) statistic. Data were analysed with the statistical package IBM® SPSS 19.

Results Significant differences in motility over time (P<0.05) were found between extenders; namely between INRA and all the others, without significant differences between the last ones (P>0.05). Table 1 summarizes results showing mean survival time for each extender. Figure 1 graphs the survival functions for the different extenders.

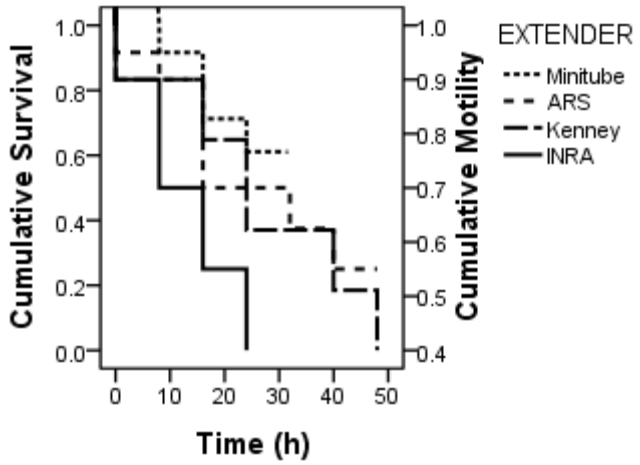

Figure 1 Survival functions for the different extenders

Table 1 Mean semen survival time (motility above 40%) for the 4 different extenders

Extender	Mean survival time (h)
INRA	12.7[a]
ARS	27.0[b]
Kenney	25.9[b]
Minitube	25.9[b]

Note: different letters in superscript indicate a significant difference (P<0.05)

Conclusions For the preservation of chilled semen for transport, INRA is less recommended based on these results compared to the other three extenders. Further research must focus on the differences in composition between these four extenders and how their ingredients may affect semen survival in order to identify the most suitable product. This will allow practioners to select the most suitable extender for the processing and transport methods used.

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The effects of equine semen storage on the success rate of embryo recovery in a commercial setting

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Introduction Equine embryo transfer has gained increasing popularity as a commercially available technique. As approaches vary between establishments, this study aimed to investigate the success rates of embryo recovery following artificial insemination with fresh, chilled and frozen semen. Freezing and thawing stallion semen successfully has proved more difficult in the equine species (Heise et al, 2010; Kirk et al; 2005, Loomis & Graham, 2008) compared to other livestock species, with variable pregnancy rates reported and different recommendations regarding insemination volume; although some guidance (Jasko et al, 1992) is available regarding semen progressive motility in relation to insemination success (Blanchard, 1998). Therefore it is important to understand how the use of frozen semen in an equine embryo transfer setting compares to the success rates when using chilled and fresh semen. The aim of this project was to compare the impact of different forms of semen preservation on flushing success of embryos for transfer in mares.

Material and methods A total of N=875 mares were inseminated with chilled (n=236), fresh (n=481) and frozen (n=158) semen, with the aim of recovering the embryo by non surgical flushing, for transplantation on day 8 after insemination. Recovery was not successful in all occasions, and these records were tested against semen processing type. A logistic regression model with recovery (yes or not) as dependent variable was fitted to the data using type of semen processing as independent variable. Data were analysed with the statistical package IBM® SPSS 19.

Results The model was found to be significant after the log likelihood ratio chi square test (P<0.001) having an Akaike's information criterion value of 24, which is very low and therefore shows a high degree of adjustment. The type of semen processing was found to be a significant variable (P<0.001) affecting the degree of success in the recovery of the flushed embryos. The full parameters of the adjusted model are stated in table 1, where the probabilities of successful recovery of embryos function of semen processing type are also stated after application of the fitted model. As can be observed the lower success rates are obtained with frozen semen, followed by chilled and fresh semen the type obtaining the highest success rates.

Table 1 Parameters of the logistic regression fitting semen processing type data against success of flushed embryo recovery. On the right hand side probabilities of successful embryo recovery function of type of semen processing are also stated.

Variable in the equation	β	SE (β)	P-value	95% CI (β)	OR (e^{β})	95% CI OR (e^{β})	Probability of successful embryo recovery
Semen			< 0.001				
chilled	0.438	0.207		0.033; 0.843	1.550	1.034; 2.323	0.581
fresh	0.760	0.186		0.396; 1.124	2.138	1.486; 3.077	0.656
frozen	0						0.472

SE: standard error; CI: confidence interval; OR: odds ratio of recovery (success/failure).

Conclusions As may have been expected when reviewing issues relating to semen preservation in relation to conception rates in the donor mares, the embryo recovery rates using frozen semen were significantly lower to those achieved when using fresh or chilled semen. This may be related to the issues that have been investigated surrounding the freezing and thawing process of stallion semen and could indicate that despite more intense monitoring of mares and insemination closer around the time of ovulation compared to chilled and fresh semen, the use of frozen semen in a commercial setting still yields a lower output in terms of pregnancies and embryo recoveries. Research findings relating to the improvement of fertility and recovery rates is available and must be communicated to industry practitioners more clearly and in a useful format.

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Factors affecting semen parameters of sixteen stallions during two breeding seasons

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Introduction Stallion fertility is affected by intrinsic and extrinsic factors which have the potential to enhance or limit the quality of stallion semen. Season influences the reproductive capacity of the stallion, due to the equine's status as a long-day breeder (Wrench *et al.,* 2010). Hormone secretion of melatonin via the pineal gland in the brain is affected by length of daylight hours, ultimately starting the equine breeding season. The stallion would naturally cover the mare numerous times in a 24 hour period. Modern management practices contradict the natural mating behaviours of the horse in many ways (Sieme *et al.,* 2003). The advent of artificial insemination and semen collection has allowed examination of stallion fertility indicators without the influence of the mare's fertility status (Turner, 2005). The factors that influence spermatogenesis may also play a role in visible parameters of the ejaculate and the environmental variable of the 57 day period prior to the ejaculate, which is the length of time of spermatogenesis, environmental variables at this time may be considered in stallion fertility (Janett *et al*., 2003). The aim of this study is to identify the extent of extrinsic influences on the semen parameters of stallions in order to maximise the reproductive efficiency of stallions in an AI programme.

Methods Semen records from 2008 and 2011 were analysed for competition stallions (n=16) in the UK.. Semen parameters including total number of sperm (billion), density (million/ml), volume (ml), motility (%) and possible number of mare inseminations per ejaculate were analysed in recognition of the effect of rate of collection frequency, timing of collection throughout the breeding season (early, mid, late) and the individual stallion variance. Variables were also measured against 24 hour minimum and maximum temperatures (°C) and 24 hour precipitation at the commencement of spermatogenesis (57 days prior to semen collection). Correlations were identified through Pearson's Correlation of transformed (ranked) data with significance set at $P < 0.05$.

Results Significant differences were found between semen parameters at different points in the breeding season (table 1), indicating lower reproductive efficiency later in the season.

Table 1 Significant differences found between semen parameters at different points in the breeding season (identified through Kruskal Wallace and Mann Whitney U-test) with P<0.05. Means that do not share letter are significantly different.

Phase of Season	Mean Density (million/ml)	Mean Volume (ml)	Mean Motility (%)	Mares/ejaculate
Early	301.7^{A}	59.3^{A}	85.1^{A}	19.14^{A}
Mid	241.2^{AB}	57.3^{A}	76.2^{AB}	14.2^{B}
Late	222.5^{B}	43.6^{B}	53.1^{B}	12.8^{B}

Significant correlations were found between semen parameters and the frequency of collection, increased time between collections (frequency) resulted in more favourable semen parameters. Pearson's correlation with transformed data, comparing the effect of frequency of semen collection with semen parameters and direction of correlation of 16 stallions in 2008 and 2011. Increasing frequency of semen collection was significantly associated (P<0001) with an increase in total number of sperm (billion) (P <0.001 R2=0.416), Density (P <0.001 R2=0316) motility (P<0.001 R2=0.299) and the number of mares/ejaculate (P<0.001 R2=0.326).
Volume and motility were correlated with temperature and precipitation, as each variable increases, so did volume and motility (table 2), density was not affected by temperature or precipitation.

Table 2 Pearson's correlation with transformed data, comparing the effect of 24 hour maximum temperature with semen parameters of 16 stallions in 2008 and 2011.

	Density (million/ml)	Pearson's Correlation	Volume (ml)	Pearson's Correlation	Motility (%)	Pearson's Correlation
24 hour maximum temperature	P =0.577	R2 -0.041	P<0.001	R2 0.570	P <0.001	R2 0.458
24 hour minimum temperature	P=0.729	R2 -0.026	P<0.001	R2 0.468	P<0.001	R2 0.439
24 hour precipitation	P=0.502	R2 0.050	P=0.397	R2 -0.062	P=0.028	R2 0.161

Conclusions Significant correlations exist between semen parameters and frequency of collection with increased collection frequencies having a positive correlation with semen parameters. Environmental influences (temperature) at the time of spermatogenesis commencing can be a potential predicting factor of semen quality, higher maximum and minimum temperatures indicate a higher volume and motility of semen. Although many of these factors cannot be controlled, recognition of their influence may aid management of the breeding stallion to produce the most effective programmes.

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Ruminant turbinate cells express CXCL8 independently of IL-1β, IL-6 and TNF-α following infection with *Chlamydia abortus*

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Introduction *Chlamydia abortus* is the most common cause of infectious abortion in sheep in the UK. *C.abortus* is an obligate intracellular pathogen which, in sheep, invades the placenta resulting in an inflammatory cascade causing pathology characterized by the production of TNF-α and CXCL8 (1). *C.abortus* has been linked to reproductive problems in cattle but in contrast to sheep has rarely been associated with abortion. The innate immune sensory mechanisms that detect *C.abortus* infection in ruminants are currently unknown. However the roles of pathogen recognition receptors (PRRs) such as toll-like receptors (TLRs) and NOD-like receptors (NLRs) have been investigated with infection by other members of the *Chlamydia* family. TLR2 has been shown to be important for the recognition of *Chlamydia* leading to the activation of NF-κB and production of inflammatory cytokines. A role for intracellular NLRs in the recognition of *Chlamydia* and activation of the potent inflammasome complex has also been elucidated (2). The inflammasome controls Il-1β production which contributes to the protective immune response to *Chlamydia* but has also been linked with pathology (3). We hypothesize that these early host-pathogen interactions determine the outcome of infection. Since *C.abortus* is transmitted oro-nasally we have compared the innate responses of ovine and bovine nasal turbinate cells to *C.abortus*.

Material and methods Ruminant turbinate cells were seeded to sub-confluence overnight. They were then infected with *C.abortus*. Controls included equivalent UV killed *C.abortus* prep and unstimulated cells. Culture supernates and cell lysates were harvested at 6, 24, 48 and 72 hours for analysis of IL-6, CXCL8, TNF-α and IL-1β protein (ELISAs) and mRNA (Taqman® RT-PCR). Data were analysed using a paired student T-test for the ELISA data and the ΔΔCT method used to analyse RT-PCR.

Results Ovine and bovine turbinate cells produce CXCL8 in response to *C.abortus* infection in a time dependant manner ($p<0.05$ for *C.abortus* infected turbinates compared to unstimulated cells at 72 hours) as measured by ELISA (Fig. 1). The protein profile matched the mRNA profile (data not shown). Production of CXCL8 was higher in ovine compared to bovine cells. Interestingly, there was no detectable production of IL-1β, IL-6 or TNF-α protein.

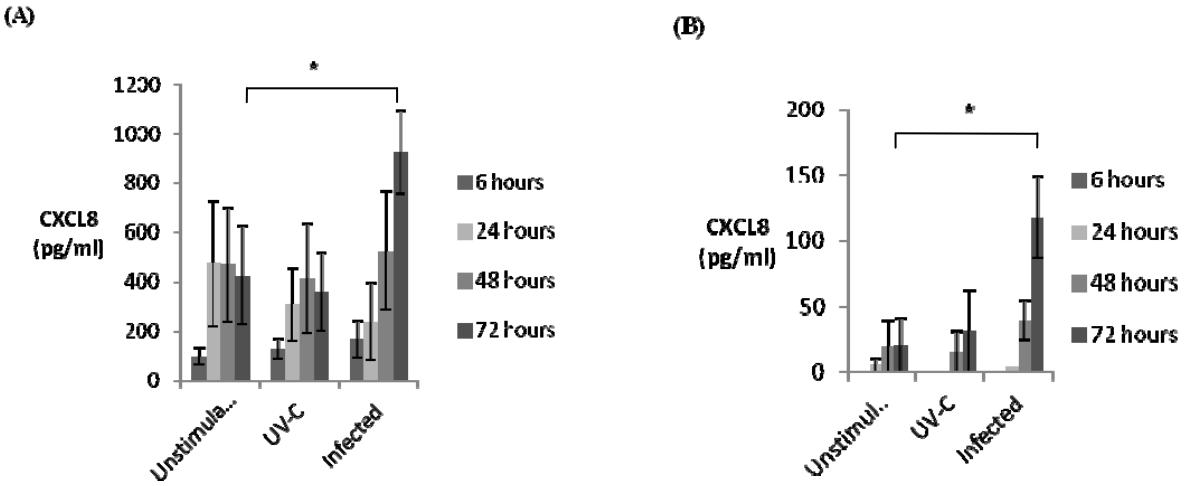

Figure 1 CXCL8 production in ovine (A) and bovine (B) turbinate cells infected with *C.abortus*. Controls included cells exposed to UV-killed *C.abortus* (UV-C) and unstimulated cells. Results are representative of three separate experiments.

Conclusions These results show that both ovine and bovine turbinate cells respond to *C.abortus* infection by producing CXCL8. Significant production is only observed 72 hours after infection. This together with the fact that cells are not responding to UV killed bacteria suggests that activation is unlikely to be due to cell surface sensory receptor and may involve intracellular recognition. Investigation of the expression of PRRs including TLR2 and intracellular NOD 1 is currently underway.

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Hydrocortisone increases, rather than inhibits, angiogenesis of equine arteries *ex vivo*

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Introduction Angiogenesis, the formation of new blood vessels from existing vasculature, is essential for tissue repair. Dysregulation of angiogenesis has been implicated in a number of important diseases (e.g. tumour growth, wound healing, endocrine disease and atherosclerosis) and consequently, manipulation of angiogenesis has potential as a therapeutic target. In the horse angiogenesis is important in wound healing but it is also thought to be dysregulated in diseases such as chronic endocrinopathic laminitis in which there is a reduction in the vascular filling and the number of branches supplying the laminae (Ackerman, Garner *et al.* 1975; Hood, Grosenbaugh *et al.* 1994). Equine Cushing's disease (Pituitary Pars Intermedia dysfunction) is associated with an increased risk of laminitis, it has been suggested that persistent cortisol elevation may be important in the pathogenesis. Synthetic anti-inflammatory glucocorticoids such as dexamethasone and prednisolone, are regularly used to treat a number of conditions in horses. Cortisol is a potent inhibitor of angiogenesis *in vivo* and *in vitro* in rodent and human models (Folkman, Langer *et al.* 1983; Small, Hadoke *et al.* 2005), but the impact of glucocorticoids on angiogenesis has not been assessed directly in equine vasculature. This study used an ex vivo assay to address the hypothesis that hydrocortisone would inhibit angiogenesis in isolated equine arteries.

Material and methods Horses that were systemically healthy with no history of endocrine disease or laminitis and no treatment with glucocorticoids in the previous three months, undergoing euthanasia at an equine referral hospital were eligible for inclusion in the study. Tissue was collected at post-mortem with permission from the owners, in accordance with University ethical guidelines. Laminar vessels were harvested from the dorsal laminar region of 6 horses (2 mares, 4 geldings) and the facial skin of five of these horses. The horses were all Thoroughbreds or Thoroughbred cross breeds. Arteries harvested were cut into 1mm rings, embedded in Matrigel and cultured for seven days under conditions designed to stimulate endothelial tube formation. Arterial rings were cultured under basal conditions or exposed to either foetal bovine serum (FBS; 3%), hydrocortisone (600 nM) or hydrocortisone (600nM) plus FBS (3%). Outgrowths from the vessels were counted on days three, five and seven. Data are mean $\pm$ sem (where n = number of horses). All exposures were performed in triplicate. Data were compared using a two-way ANOVA with a Bonferroni post-hoc test.

Results Tube formation was observed with laminar and facial arteries from day two in culture. Tube formation appeared to increase after exposure to FBS (Control = 6 +/- 1.7 v FBS = 16 +/- 5.5 P >0.05) but this did not achieve significance at any time point. Exposure to hydrocortisone alone (Control = 6 +/- 1.7 v hydrocortisone 90 +/- 23, P<0.001), or in combination with FBS (Control = 6 +/- 1.7 v hydrocortisone and FBS = 143 +/- 17.9, P<0.001), dramatically increased tube formation in both types of artery at day five and day seven (Figures 1 and 2).

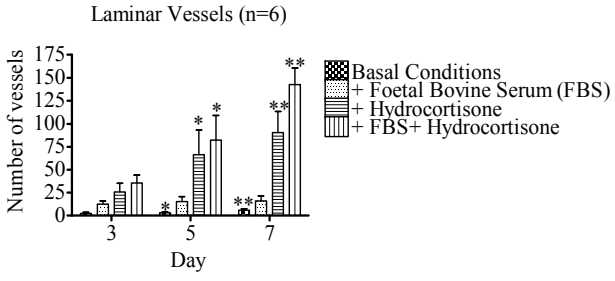

Figure 1 Graph showing outgrowths from laminar vessels at day 3, 5 and 7 in four differing media

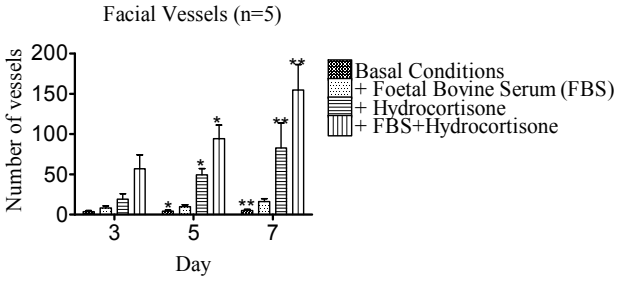

Figure 2 Graph showing outgrowths from facial vessels at day 3, 5 and 7 in four differing media

Conclusions Adaptation of the *ex vivo* angiogenesis assay for use with equine arteries demonstrated that hydrocortisone increased, rather than inhibited angiogenesis. This was seen with both facial and laminar arteries and appears to be species specific since glucocorticoids consistently inhibit tube formation when this model is applied to rodent arteries. Glucocorticoid inhibition of angiogenesis in rodents appears to be mediated by glucocorticoid receptors on the arterial endothelium. The mechanism responsible for increased angiogenesis in response to hydrocortisone in the current study remains to be determined. Whatever the mechanism, a similar pro-angiogenic response to hydrocortisone in horses *in vivo* could have considerable implications for therapeutic use of glucocorticoids in these animals.

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Characterisation of the equine macrophage/monocyte – a step in understanding equine pulmonary innate immunity

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Introduction Although inflammatory airway disease (IAD) is recognized as the most common performance-limiting respiratory disorder in young racehorses, its aetiopathogenesis remains incompletely elucidated. As a pre-requisite to investigating the role of the alveolar macrophage (AM) in IAD, the purpose of the current study was to make functional and phenotypic comparisons between AMs and macrophages derived from other anatomical sites, thus characterising this important innate immune cell at the tissue level. We hypothesised that the tissue of origin (i.e. the lung environment) determines a unique phenotype of AMs, which may constitute an appropriate therapeutic target cell in IAD.

Material and methods Macrophages (lungs, peritoneal cavity and spleen) and monocytes (peripheral blood) were isolated from 14 healthy horses according to different protocols. Fresh and cryopreserved AMs and peritoneal macrophages (PMs) were cultured with fetal calf (FCS) or horse serum (HS). Following stimulation with various TLR ligands, the production of tumour necrosis factor alpha (TNF-alpha) and interleukin (IL)-10 were measured by ELISA and nitrite production was measured by the Griess reaction. Additionally, comparisons between cell types were made on the basis of CD14, CD163 and TLR4 expression and phagocytic-capacity, all measured by flow-cytometry. Finally, RNA was extracted from LPS treated and untreated AMs and hybridized on the new equine Affymetrix microarray to investigate LPS-induced alterations in gene expression. Two way ANOVA statistical analysis was performed on the whole gene list and an unadjusted p-value of 0.05 was set. Peripheral blood monocytes (PBMCs) were differentiated to macrophages over 5 days following either treatment with recombinant human CSF1 or supplementation of the culture media with 40% HS. LPS tolerance was also investigated on both AMs and peripheral blood monocytes (PBMCs) by treatment with the same dose of LPS on two consequent days.

Results We demonstrated excellent cell recovery associated with good viability following cryopreservation. Thawing cells did not affect viability, although LPS responsiveness was slightly attenuated. The inflammatory response (TNF-alpha release) following LPS stimulation was greater in the presence of HS (6.6 ng/ml $^{+}/_{-}$ 1.6) compared with FCS (3.6 ng/ml $^{+}/_{-}$ 1.4). AMs produced TNFa when stimulated with LPS (2.1 ng/ml $^{+}/_{-}$0.8), Poly I:C (0.5 ng/ml $^{+}/_{-}$ 0.3) and heat-killed *Salmonella typhinurium,* (4.9 ng/ml $^{+}/_{-}$ 1.9) 6h post incubation. In contrast, PMs failed to exhibit any specific response to these inducers (0.2 ng/ml $^{+}/_{-}$ 0.6, 0.01 ng/ml $^{+}/_{-}$ 0.01, 0.1 ng/ml $^{+}/_{-}$ 0.1, respectively) or any phagocytic activity. In contrast to AMs that showed high expression of the specific macrophages markers CD14, CD163 and TLR4, PMs showed high expression of TLR4 only, with very low expression of CD14 and CD163. Moreover, LPS pre-treated AMs and PBMCs markedly attenuated the response to a subsequent LPS stimulus, indicating the development of LPS tolerance. Preliminary results from microarray analysis showed a statistically significant change in the expression of 173 genes following LPS treatment of AMs; 163 up-regulated and 10 down regulated. Those that were up-regulated included inflammatory genes such as TNF-alpha, IL-1a, IL-6 and CXCL6. Finally, horse AMs share certain characteristic similarities with human and pig macrophages and certain differences with mouse macrophages. These include the expression of STAT4 genes after LPS stimulation and the failure to produce nitrite in response to LPS.

Conclusions We have shown that large numbers of macrophages can be isolated from different anatomical sites of the horse and cryopreserved for future studies, thus facilitating the execution of multiple experimental protocols on samples derived from the same individual. Alveolar macrophages are more active compared to PM when stimulated with LPS, heat-killed *S. typhimurium* or Poly I:C, thus demonstrating the importance of the local microenvironment in the activation status of the macrophage. This comparative data and that derived from the microarray analysis has provided us with a better understanding of the specific inflammatory response of the horse AMs, particularly to an LPS stimulus. We consider this information to provide a valuable knowledge base on which to improve our understanding of the mechanisms that might be involved in IAD pathogenesis. qPCR and further microarray analyses are currently being processed and will shed more light on the list of LPS-activated genes in AMs. Finally, we have demonstrated a closer similarity between horse and human compared with mouse in AM inflammatory gene regulation, thus supporting a role for equine studies in providing comparative data in the field of exercise immunology.

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The effect of immunization against gonadotropin-releasing factor (GnRF) on circulating testosterone and the development of sexual behaviour in ram lambs

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Introduction Immunization against gonadotropin releasing factor (GnRF) is an alternative to traditional physical methods of castration, which cause pain and distress in castrated animals (Molony *et al.*, 2002; Thornton & Waterman-Pearson 2002) that may last for a few days after the procedure (Thornton & Waterman-Pearson 1999). Several studies in other species (i.e. porcine, caprine, bovine) have shown that active immunization against GnRF temporarily suppresses testosterone, and reduces sexual and aggressive behaviours. Immunization against GNRF can also improve feed efficiency, carcass conformation and meat quality (Thompson 2000). However, there is little information on the effects of immunization against GnRF in lambs, and also very little or no information on how the anti-GnRF vaccine influences their behaviour (i.e. sexual, dominance/hierarchy, aggression). Our objective was to determine efficacy of an experimental anti-GnRF vaccine developed by Zoetis as a more welfare friendly method of castration in reduction of circulating testosterone, scrotal circumference, testes consistency and the development of sexual behavior in ram lambs.

Material and methods Sixty four, one day-old Mule (Scottish Blackface x Bluefaced Leicester) x Suffolk or Texel lambs were used to conduct 2 studies. Lambs were single (n=6), twin (n=50) or triplets (n=8). At 24 h of age, lambs were allocated to one of 2 treatment groups: Controls (C; n=32): entire male lambs which were handled only; or Vaccinated (Vac, 32) lambs were vaccinated at 6 and 12 weeks (in year 1, Vac1, n=20) or at 6, 12, and 22 weeks of age (year 2, Vac2, n=12) with 0.5 ml of an experimental GnRF vaccine. From 6 weeks of age, blood samples were collected by jugular venepuncture at 4 week intervals for determination of testosterone concentrations. Scrotum circumference and testes consistency data (scored from 1 – 4 by manual palpation) were also collected at the same time. At 7 months of age lambs were tested for the expression of sexual behaviour by exposure to an oestrus female for 20 minutes on 6 consecutive occasions. After the last behavioural testing, lambs were euthanized and the testes collected. Proximal, medial and distal transverse sections were taken and fixed in freshly prepared methanol, chloroform and acetic acid solution (60:30:10) and subsequently changed to 70% ethanol after 24 hours. All samples were then washed in ethanol, embedded in the liquid paraffin, and stained in haematoxylin and eosin. Histological assessment of seminiferous tubules and cell types was carried out. Testosterone concentrations were not normally distributed and were assessed by Kruskal Wallis non-parametric tests. Scrotal circumference and consistency were analyzed as a repeated measures REML variance components analysis fitting year, age, sire breed and litter size.

Results Vaccination against GnRF significantly suppressed the age-related increase in circulating testosterone (Table 1), scrotal circumference (average scrotal circumference, cm): C=23.99, Vac=19.14, s.e.d.=0.66, P<0.001) and testicular consistency (C=3.55, Vac=2.70, s.e.d.=0.10, P<0.001). At 30 weeks of age scrotal measures were still suppressed in Vac2 lambs, but not in Vac1 lambs (scrotal circumference: C=34.3, Vac1=31.0, Vac2=22.5, P<0.001; testicular consistency: C=4.0, Vac1=3.0, Vac2=2.0, P<0.001). At 7 months of age 75% of Vac1 lambs showed sexual interest in oestrus females, whereas only 16.7% of Vac2 lambs showed interest, compared to 90% of C lambs (Chi-Sq. = 11.733, DF = 1, P<0.001). Effective vaccination resulted in markedly altered testicular histology (fig. 1).

Table 1 Effects of GnRF vaccination on circulating testosterone (measured in ng/ml) for intact males (C) or lambs immunized against GnRF (Vac) to 22 weeks of age (before the third vaccination in the second study).

Group	6 weeks old	10 weeks old	14 weeks old	18 weeks old	22 weeks old
C	0.38	0.53	0.50	0.84	1.45
Vac	0.68	0.28	0.00	0.00	0.91
Kruskal-Wallis test	H=0.01,d.f.=1 P =0.908	H=0.69, d.f.=1 P = 0.41	H=27.33,d.f.=1 P < 0.001	H=26.14,d.f.=1 P =<0.001	H=3.98,d.f.=1 P = 0.046

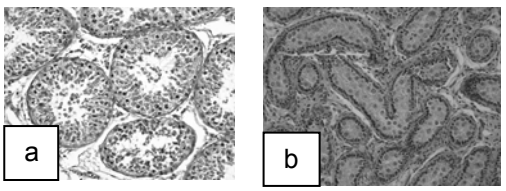

Figure 1 Representative sections of testes from (a) entire males (Control) or (b) males immunized against GnRF. The size of tubules and different cell types are reduced in the vaccinated male lambs.

Conclusions Vaccination against GnRF suppressed testicular development and the display of sexual behaviour in male lambs., with 3 injections providing for a longer-lasting suppression of testicular development and sexual behaviours (until 30 weeks of age). Our preliminary data suggest that vaccination against GnRF may be a more humane method of castrating male lambs.

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Continual environmental exposure of pigs to highly pathogenic avian influenza virus via infected ducks or chickens failed to establish infection in the pigs

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Introduction Pigs are thought to acts as mixing vessels for avian, swine and human influenza viruses since their respiratory tract contain the receptors used by both avian (α2-3 sialic acid) and mammalian (α2-6 sialic acid) influenza viruses. However, it has not been shown conclusively that birds infected with HPAI virus can transmit this virus to pigs. The aim of this study was to determine, for the first time under experimental conditions whether infected birds can transmit H5N1 HPAI virus to pigs when the animals are co-housed.

Methods To study the transmission of avian influenza (AI) viruses to pigs, five uninfected 6-week-old pigs were housed in an enclosure adjacent to another containing five 3-week-old Pekin ducks or chickens each infected with 10^6 EID_{50} A/turkey/Turkey/1/2005 H5N1 highly pathogenic AI (HPAI) virus which allowed for the sharing of circulating air. To maintain the levels of virus within the environment; when each group of birds succumbed to the virus, a new group of five, infected birds were added. To increase the potential exposure of pigs to virus, the animals swapped enclosures daily to allow contact between the pigs and the potentially infectious bedding and water that the birds had access to during the previous 24 hours. Pig temperatures were measured daily via Biothermal microchips. Cloacal and oropharyngeal swabs from birds, nasal swabs from pigs, and environmental samples (water and swabs from bedding) were taken daily. Influenza virus was detected by matrix gene realtime RT-PCR. Blood was collected from the pigs at 7 and 14 days post co-housing with the first group of infected birds (DPC) for serological analysis. Antibodies to A/turkey/Turkey/1/2005 H5N1 HPAI virus were detected by haemagglutination inhibition (HI) assay.

Results All ducks and chickens became infected and shed virus. The peak shedding in the duck groups (20/20) was identified at 48hpi - 1.00 $\pm$ 0.18 (mean $\pm$ SEM) $\log_{10}$ relative equivalent units (REU) of viral RNA. The viral shedding for the chickens at 24hpi (25/25) was similar to that of the ducks (0.92 $\pm$ 0.12 $\log_{10}$ REU), but peaked at 48hpi in those that survived (2/25, 8%) which shed in excess of 3.0 $\log_{10}$ REU. Despite these substantial levels comparatively little virus was detected in the bedding or water of either bird species. Viral RNA from the chicken bedding was detected only twice (DPC-3 and 10 at 0.51 and 1.00 $\log_{10}$ REU respectively) and from the pig bedding on a single day (DPC-13 at 1.26 $\log_{10}$). The water used by the chickens was positive for viral RNA 5 out of 14 times (DPC-4, 8, 10, 11 and 12 at 0.77, 0.93, 1.25, 1.70, 1.00 $\log_{10}$ REU). Viral RNA was detected in the duck bedding twice (DPC-4 and 14 at 0.62 and 0.93 $\log_{10}$ REU respectively), while the water used by the ducks and the pig bedding remained negative. No clinical signs or pyrexia (>39^{o}C) were observed in the pigs. Viral RNA was detected from a single pig co-housed with chickens on one day (DPC-10 at 0.91 $\log_{10}$ REU). No antibodies to the virus were detected by HI assay. However, since the pigs were killed humanely on DPC-14, seroconversion may not have been detected if a successful infection had occurred after day 7 post contact.

Conclusion Direct inoculation of pigs with a high dose of HPAI H5N1 virus has been shown previously to produce low levels of viral shedding, minor pathology and limited evidence of influenza like disease. In the present study, prolonged (14d) exposure of pigs to a low dose of HPAI H5N1 virus via infected birds failed to produce infection and/or disease in the pigs. This observation is similar to that observed in the field. This may, therefore, minimise the extent to which pigs may act as mixing vessels for these particular variants of H5N1 HPAI viruses.

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Associations between host characteristics and the response to equine influenza vaccination in donkeys

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Introduction Many equids are vaccinated against equine influenza annually as it causes a highly contagious respiratory infection. In horses, both age and adiposity contribute to increased levels of inflammatory markers, which could affect the response to vaccination. In humans, a chronic inflammatory state associated with obesity can impair response to vaccination or infection (Sheridan *et al.,* 2012). The objective of this study was to determine host factors associated with the response to the equine influenza vaccination in donkeys.

Material and methods Surplus to diagnostic requirement serum samples were obtained from 55 donkeys that had recently (within 7–80 days) received a booster dose of a commercially-available inactivated virus equine influenza vaccine. Antibody levels against a component strain of the vaccine (influenza A/equine/Newmarket/2/93 [H3N8]) were measured using the single radial haemolysis assay. High molecular weight (HMW) adiponectin was measured using a human ELISA kit (Millipore) previously shown to be relevant for the horse (Woolridge *et al.,* 2012). Non-esterified fatty acid (NEFA) levels were measured using a kit from Randox with some modifications to the manufacturer's instructions. Additional data including age, weight, body condition score (BCS), and total cholesterol and triglyceride serum levels were kindly provided by the Donkey Sanctuary. Univariate analysis was conducted using Pearson correlation for normally distributed variables and Spearman's rank correlation for variables that were not normally distributed. To evaluate the effect of gender, comparisons were made using a t-test for normally-distributed variables or a Mann-Whitney test for variables not normally distributed. Significant differences were determined at the level of $p<0.05$.

Results There was no significant association between antibody level and time since vaccination. In this study, the factor with the greatest influence on the response to vaccination was gender, with females having significantly higher antibody levels than males (Table 1). In addition, the mean age of the female donkeys was significantly greater than that of the male donkeys. There was no correlation between BCS or weight and antibody levels, but NEFA levels were negatively correlated with antibody levels ($p=0.044$). Associations between NEFA and age, weight and days since vaccination also reached statistical significance. Triglyceride levels were also positively correlated with days since vaccination although antibody levels were not. Positive correlations were seen between serum adiponectin and age as well as triglyceride and cholesterol levels.

Table 1 Influence of gender on variables measured

Variable	Mean ±SD (Range)		p-value
	Males (n=28)	Females (n=27)	
Age (years)	19.2 ±11.8	25.0 ±6.4	.028
Antibody (area of lysis - mm^2)	178.9 ±39.3	208.9 ±42.3	.009

Conclusions Negative correlations were expected between antibody levels and both age and BCS, but were not seen. This may have been confounded by the narrow distribution of BCS in the study population (the majority had a BCS score of 2.5–3.5 on a scale of 1–5) and the overwhelming influence of gender with female donkeys having a higher antibody response despite a greater mean age. It has been demonstrated in human subjects that influenza vaccination can cause alterations to the lipid profile (Tsai *et al.,* 2005). The correlation between NEFA and serum antibody levels warrants further investigation as does the finding that gender has a significant impact on response to equine influenza vaccination in donkeys.

Acknowledgments The authors gratefully acknowledge the Donkey Sanctuary for providing surplus to diagnostic requirement serum samples and data, WALTHAM Centre for Pet Nutrition for funding the study and Dr Marnie Brennan for assistance with the statistical analysis.

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The effects of substituting two different levels of canola seed with cotton seed meal and barley grain on milk properties of lactating cows

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Introduction Canola seed (CS) is utilised as an energy and protein source in ruminant diets. It contains approximately 210 g/kg crude protein (CP) and 430 g/kg oil and has an amino acid composition well suited for Ruminants. High producing dairy cows and rapidly growing ruminants requires more energy and CP in their diets. The objective of this study was to determine effects of full fat in the diet of mid lactation cows on milk yield and fractional composition of milk. The study was designed to identify the optimum substitution level of cotton seed meal and barley grain with canola seed.

Material and methods The canola seed samples were obtained from Oilseed Developing and Cultivation Company in Tehran, Iran.
Twelve multiparous cows (6 cows second lactation and 6 cows third lactation and above) in mid lactation with 149±12 days in milk (DIM) and 24±2.7 litres milk yield were assigned to treatments a randomised complete block design. Diets consisted of 24.4% alfalfa hay, 17.1% corn silage, and 58.5% of the concentrate mixtures (on the DM basis). The effects of feeding three levels (0, 3/7, and 7/5% of DM diet) of canola seed compared. Water was provided for each cow *ad libitum*. Cows were adapted to the basal diet for 10 d before initiating the trial. Cows fed twice a day with a completely mixed ration and three times a day milking was done within 8 hours. Milk yield was recorded daily. Milk composition and somatic cell count (SCC) were determined every week. Urea nitrogen in cows milk determined by Spectrophotometric every week. The various milk yield and fractional composition of milk parameters data were analysed as a randomised complete block design, using parity as blocks (second lactation or third lactation and above). The analysis was carried out using the general linear model procedure of Statistical Analysis System (SAS, 1996). When a significant difference was found, the means were separated using Duncan's multiple range tests.

Results Feeding of canola seed had a significant effect (P<0/05) on milk yield and milk urea nitrogen (MUN). Using canola seed in the diets did not have statistically significant effects on the milk composition percents and feed intake (Table 2).

Table 1 Substitution of two different levels of canola seed in diets

feeds	Diet 1	Diet 2	Diet 3
Canola Seed (%/DM)	0	3.7	7.5
Barley Grain (%/DM)	31.5	27.0	22.3
Cottenseed Meal (%/DM)	7.1	3. 4	0

Table 2 The average yield of milk and feed intake between treatments

Parameters	Diet 1	Diet 2	Diet 3	SEM	p
Milk Production (kg/d)	28.96	24.08	25.35	0.59	0.033
4% Milk Fat Corrected (kg/d)	26.17	22.70	24.28	0.43	ns
Milk Fat (%)	3.36	3.54	3.72	0.09	ns
Milk Protein (%)	3.32	3.22	2.96	0.29	ns
Milk Lactose (%)	4.43	4.57	4.44	0.17	ns
Total Milk Solides (%)	12.03	12.66	12.31	1.11	ns
Somatic Cell Count	708	749	652	18.50	ns
Milk Urea Nitrogen (ppm)	19.10	21.73	24.14	0.22	0.016
Dry Matter Intake (kg/d)	22.00	19.30	21.32		ns

Conclusions Milk production with whole CS consumption in diet3 trials decreased due to 10- *trans* 12- *cis* conjugated linoleic acid production in the rumen (Delbecchi *et al.*, 2001). The results of this study revealed that increase consumption of canola seed in the diet, increased the amount of milk urea nitrogen. It seems that increased canola seed consumption will lead to an increase in rumen dissolved nitrogen and ultimately increased nitrogen absorption from the rumen wall because of the high fat level and a lower energy fermentation (Hussein *et al.*, 1996). The results of this study revealed that high-fat diet with high levels of canola is better be processed.

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Finishing pig performance and eating behaviour when using up to 210 g/kg rapeseed meal in the diet

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Introduction Rapeseed meal (RSM) is a highly available home grown protein source for the UK pig industry. However, concerns exist with regard to RSM inclusion in pig feed since some historical studies found a decrease in pig performance when it was used. This decrease in performance was often a result of decreased feed intake due to the presence of anti-nutritive factors, especially glucosinolates. However, in recent years the level of glucosinolates has been reduced in RSM (Schone *et al.*, 1997). As such, inclusion of RSM at high levels in pig feed should now be possible. The current study aimed to investigate the performance and eating behaviour response of finishing pigs when offered diets containing increasing levels of RSM to a maximum level of 210 g/kg.

Material and methods A total of 64 pigs (Tempo x (Landrace x Large White)) were offered one of four isoenergetic (13.6 MJ/kg digestibly energy), isonitrogenous (170 g/kg crude protein; 11 g/kg total lysine) diets containing either 0, 70, 140 or 210 g/kg RSM. Pigs were housed in groups of 8 which were balanced for gender and weight. The feed intake and eating behaviour of individual pigs was recorded continuously using ACEMO electronic feed stations. Each pig represented one replicate. Pigs were weighed at 12, 15 and 18 weeks of age (Wks) and at slaughter (153 days of age). The composition of the control diet (0 g/kg RSM) was (g/kg) wheat 398; barley 250; soya 192; pollard 53; maize 50; vegetable oil 19.7; limestone 11.0; dicalcium phosphate 10.5; minerals and vitamins 5.0; salt 4.0; lysine 4.0; methionine 1.3 and L-threonine 1.2. As the inclusion of RSM increased the levels of wheat and barley remained constant but the level of soya was reduced to 149, 110 and 65 g/kg in the 70, 140 and 210 g/kg RSM diets respectively. Pollard was included at 70 g/kg in the 70 g/kg RSM diet and was absent from the 140 and 210 g/kg RSM diets. Vegetable oil was increased to 16.5 and 20.6 g/kg in the 140 and 210 g/kg RSM diets. Lysine inclusion remained similar across all diets but methionine and L-threonine inclusion decreased as RSM inclusion increased (methionine 1.1, 0.9 and 0.7 g/kg; L-threonine 0.9, 0.7 and 0.6 g/kg for the 70, 140 and 210 g/kg RSM diets respectively). The raw RSM was analysed for total glucosinolate content using an HPLC method conforming to BS4325 Part 12 (NIAB Labs, England). Pig performance (average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR)) and eating behaviour (average number of visits per day (ANV/D), average feed intake per visit (AFI/V) and the average time spent per feeding visit (AT/FV)) was calculated on a per pig basis and data were statistically analysed using analysis of variance (Genstat version 10) for linear and quadratic effects of treatment at the probability level of 5%. The 12-week weight of pigs was used as a covariate.

Results The total glucosinolate level of the RSM was 4.8 µmol/g. The 12-week weight of pigs averaged 37.8 kg and as RSM inclusion increased the finish weight, ADG and ADFI of pigs decreased linearly (all $P < 0.01$) (Table 1). There was no effect of RSM inclusion on the FCR of pigs at any stage of growth and FCR between 12 wks and finish averaged 2.49 ($P>0.05$, SEM 0.056). There was a quadratic effect of treatment on ANV/D ($P<0.05$) with the ANV/D decreasing as RSM inclusion increased from 0 to 140g/kg but increased again when pigs were offered feed containing 210g/kg RSM. As RSM inclusion increased the AFI/V decreased linearly ($P<0.05$) as did the AT/FV ($P<0.01$) (Table 1).

Table 1 Effect of RSM inclusion on pig performance and eating behaviour between 12 weeks of age and finish

	RSM inclusion (g/kg)					P value		
	0	70	140	210	SEM	Treatment	Linear	Quadratic
Finish weight (kg)	105.0^{c}	100.4^{b}	101.6^{b}	97.3^{a}	1.05	<0.01	<0.01	NS
ADG (g/kg)	987^{c}	919^{ab}	936^{bc}	873a	21.5	<0.01	<0.01	NS
ADFI (g/kg)	2464^{c}	2279^{ab}	2327^{bc}	2144a	60.3	<0.01	<0.01	NS
ANV/D	9.1	7.8	6.7	9.5	0.90	NS	NS	<0.05
AFI/V (g)	386^{b}	337^{ab}	394^{b}	286^{a}	26.5	<0.05	<0.05	NS
AT/FV (mins)	13.5^{c}	10.1^{ab}	11.8^{bc}	8.1^{a}	1.07	<0.01	<0.01	NS

Conclusions The inclusion of RSM negatively affected the eating behaviour of pigs with the overall result that ADFI and ADG decreased as RSM inclusion increased. Since the inclusion of RSM affected eating behaviour it is suggested that its inclusion affected the palatability of the feed. Furthermore, the significant increase in the number of visits per day when the diet containing 210 g/kg RSM was offered may be due to hunger experienced by these animals since they ate the least within each visit. The animal response found in this trial is surprising since the total level of glucosinolates was considered low in the raw RSM which would have resulted in a level in the finished diet well below the perceived 'safety' threshold of 2 mmol/g. However, it demonstrates the fact that a risk, with regard to reducing pig performance, remains when including RSM in pig diets.

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Behavioural responses of intact and castrated pigs to quantity of feed offered

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Introduction Feed restriction is a common management practices done mostly in pig production. It involves portioning of the pig's daily ration into two or three meals or a reduction in the total amount of feed offered daily. Feed restriction is commonly practiced in order to improve carcass quality and feed efficiency while decreasing production cost (Raemaekers *et al*., 1999). Pigs whose feed intake is restricted develop certain behaviours which were different from those on *ad libitum* feeding (Terlouw *et al*., 1991). Few studies have been carried out to evaluate the behavioural responses of intact and castrated pigs when offered different quantity of feed. To this effect, this study was carried out.

Material and methods A total of forty eight Large White male pigs of eight weeks old with initial average weight of 7.16±0.45 were assigned to two groups (castrated and intact boars). The pigs were allotted to 6 treatments with 4 replicates of 2 pigs each. Treatments 1, 2 and 3 consisted of intact boars fed 2.5, 2.0 and 1.5 kg feed daily. While, Treatments 4, 5 and 6 consisted of castrated boars fed 2.5, 2.0 and 1.5 kg feed daily, respectively. Pigs on each treatment group received ½ of their daily ration at 08:00hr and the remaining portion at 14:00 hr. Methodology for behavioural observation was based on modification of Hessel *et al.* (2006). Two digital cameras were used to capture the various behaviours exhibited by the pigs. Observation of the behaviour was made during three 5-minute sessions (07 hours, 13 hours and 18 hours) of the 24-h period at weeks 10, 16 and 22 of growing-finishing phase. Two pigs were selected from each treatment group and marked for observation. Continuous observation of the focal pigs was used to categorize the feeding behaviour and evaluate how quantity of feed offered affected the behaviour of pigs. Behaviours of focal pigs observed include: standing, sitting, feeding, lying sterna, lying inclined, lying lateral, walking, rooting, nosing and agnostic behaviours (aggressive and displacement activities). Based on the video observations, frequency and duration of exhibited behaviours were calculated. One-way Analysis of variance using SAS (2000) in 2x3 factorial experimental layouts was used to determine the behavioural responses of intact and castrated boars to quantity of feed offered.

Results Pigs on treatment 2 had highest value (11.11%) in standing behaviour while the least mean value (2.78%) was documented for pigs on treatment 4. The values for sitting behaviour ranged from 4.17% (treatment 4 and 5) to 12.50% (treatment 6). Lying sterna had highest value of 16.08 obtained by pigs on treatment 5 while the least observed value (3.57) was noted for pigs on treatment 2 and 6. The mean values for lying inclined ranges from 5.75% (treatment6) to 17.31% (treatment 1). Pigs on treatment 3 had the highest recorded value (14.59%) for rooting behaviour while 4.17% was noted for pigs on treatment 1. The value obtained for walking had the highest recorded value of 12.50% found in pigs on treatment 3 while the least recorded value of 4.55% was recorded for pigs on treatment 4.

Table 1 Details interaction of state of boar and quantity of feed offered on behavioural characteristics of finishing pigs

	Intact			Castrated			SEM
Parameters\Quantity offered (kg)	1.5	2.0	2.5	1.5	2.0	2.5	
Standing	5.56^{b}	11.11^{a}	10.19^{a}	2.78^{b}	10.19^{a}	10.19^{a}	1.07
Sitting	10.42^{ab}	6.25^{ab}	12.50^{a}	4.17^{b}	4.17^{b}	12.50^{a}	2.08
Feeding	11.29	6.45	8.07	8.07	6.45	9.68	3.16
Lying sterna	10.72^{ab}	3.57^{b}	7.14^{b}	8.93^{ab}	16.08^{a}	3.57^{b}	2.31
Lying inclined	17.31^{a}	5.78^{b}	5.77^{b}	9.62^{b}	5.77^{b}	5.75^{b}	1.92
Lying lateral	7.90	5.26	5.26	18.43	7.90	5.26	3.56
Rooting	4.17^{b}	8.34^{ab}	14.59^{a}	6.25^{ab}	8.34^{ab}	8.33^{ab}	2.69
Nosing	9.38	12.50	9.38	6.30	6.28	6.25	3.13
Mounting	7.02	8.14	11.91	6.14	7.10	7.14	2.11
Agonistic	9.52	9.52	7.14	4.76	9.55	9.55	2.17
Walking	4.57^{d}	7.96^{c}	12.50^{a}	4.55^{d}	9.09^{bc}	11.36^{ab}	0.66

^{abcd}- means within rows followed by different superscripts are significantly (P<0.05) different

Conclusion It can be concluded from these results that boar type and quantity of feed offered influenced (standing, sitting, lying sterna, lying inclined, rooting and walking) active behaviours of pigs. These can be used as management tool in order to modify the behaviours of growing pigs.

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Chemical and phytochemical analysis of *Basella Rubra* leafmeal as potential feed for non monogastric animals

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Introduction *Basella rubra* (Indian spinach) is a plant that is normally found in the backyard gardens belonging to the family of Basellaceae and it is a wildly cultivated, cool season vegetable in India (Kumar, 2010.) with climbing growth habit. It is a succulent, branched, smooth, perennial, sprawling twining herbaceous vine, several meters in length. The purplish, fleshy, ovate or heart-shaped, leaves and stems are used for medicinal purpose, (Bailey: 1997) and as vegetable. Despite all its Nutraceutical potentials, there is scanty information on the bioactive compounds, anti-nutrients, and micronutrients composition of this plant's leafmeal. Therefore, the evaluation was undertaken to examine the proximate, amino acid, mineral constituents and phytochemicals profiles in furtherance to ascertaining its potentials as a leafmeal to non-ruminant livestock

Material and methods *Basella. rubra* leaves were collected from a garden at Agudama located in Yenagoa, Bayelsa State, Nigeria, cleaned and spread on the already cleaned laboratory bench for 5 days in the Livestock Production Technology Laboratory, Niger Delta University. Dried leaves were milled and a portion (50 g) of the powdered sample was processed for various parameters according to the following procedures: The proximate analyses of the plant leaf were determined by the method described by AOAC, {1990}. Atomic Absorption Spectrophotometer (AAS), bulk scientific model AVG 210 was used for elemental composition and appropriate working standard solution was prepared for each element and calibration curves were obtained for concentration versus absorbance. Total phenolics were determined by method described by Mole and Waterman (1987), Saponins by the spectrophometric method of Brunner as described by Akinmutimi (2006), Alkaloids by gravimetric method of Harbone (Harbone, 1998), Tannins was determined by method of Maga as described by Akinmutimi (2006) and Phytate by Lucus and Markakas method as described by Akinmutimi (2006). All data were expressed as mean ± SD and GraphPad Instat (Data set LSD) were used.

Result The results showed that *Basella rubra* leafmeal (BRLM) contained Dry Matter 91.66 ± 1.40% Crude Protein,18.16± 1.10% Nitrogen Free Extract, 58.73± 0.40% Crude fat 6.27 ± 0.05% Ash 13.28. ± 0.07% Crude fiber 21.78± 0.04% and Gross Energy of 3.412Kcal-^{g}. The macro/micro elements observed in BRLM are Ca:3.36±1.40mg-^{100g,} Mg: 1.02±0.46mg-^{100g}, P: 0.18±0.1mg-^{100g}, Mn:22.0±0.01mg-^{kg}, and Zn:192.00±0.02mg-^{kg}. The phytochemicals found were Tannins, 0.06±0.26mg-^{100g} Saponins 0.79±0.60mg-^{100g}, Phytate 1.47±0.10mg-^{100g} The phytochemical component studied revealed that BRLM has substantial amount of tannin, saponnins, phytate, oxalate, and glycoside. which are known to exhibit medicinal activity as well as physiological activity (Sofowora, 1993) though consumed by Nigerians in the South West but unknown nor used by the people of the South South hence if massively planted in Bayelsa will serve effectively as feeding stuff

Table 1 Proximate, Mineral & Phytochemical Analysis of *Basella rubra*

Proximate Analysis	g-^{100gm}	Phytochemicals	(mg^{-Kg})	Mineral Content	
Crude Protein	18.16	Tannins	0.06	Ca	3.36mg^{-100g}
Crude fat	6.27	Saponins	0.79	Mg	1.02 mg^{-100g}
Crude Fibre	21.78	Phytate	1.47	P(Tot)	0.18 mg^{-100g}
Ash	13.28	Oxalate	0.94	Mn	22.0 (mg/^{Kg})
NFE	58.73	Glycoside	0.09	Cu	-
Dry Matter	91.66			Zn	192.00 (mg/^{Kg})
G. Energy	3.412Kcal$^{/-g}$				

Conclusion All these results indicate that the BRLM contained nutrients and mineral elements that may be useful in both human and monogastric animal nutrition while the Phytochemical compounds explained the medicinal action of the plant leaves encountered in its therapeutic uses and or which provide scientific basis for its use in folk medicine.

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Association of cow locomotion score and lameness with their sires' estimated breeding values for conformation traits

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Introduction Lameness is a painful and debilitating condition that deteriorates animal welfare and productivity. Poor locomotion is connected with economic losses, direct and indirect (Boettcher *et al*., 1998). Total cost per lameness case has been estimated to range from 48 to 886 € (Noordhuizen, 2012). Heritability estimates of locomotion scores for diagnosing lameness or as a linear trait range from 0.05 to 0.14. Heritability of lameness itself has been estimated to range from 0.07 to 0.10. Locomotion traits are important criteria for sire selection by dairy breeders, affecting longevity (Booth *et al*., 2004). High prices of semen for sires with high breeding values; for feet and leg conformation reflects the importance given to this trait by dairy cow farmers. Significant genetic correlations have been reported between lameness, locomotion scores and specific diseases/lesions with feet conformation traits (feet and leg composite, rear leg side view, rear leg rear view and foot angle) based on cow records (Laursen *et al*., 2009; Zink *et al*., 2011). The objective of this study was to derive cow locomotion score and lameness predictions from their sires' breeding values for conformation traits.

Material and methods The study was carried out in a large commercial farm located in Northern Greece and included 237 first lactation Holstein cows that calved between 2008 and 2010 and were daughters of 115 proven sires. Cows were locomotion scored weekly on a five-point scale; starting six weeks before calving and throughout lactation. Cows with a score greater than or equal to two were considered as lame on the particular week. Total number of repeated scoring records amounted to 9,643. A univariate random regression model was used to analyse this data, including year-season of calving, country of origin, calendar month, barn, age at calving, fixed regression on week from calving, and random regressions on week from calving associated with the additive genetic effect and the permanent environment effect of cow. The model also included a linear regression on cow sires' estimated breeding value for conformation traits (rear leg rear view, overall conformation, overall feet and leg, overall feet and leg, locomotion, body condition score). A quadratic regression was fitted for traits with an intermediate optimum, namely rear leg side view and foot angle.

Results Table 1 summarises the regression results. Since the cows considered in the present study had not been included in the estimation of their sires' breeding value, these regression coefficients represent an authentic association of the two variables based on independent datasets. Optimum (intermediate) foot angle conformation is indeed related with improved locomotion and lower lameness frequency, with steeper angles being preferable to lower ones. On the other hand, the quadratic relationships of rear leg side view, which is also an intermediate optimum trait, were not statistically different from zero ($P > 0.05$); in this case, the significant linear regression suggested that straight rear legs are associated with improved locomotion and fewer lameness problems. Overall conformation score showed a statistically significant linear relationship with locomotion. There was also a favourable and expected relationship of the sires' evaluations for locomotion and body condition score with their daughters' locomotion score and lameness.

Table 1 Regression coefficients of cow locomotion score and lameness on their sires' estimated breeding values for conformation.

	Locomotion score			Lameness		
Trait	tercept	Linear	Quadratic	Intercept	Linear	Quadratic
Rear leg side view	0.68*	0.0251*	-0.0021	-0.12	0.0143*	-0.0056
Rear leg rear view	0.68*	0.0057		-0.13	0.0107*	
Foot angle	0.65*	-0.0031	0.0193*	-0.15	0.0113	0.0150*
Overall conformation	0.64*	-0.0383*		-0.15	-0.0206	
Overall feet and leg	0.67*	-0.0117		-0.14	-0.0088	
Locomotion	0.67*	-0.0580*		-0.14	-0.0328*	
Body condition score	0.75*	0.0473*		-0.09	0.0273*	

*Statistically different from zero ($P < 0.05$).

Conclusion There is scope for inclusion of specific claw health traits in a "lameness index" of sires that would result in faster genetic progress.

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Using spent tea leaves to improve *in vitro* degradability but reduce rumen ammonia from rice straw-based diets

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Introduction Spent tea leaves (STL) are industrial waste products that contain high crude protein (CP) and secondary metabolites such as tannins (Ramdani *et al.,* 2012). Tannins are known to reduce solubility and degradability of most leaf proteins in the rumen resulting in reduced rumen ammonia (NH_3) and increased rumen by-pass protein for its absorption in small intestine. Although NH_3 is an important source of N for rumen microbes, its over or fast production may exceed the ability of microbes to utilize it. This can lead to an excessive NH_3 supply that after absorption through rumen wall can enter the blood stream, liver and eventually be lost in urine as a waste. Thus, this study hypothesises that addition of green or black STL (SGTL or SBTL) to partly substitute rice straw (RS, variety IR50) in a ruminant diet would improve *in vitro* degradability while reduce rumen NH_3 production in ruminant animals.

Material and methods A 5x5 factorial design, in triplicate, was used to compare 5 different diets, all containing (DM basis) 70% concentrate with 30% RS (T0), 20% RS + 10% SGTL (SGTL10), 10% RS + 20% SGTL (SGTL20), 20% RS + 10% SBTL (SBTL10) and 10% RS + 20% SBTL (SBTL20) and 5 different incubation times (0, 6, 24, 48 and 72h). Both SGTL and SBTL were obtained after boiling about 2.8 g tea leaves in 300 ml water by using the method of Ramdani *et al.,* (2012). *In vitro* dry matter degradability (IVDMD) was completed according to Khan and Chaudhry (2010) by using rumen fluid (RF) from freshly slaughtered grass-fed-lambs (Texel cross) from an abattoir. The centrifuged and acidified RF after each incubation time was analysed for NH_3 by Pentra 400 (Horriba Ltd, Japan). The GLM on Minitab 16 was used to examine the statistical effects of different STL and inclusion level combinations in the diets as well as different incubation times alongside their interaction on IVDMD and NH_3 production.

Results Main effects of diets, incubation times and their interaction were significant (P<0.001) for IVDMD and NH_3 production (Tables 1 and 2). Across incubation times, all STL-containing diets had significantly higher IVDMD, but lower NH_3 compared to the control (T0). There was no statistical difference between STL-containing diets for IVDMD but SGTL20 had significantly lower NH_3 than other diets. Across the diets, both IVDMD and NH_3were increased as the incubation times were increased.

Table 1 Effect of SGTL and SBTL inclusions into ruminant diets on IVDMD at different incubation times (g/kg DM)

Diets	0h	6h	24h	48h	72h	Means	SEM
T0	25.6^{h}	37.4^{gh}	171.9^{f}	447.4^{cd}	543.5^{ab}	245.2^{b}	5.994
SGTL10	74.7^{gh}	105.8^{fg}	307.4^{e}	443.2^{cd}	576.6^{a}	301.5^{a}	5.715
SGTL20	65.1^{gh}	67.6^{gh}	419.2^{d}	454.4^{cd}	583.0^{a}	317.9^{a}	5.994
SBTL10	59.8^{gh}	46.7^{gh}	413.8^{d}	505.4^{bc}	574.8^{ab}	320.1^{a}	5.994
SBTL20	59.1^{gh}	70.2^{gh}	377.2^{de}	423.9^{d}	560.6^{ab}	298.2^{a}	5.994
Means	56.9^{D}	65.54^{D}	337.9^{C}	454.9^{B}	567.7^{A}		P<0.001
SEM	5.715	5.994	5.994	6.261	5.715	P<0.001	

Table 2 Effect of SGTL and SBTL inclusions into ruminant diets on NH_3 production at different incubation times (mg/L)

Diets	0h	6h	24h	48h	72h	Means	SEM
T0	45.5^{i}	80.1^{efgh}	184.0^{acb}	200.0^{ab}	206.4^{a}	143.2^{a}	2.556
SGTL10	47.5^{i}	63.7^{ghi}	102.7^{de}	173.0^{bc}	185.5^{abc}	114.5^{b}	2.556
SGTL20	42.2^{i}	51.9^{hi}	89.4^{defg}	158.3^{c}	158.0^{c}	100.0^{c}	2.680
SBTL10	40.8^{i}	67.9^{fghi}	112.9^{d}	179.2^{abc}	194.3^{ab}	119.0^{b}	2.556
SBTL20	40.8^{i}	64.9^{ghi}	101.2^{def}	172.6^{bc}	192.3^{ab}	114.4^{b}	2.680
Means	43.4^{E}	65.7^{D}	118.1^{C}	176.2^{B}	187.3^{A}		P<0.001
SEM	2.556	2.556	2.800	2.556	2.556	P<0.001	

Means with different letters (Tables 1 and 2) either in the same column for the diets (small letters) or row for incubation times (capital letters) or both for their interaction (Italic small letters) are significantly different; SEM=standard error of mean.

Conclusion Both SGTL and SBTL inclusions can improve IVDMD while reduce NH_3 production in RS based ruminant diets. Lower NH_3 production may result in increased availability of rumen by-pass protein supply to be absorbed in the small intestine. Based on IVDMD, SGTL can be included up to 20% but SBTL should not exceed 10% of a diet.

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The effect of lactation housing system and grouping at weaning on post weaning performance of pigs

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Introduction Weaning involves many nutritional, environmental and social changes which can adversely affect the welfare, health and growth of piglets. Piglets reared in more enriched conditions, for example outdoor paddocks, adapt better to these changes and some of this may be due to less aggression when mixed with other pigs (Chaloupková *et al.*, 2007). The objective of this experiment was therefore to investigate the post weaning growth of piglets raised in a novel loose-farrowing system (the PigSAFE system, Edwards *et al.*, 2012) with those raised in a conventional farrowing crate system, and to determine whether any difference was attributable to reduced aggression when mixed with unfamiliar piglets.

Material and methods The experiment was a 2 x 2 factorial design using 32 litters of piglets in 4 replicates over time. Half of the litters were born and nursed in conventional part-slatted pens (1.90 x 2.38m) with a farrowing crate and a heated creep area with sawdust bedding. The other litters were born and nursed in a novel design of loose farrowing pen, the PigSAFE system (2.36 x 3.35m) with a lightly strawed nest area, heated creep and separate slatted sow feeding and dunging areas. At weaning at 4 weeks of age, half of the litters from each system were maintained as single litter groups of 10 piglets, and the others housed as groups of 20 piglets made up from two litters. Litters within a replicate were housed in the same controlled-environment room with fully slatted pens providing 0.3 m^2 per pig. Standard commercial weaner diets were offered *ad libitum* and water was freely available from 2 nipple drinkers per 10 piglets. Pigs were individually weighed at weaning and weekly thereafter for a period of 5 weeks. Food offered and residuals remaining in the hopper were recorded weekly for each pen. Lesions on the integument of each individual piglet were scored before weaning and weekly thereafter. The body was divided into 3 sections: head and ears, shoulders and flank, and hind quarters. Damage level for each region was scored as: 0= no damage, 1= 1-10 superficial marks, 2 = more than superficial damage with evidence of bleeding or >10 superficial marks. Scores for the 3 areas were totalled for each pig. Data were analysed on a group mean basis by ANOVA using Minitab version 16, with lactation housing, post weaning group size and their interaction as factors. Replicate was included as a blocking factor, and mean weaning weight as a covariate.

Results Litters nursed in the PigSAFE system had higher daily liveweight gain in the first week after weaning, but this difference was not maintained and feed intake, growth and feed efficiency did not differ between treatments over the full 5 week post weaning period (Table 1). There was no effect on performamce of grouping litters or leaving litters intact, and no interaction between lactation housing and post-weaning grouping treatment. Grouped pigs had higher lesion scores after the first week, but the interaction with lactation housing system was not significant.

Table 1 Effect of lactation housing system (crate or PigSAFE) and post weaning grouping treatment (single litter or two litters combined) on daily gain (DLWG), feed intake (FI) and feed conversion ratio (FCR) of pigs during 5 weeks after weaning

Housing treatment	Crate		PigSAFE		RSD§	Significance	
Grouping treatment	Single	Combined	Single	Combined		Housing	Grouping
DLWG in first week (kg)	0.278	0.279	0.355	0.335	0.0634	0.04	ns
DLWG overall (kg)	0.411	0.438	0.426	0.428	0.0286	ns	ns
FI overall (kg)	0.635	0.652	0.626	0.635	0.0458	ns	ns
FCR overall (feed/gain)	1.55	1.50	1.48	1.49	0.11	ns	ns
% of piglets with lesion score >4 after 1 week	0	17.2	0	7.2		ns	0.02

§N=8 per treatment for single litters and 4 for combined litters.
There were no significant Housing x Grouping interactions

Conclusions A more complex housing system during the nursing period reduced the immediate post-weaning growth check but gave no long term benefit to pig performance. This effect occurred in both single and grouped litters, so was not dependent on better social skills of the piglets.

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Influence of bull exposure on resumption of ovulatory activity and reproductive performance of *post partum anoestrous* dairy cows

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Introduction The prolonged calving interval of high yielding cows is a significant problem for dairy cow production. Studies in swine, sheep and beef cattle indicate that exposure to a male can improve *post partum* reproductive performance (Berardinelli *et al*., 2005; Fike *et al.*, 1996). Therefore this study aimed to examine the biostimulatory effects of fenceline bull exposure from the time of calving on resumption of ovulatory activity and subsequent conception in lactating dairy cows.

Material and methods In this study, 41 freshly calved high-yielding Holstein-Friesian dairy cows, with average parity 3.05±0.5, were allocated to two treatment groups; 20 cows with no bull contact (NBC) formed the control group and 21 matched cows formed the bull contact group (BC). The cows were housed in cubicles with rubber mats and sawdust bedding. At one end of the cubicle house, the bull was placed within a pen which was separated from the cows in the bull contact (BC) group by a barred fence. The cows in the BC group had unlimited time of access to the bull throughout the trial period. A sexually active Friesian-Holstein bull aged approximately 13 months old was used as the stimulus animal. The cows in the no bull contact (NBC) group were housed at the other end of the cubicle shed, distant from the bull pen. For each cow, calving number, date of previous calving, body condition score, milk yield and date seen bulling were recorded. A sensor (Icetag) was attached to each cow above the fetlock to continuously monitor stepping behaviour and record changes in activity level. Visual observation was done to observe mounting activity indicative of oestrus. Milk samples were collected 3 times a week and analysed to determine progesterone concentrations to measure the resumption of ovarian cyclicity. Cows detected in oestrus were served with artificial insemination (AI) semen approximately 12 hours after showing oestrus. Cows that showed difficulty to conceive received a PRID treatment. PRID device was inserted for 12 days; on day 10 of treatment PFG2a was injected. After PRID removal, cows were observed for oestrus behaviour and were AI as a batch on day 14 to 15 of PRID treatment. Progesterone concentrations were normally distributed and analysed by repeated measures ANOVA. The pregnancy rates were analysed as categorical counts using chi-square tests.

Results The interval before cows resumed cyclicity was similar for cows either exposed or not exposed to bull stimulation; the average days *post partum* for resumption of ovulatory activity was 30±2 dpp, based on progesterone concentration profiles. The *post partum* increase in concentration of progesterone was slightly more rapid for BC cows, however the difference was small and not statistically different (P=0.24) compared to NBC cows. The pregnancy rate to first service of BC cows was higher (P=0.011) compared to NBC cows, especially following a PRID treatment (Table 1). The average number of services per conception was lower in BC cows compared to NBC with 1.5 and 2.4 services, respectively (P=0.003). Consequently, the mean calving interval for BC cows was significantly shorter (P=0.007) compared to NBC cows with this effect coming mainly from the PRID treated cows (P=0.009) (Figure 1).

Table 1 The pregnancy rates to first service for cows from the NBC and BC groups.

	NBC	BC	P value
Pregnancy to first service	20.0% (4/20)	61.9% (13/21)	0.011
Natural Oestrus	27.3% (3/11)	53.85% (7/13)	0.24
PRID	11% (1/9)	75% (6/8)	0.015

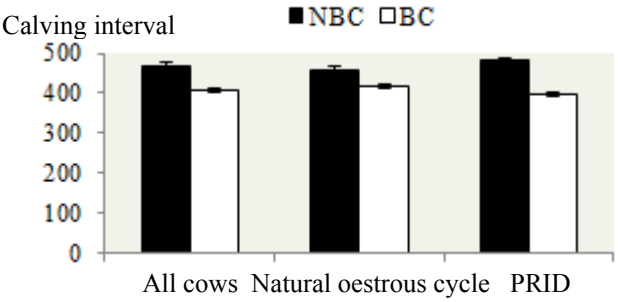

Figure 1 The mean calving interval (days), as predicted based on a 280 day gestation period.

Conclusions The biostimulatory effects provided by close physical contact with a bull through continuous fenceline exposure improved the breeding performance of cows, particularly cows treated with a PRID. Since the control group were in the same building, they may have received some pheromonal effect but this would have been greatly attenuated compared to the treatment group. Further study is on-going to replicate the comparisons in different groups.

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The feed intake of individually housed finishing pigs when offered diets containing between 0 and 210 g/kg of rapeseed meal and balanced or not for fibre

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Introduction Magowan *et al*. (2013) reported a reduction in pig performance when rapeseed meal (RSM), with a low glucosinolate level (4.8 µmol/g), was included in finishing pig feed between the levels of 70 and 210 g/kg. The formulation of diets used by Magowan *et al*. (2013) did not control fibre content. The aim of the current experiment was to investigate the effect of diets containing increasing levels of RSM, balanced for fibre content or not, on the feed intake of finishing pigs.

Material and methods A total of 64 pigs (Tempo x (Landrace x Large White)) were individually housed and used in a changeover design experiment within one six week time period. A total of 16 diets were prepared representing a 2 x 8 factorial design with diets being balanced or unbalanced for fibre content and containing 0, 30, 60, 90, 120, 150, 180 or 210 g/kg of RSM. Each pig was offered each diet for two weeks and therefore a total of three diets over the six-week period. Pigs were weighed at 12 weeks of age and assigned to treatments within the changeover design according to their weight and sex. Pigs were weighed again at 14 and 16 weeks of age. Pig feed intake was recorded daily (ADFI) and the dry matter (DM) of the feed and feed refusals were measured by drying feed samples at 105°C for 48 hrs. The DM intake on an individual pig basis over the latter 10-day period of each two-week period was calculated and used in statistical analysis. Three diets were manufactured (0 g/kg RSM fibre balanced, 0 g/kg RSM fibre unbalanced and 210 g/kg RSM fibre balanced) from which the 16 diets were prepared by mixing appropriate proportions of these three diets. All three diets were formulated to contain 171 g/kg crude protein, 11 g/kg lysine and 13.8 MJ/kg digestible energy (fresh basis). The diets balanced for fibre were formulated to contain 42 g/kg DM fibre and the 0 g/kg RSM unbalanced fibre diet was formulated to contain 29 g/kg DM fibre. After mixing the target fibre content of all the 'fibre balanced' diets was 42 g/kg DM and that of the unbalanced fibre diets was 31, 33, 35, 36, 38, 40 and 42 g/kg DM fibre for the 30, 60, 90, 120, 150, 180 and 210 g/kg RSM diets respectively. The main ingredients in the 0 g/kg RSM diet balanced for fibre was (g/kg) maize 393; wheat 283; soya 246; soya hulls 47; vegetable oil 6; limestone 4.7; dicalcium phosphate 7.0; minerals and vitamins 5; salt 4.6; lysine 3.3. The main ingredients in the 0 g/kg RSM diet unbalanced for fibre was (g/kg) maize 196; wheat 450; barley 71; pollard 48.8; soya 196; vegetable oil blend 10; limestone 6.1; dicalcium phosphate 5.6; minerals and vitamins 5; salt 4.1; lysine 4.0. The main ingredients in the 210 g/kg RSM diet balanced for fibre was (g/kg) maize 206; wheat 460; barley 16.1; RSM 210; soya 61.8; vegetable oil blend 22.5; limestone 5; dicalcium phosphate 3.4; minerals and vitamins 5; salt 4.0; lysine 4.4. The raw RSM was analysed for total glucosinolate content using an HPLC method conforming to BS4325 Part 12 (NIAB Labs, England). Data were statistically analysed according to the 2 x 8 factorial experimental design, blocking for changeover period, using analysis of variance in Genstat V10, at a probability level of 5%.

Results The total glucosinolate level of the RSM was 10.1 µmol/g. The average fibre content of the balanced diets was 43.1 g/kg DM (SD 0.51) and the average fibre content of the unbalanced diets was 41.0 g/kg DM (SD 0.52). The average weight of pigs was 44.3 kg (SD 1.63) at 12 weeks of age, 59.8 kg (SD 1.96) at 14 weeks of age, 75.9 kg (SD 2.40) at 16 weeks of age and 90.9 kg (SD 4.03) at 18 weeks of age. There was no significant interaction or effect of fibre content or RSM inclusion on the average daily feed intake of pigs (Table 1).

Table 1 Interaction between RSM inclusion and fibre content on ADFI of pigs (g/day DM) between 12 and 18 weeks of age.

	Rapeseed inclusion								Interaction Statistics[1]	
	0%	3%	6%	9%	12%	15%	18%	21%	SEM	P value
Balanced	2105	2109	2207	2152	2207	2137	2144	2111	83.7	0.878
Non Balanced	2175	2118	2150	2169	2101	2106	2136	2049		

[1] Effect of balancing: SEM = 30.2, P>0.05, Effect of RSM inclusion: SEM = 59.6, P>0.05

Conclusions The actual level of fibre in the balanced and unbalanced diets was similar with a similar variance between diets. This may explain the non significant effect of 'fibre content' on feed intake. In contrast to Magowan *et al.* (2013), the inclusion of RSM at high levels did not significantly reduce the feed intake of pigs in this study. Being individually housed there were no social limitations to affect eating behaviour in this study and the feed intake of pigs offered the control diet (0 g/kg RSM) was approximately 2432 g/day on a fresh matter basis. Assuming that the intake of pigs' increases by at least 10% when they are individually housed compared with group housing, it is suggested that the same diet offered to a group of pigs would yield an ADFI of approximately 2188 g/day. It is interesting to note that this predicted group feed intake value is more in line with the ADFI of pigs offered the RSM diets (average of 2250 g/day) than that of the control diet (2464 g/day) in the study by Magowan *et al*. (2013). In conclusion, this study suggests that the inclusion of up to 210 g/kg RSM will not affect the average daily feed intake of finishing pigs.

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Growth response and nutrient digestibility of growing pigs fed varying levels of maize offal in diets containing chicken offal meal

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Introduction The feed deficit situation is more serious with protein deficiency when compared to the availability of calories and microelements. Poultry and pigs represent the fastest means of correcting the shortage of animal protein in Africa (Adesehinwa, 2004). The cost of feed conversion by these species of livestock may be very high. This is as a result of the increased costs of the ingredients required to formulate their concentrate feeds, hence, the use of agro-industrial by-products (AIBPs). These AIBPs or non conventional feed sources of plant origin are mostly fibrous in nature. The ability of pigs to utilize fibrous feed resources for growth is confined in the lower part of the GIT (Adesehinwa, 2008) and can to contribute significantly (5-30%) to the net energy requirement of pigs (Rerat et al 1987). The influence of crude fibre on organic matter digestibility varies from feed to feed, depending on the special characteristics of the crude fibre in individual feeds (Kidder and Manners, 1978). It was therefore the aim of this study to evaluate the growth response and nutrient digestibility of growing pigs fed varying levels of maize offal (MO) in diets containing chicken offal meal (COM) as animal protein source formulated to contain approximately 18% crude protein.

Material and methods Sixty crossbred (Largewhite x Landrace) growing pigs (averaging 29.51±1.60kg) were used in the 42-day study. The dietary treatment groups comprised diets containing 0, 14.27, 28.54, 42.53 and 56.81% maize offal representing 0, 25, 50, 75 and 100% replacements of the maize content in the diet, with chicken offal meal added to supply 20% of the crude protein provided by the 16.19% soybean content. Each treatment/diet was replicated four times in a completely randomized design and 3pigs/pen representing a replicate. Feed and water were provided *ad libitum* for the 42-day duration of the study. Proximate composition of the test diet was done according to the methods of A.O.A.C. (1990). One pig/replicate for the 4replicates/treatment was used in the digestion trial (Adesehinwa *et al.*, 2011) in a complete randomized design. Data generated on the growth performance and nutrient digestibility of the pigs were subjected to ANOVA using SAS software version 9.2 (SAS Institute Inc. Cary., N.C. USA).

Results The result shows that the crude fibre content of the diets increased numerically with increasing inclusion of MO resulting in the reduction of the ME/kg diet. The DM, protein and energy contents of the diets were efficiently utilized, bringing about comparable weight gains, even up to the total replacement of the entire maize containing e with MO in the diet containing COM. The efficient utilization of the nutrients, especially the protein and fibre in the diets and the favourable feed conversion efficiency were significant explanatory variables for the comparable gains across the groups.

Table 1 Proximate, Growth performance and nutrient digestibilties of growing pigs fed varying levels of maize offal

Parameters	0% MO	14.27% MO	28.54% MO	42.53% MO	56.81% MO
Crude protein, %	18.52	18.38	18.31	18.72	18.98
Crude fibre, %	5.49	6.79	7.44	8.05	9.31
Ether extract, %	7.13	6.06	6.77	6.61	7.01
ME (Kcal ME/kg)*	3487.12	3361.81	3304.36	3232.80	3189.47
Daily weight gain, kg	0.67±0.05	0.68±0.05	0.69±0.05	0.66±0.04	0.57±0.04
Daily DM intake, kg	1.30±0.04	1.27±0.03	1.28±0.05	1.28±0.04	1.25±0.03
Daily protein intake, kg	0.23±0.01	0.22±0.01	0.23±0.01	0.23±0.01	0.23±0.01
Feed:Gain	2.16±0.17	2.04±0.26	2.07±0.21	2.05±0.13	2.43±0.20
Protein efficiency ratio	2.91±0.23	3.29±0.27	3.08±0.23	2.90±0.17	2.59±0.21
Apparent digestibilities (%)					
Dry matter	77.33±4.00	75.49±5.07	75.63±1.34	78.57±1.65	79.00±0.49
Crude protein	81.27±3.31	80.41±4.05	81.70±1.01	85.48±1.12	86.17±0.32
Crude fibre	73.95±4.60	76.95±4.76	78.74±1.17	81.69±1.41	84.33±0.37

*Estimated by Morgan *et al.* (1975) prediction equation

Conclusion It can thus be concluded that this class of pigs can tolerate complete replacement of maize with maize offal in diets of growing pigs containing chicken offal meal without any adverse effect on the performance and nutrient digestibility of the pigs.

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A partial budget for changing from Holstein-Friesian to Holstein-Friesian/Norwegian Red crossbred dairy cows on grazed and high input systems

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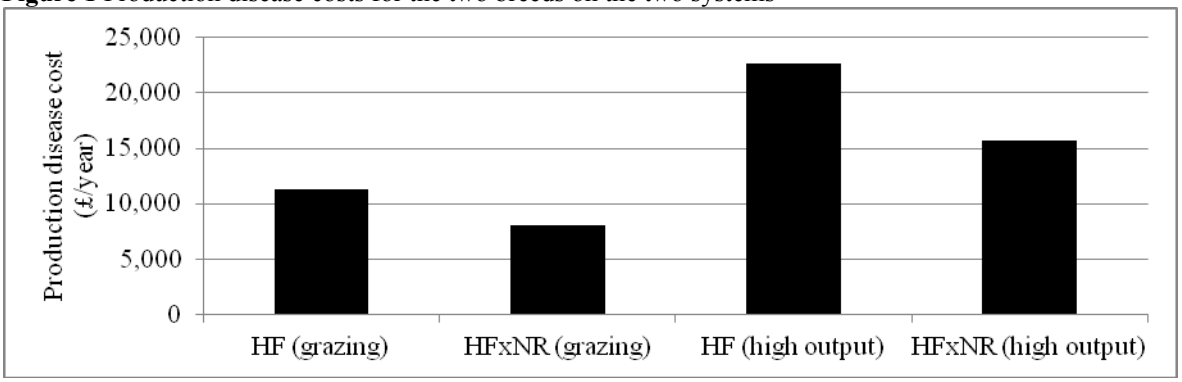
Introduction A recent publication from DairyCo (2012) identified three different milk production systems in the UK, stating that profitable milk production was possible from each system regardless of herd size, the key issue being minimising production costs. The three systems were grass based (grazing), composite systems and high output systems (DairyCo, 2012). This paper outlines a partial budget analysis for two different breeds, the Holstein Friesian (HF) and the Holstein Friesian Norwegian Red crossbreed (NRxHF) under the grazed and high output systems.

Materials and methods Data sets for the two breeds for the two different systems were supplied by Geno Global of Norway and owing to low uptake of HFxNR in the UK, the data for grazed systems came from the Republic of Ireland and Northern Ireland and the data for high output systems from California and Israel, thus making the data for the two breeds comparable on identical systems. The data supplied included figures for mastitis incidence, lameness incidence, average milk yield, calving index, replacement rates, forage utilisation, longevity, calf mortality and dystocia but not necessarily for all sites, systems and breeds. Financial values for the UK were then applied to this data, sourced from published literature such as Esslemont, and Kossaibati (1997), standard farm management data (Nix, 2012), and current market values at the time. Where necessary costs were inflated to current prices. A partial budget analysis was then carried out to assess the cost or benefit of changing from HF to NRxHF on grazed and high output systems.

Results After compilation of the figures, the final analysis was based on a 100 cow dairy herd for each of the two breeds on the two systems. The final partial budget analysis revealed that there was a net annual benefit of £9,727 per annum to be gained by moving from HF to HFxNR on grazed systems. The change from HF to HFxNR on high output systems showed a benefit of £3,529. The biggest difference between the two breeds was the total costs of the production diseases, lameness, mastitis and infertility illustrated in Figure 1. On grazed systems this was an annual cost of £11,324 per herd per year against £8,302 for the HFxNR, a difference of £3,292 (£32.92/cow). On high output systems the costs of production disorders for HF were £22,612 per herd per year and £15,731for HFxNR, a difference of £6,881 (£68.81/cow).

Figure 1 Production disease costs for the two breeds on the two systems



Overall, the greater income from milk sales owing to the higher yields of the pure HF (3 to 5% over both systems) did not compensate for the lower disease cost, reduced replacement costs, increased value of bull calves and lower calf mortality of the HFxNR.

Discussion The analysis suggests that there is a positive benefit for changing from HF to HFxNR over both systems. Sources of suitable production data on identical systems for crossbreeds are limited and may not necessarily reflect figures for comparable UK systems, for example, the milk yields on the high output systems were higher for HF cows than those observed in the UK. Milk producers with pure HF cows would be aware of disease, replacement and calf mortality levels alongside market values for bull calves, but maybe not aware of the high costs or benefits associated with these issues at herd level. Changing production systems or breeding programmes can be expensive but as cereal prices continue to increase, margins on high output systems will get smaller leading to a possible shift towards grazed systems. As all dairy farmers seek to reduce production costs, lower yielding cross breeds are becoming ever more popular and the HFxNR may well become a viable alternative for UK farmers.

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The source of alkaline phosphatase activity in bovine nasal secretion is the nasal mucosa

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Introduction The clinical biochemistry of bovine nasal secretion (NS) has not been well characterized but may provide useful indicators of immune function and health status of the animal. Our work aims to determine the biochemical composition of NS in healthy cows, and following the discovery of a high level of alkaline phosphatase (ALP) activity in NS, a further aim was to identify the origin of the enzyme in the secretion.

Material and methods Nasal secretions were collected from thirty eight clinically healthy Holstein-Friesian cows aged 2-5 years on the University of Glasgow Cochno Farm. The cows were restrained in a cattle crush during the procedure. A commercially available tampon was inserted into one nostril and slid gently in an upwards and backwards direction about 2-3 inches deep and was left in place for up to 15 minutes. The tampon, saturated with NS was removed from the nostril by gently pulling on the string attached to the end of the tampon. Each collected tampon was weighed before being inserted into a collecting tube and centrifuged at 3000 rpm at 4°C for 10 minutes. Nasal secretions were assayed for biochemical composition using an Olympus A640 analyzer (Olympus, Tokyo, Japan) at the Veterinary Diagnostic Services, University of Glasgow. Protein concentration was determined using the Bradford assay, and immunoglobulin A and G concentrations using specific ELISAs (Bethyl Labs, Texas, USA). Tissue samples were obtained at *post mortem* from six further cows (Holstein-Friesian, 2-6 years) free from infectious and respiratory disease. Two grams of nasal mucosa, small intestine, heart, liver and kidney, obtained from each animal were washed with isotonic saline solution and homogenized mechanically in 5ml saline containing 20% (v/v) n-butanol. The alkaline phosphatase (ALP) activity in tissue extractions were measured using para-nitrophenyl phosphate (pNPP) enzymatic reaction. Histochemistry of the bovine nasal mucosa was performed using snap freezing technique, tissue was dissected at 0.7µm, were fixed with acetone and stained with Vector® Blue Alkaline Phosphatase Substrate Kit (Vector Labs, California, USA). Isoelectric focusing for the separation of ALP isozymes were undertaken with Invitrogen Novex® pH 3–7 IEF Gel (Life Technologies, Carlsbad, California, USA) and stained with Pierce 1-Step™ NBT-BCIP ALP substrate solutions (Thermo Fisher Scientific Inc, Illinois, USA). ALP mRNA levels in nasal mucosa was analyzed by end-point semi quantitative RT-PCR, using primers designed to amplify specific regions of the bovine intestinal and non intestinal ALP gene using an interactive web-based primer program algorithm, GeneFisher software version 1.2.2 (BiBiServe, Germany). The 500bp PCR products were visualized by ethidium bromide stained agarose gels.

Results 5 to 12 ml volumes of nasal secretion were collected per tampon from the cows. Protein concentrations in bovine nasal secretion ranged from 8.21 to 33.7 g/L. ALP concentrations were up to 10 fold higher than the reference range for serum from healthy cattle (Table 1). Other biochemical analytes were within or near the reference range for serum. The concentrations of immunoglobulin A and immunoglobulin G in nasal secretion were between 0.45 to 1.82 g/L and 0.17 to 1.88 g/L respectively. The nasal mucosa had the highest ALP concentration in the tissue extracts suggesting that the ALP in the NS is locally produced. Histochemistry showed strong ALP activity in the nasal epithelium and serous glands. Isoelectric focusing demonstrated that ALP from the nasal mucosa has a higher pI (pH 5.5) than ALP from bovine liver (pH 4.5) and bone (pH 5.2) but lower than ALP from the intestine (pH 6.5), suggesting that they are different isozymes. A single robust PCR product at the predicted size of 500bp was detected in the nasal mucosa with the non intestinal ALP specific primers but not with the intestinal ALP primers (Figure 1). Liver and intestine samples served as positive and negative controls for each primer pair.

Table 1 ALP activity in various tissues

Tissue	U/L	Mean	Standard Deviation (s)	Median	Range	Reference (Bovine serum)
Nasal secretion	U/g	1192	500	1236	144 - 2392	20 – 280[a]
Nasal mucosa	U/g	10.0	9.6	8.4	1.0 - 27.6	
Intestinal mucosa	U/g	8.3	8.7	2.3	2.2 - 24.0	
Heart	U/g	0.6	0.4	0.6	0.2 - 1.1	
Kidney	U/g	7.3	4.5	7.4	3.9 - 16.2	
Liver	U/g	9.5	10.0	7.0	1.04 - 27.2	

[a]laboratory reference range

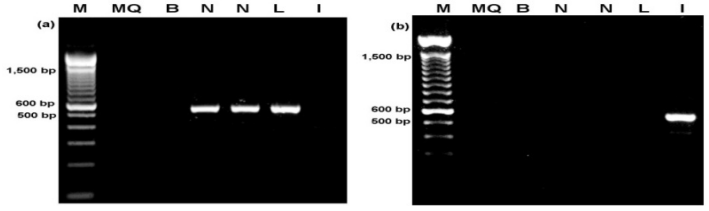

Figure 1 PCR analysis of ALP from bovine nasal mucosa. The presence of a single 500bp DNA fragment with bovine non intestinal ALP primer (a) and were absent with the intestinal ALP primer (b). Lanes M contain molecular mass standards from 100- 2072bp (100bp DNA ladder).

MQ=water; B=blank; N=nasal mucosa; L=liver; I=intestine

Conclusions Samples of bovine NS were collected from healthy animals using a simple non-invasive collection method. The biochemical and immunological results provide baseline data on the composition of a normal bovine NS. High concentration of ALP in the nasal secretion and nasal mucosa extraction along with the histochemical findings indicate that nasal ALP is derived from local synthesis and secretion of the enzyme. The RT-PCR data indicates there is homology with non-intestinal ALP mRNA. However, isoelectric focusing of ALP also verified that the ALP from nasal mucosa differs from other isozymes from other tissues perhaps indicating differences in post translational modifications. Further study will determine if ALP and other biomarkers in this fluid can be used to characterize the pathophysiological responses of the host against respiratory diseases.

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Functional characterization of bovine interleukin eight promoter haplotypes in uterine epithelial cells

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Introduction The uterine lumen of almost all cows is exposed to microbial contamination during the first two weeks after calving, and 25 to 40% of animals develop clinical metritis. Clinical and sub-clinical uterine diseases are associated with subfertility and infertility and impose one of the most expensive challenges in dairy industry (Sheldon *et al.* 2008). Upon bacterial infiltration, uterine epithelial cells respond by secreting proinflammatory molecules. The key anti-bacterial defensive mechanism during parturition is the attraction and recruitment of neutrophils, which is regulated by interleukin 8 (IL8).
Previously, our group has identified novel polymorphisms in the promoter region of IL8 spanning 2.1 kb upstream of the transcription start site, which may confer functional differences in an animals ability to mount an effective immune response and control infection. We also showed that these polymorphisms segregate into two distinct haplotypes, which were functionally different *in vitro* (Meade *et al.,* 2012). The aim of the current study is to analyse the expression of two IL8 haplotypes in bovine endometrial cells, upon stimulation with gram-negative (LPS) stimuli.

Material and methods ***IL8 promoter reporter vectors*** Reporter constructs from two IL8 haplotypes were previously cloned in pGL4.17 vector (Meade *et al.*, 2012). Plasmids were PCR amplified and commercially sequenced for verification of the correct insert.
Cell culture The bovine endometrial cell line BEND was purchased from ATCC (LGC Standards, U.K.) and cultured in 75 cm^2 flasks in 5% CO_2, in a 37°C incubator. Complete growth medium consisted of 1:1 mixure of Hams F12 medium and minimal essential medium MEM Glutamax^{TM}, with 34 mg/ml D-Valine, 10% heat-inactivated fetal calf serum, 10% heat-inactivated horse serum and 1% Pen/Strep.
Transient transfections, treatments and luciferase reporter assays Cells were seeded in 12-well plates at a density of 2.5 X 10^4 cells per well in 1ml of media. Transient cotransfection treatments for luciferase reporter assays were carried out in six replicates using 100 ng of each of the pGL4.17 maxipreparations (Hap 1, Hap 2 and empty vector) together with 10 ng of a renilla control vector. The transfection mixure was removed after 16h and replaced with either complete growth media or media supplemented with serial dilutions of LPS. Medium was removed and cells were lysed with freshly prepared passive lysis buffer solution. Lysates were analysed for luciferase and renilla levels using the Dual Luciferase Reporter Assay system. Data was analysed using 2 sample t-test as implemented in version 15 of Minitab. Probabilities of less than 5% ($p<0.05$) were considered significant.

Preliminary results Both haplotypes showed increased functional activity after treatment with LPS. The difference was significant for the treatments with 2 and 20ug/ml of LPS in haplotype 2 ($p<0.05$). Maximal response was induced by 200ng/ml and 2ug/ml for haplotype 1 and 2, respectively. The significant difference in functional activity between haplotype 1 and 2 was detected for 2ug/ml LPS treatment, while the trend of higher haplotype 2 activity ($p<0.1$) was observed in 20ug/ml LPS treatment (Figure 1).

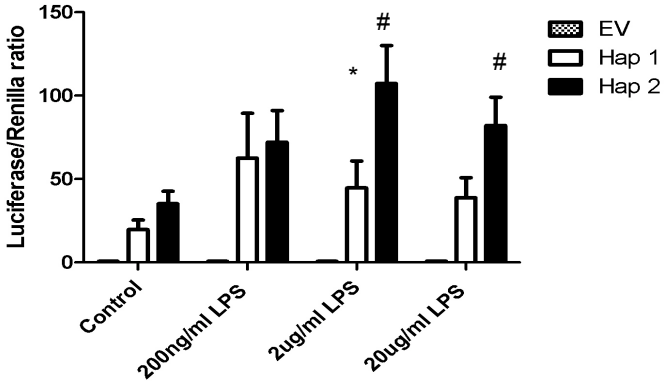

Figure 1 Functional analysis of IL8 promoter haplotypes fused with pGL4.17 luciferase reporter plasmid. Both haplotypes were treated with various concentrations of LPS. Relative luciferase activity was calculated as a ratio of luciferase activity over renilla control. Results are means ± SEM from six replicates per treatment. Statistically significant differences were found between haplotypes (*) and between control and LPS treatments (#)

Conclusions In summary, we demonstrated that two IL8 promoter haplotypes in uterine epithelial cells show differential response to LPS *in vitro*. Different IL8 expression between animals of two haplotypes may confer differential ability to clear post-partum infection, which could be exploited via selection.
Ongoing work will characterize the expression profiles of full and deletion constructs of two IL8 haplotypes in response to LTA and TNFα, and examine the consequences of IL8 haplotype for uterine infection *in vivo*.

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An enzyme linked immunosorbent assay to determine haptoglobin concentrations in milk from a dairy herd

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Introduction Haptoglobin (Hp) is a positive acute phase protein (APP) involved in haemoglobin transport that also acts as an antioxidant. It increases in concentration not just in the serum, but also in the milk of cows during episodes of mastitis (inflammation of the udder) (Eckersall et al, 2001). Recently it has been established that Hp levels in milk are associated with somatic cell counts in milk (SCC) and other indicators of mastitis, hence the assay of milk Hp may become an important and consistent marker for the detection of clinical and subclinical mastitis in dairy farms. Direct and indirect methods have been developed for the assay of Hp, based on either its binding ability to haemoglobin or via immunoassays using anti-Hp antibodies. The latter methods have the benefit of greater sensitivity, which is required to measure Hp in milk. The aim of the present study was to develop, optimise and validate a reliable and specific sandwich ELISA using direct conjugation of alkaline phosphatase to an anti-bovine Hp antiserum and use the assay to determine Hp concentrations in milk from a commercial West of Scotland dairy herd.

Materials and methods Purified rabbit anti-bovine Hp (Life Diagnostics, USA*)* was conjugated with alkaline phosphatase (Innova Biosciences, Cambridge, UK). A sandwich ELISA procedure was carried out using the same anti-bovine Hp as capture antibody and the alkaline phosphatase conjugated antibody as signal with absorbance read at 595 nm using Fluostar OPTIMA plate reader (BMG Labtech UK.) and FLUOstar OPTIMA Software V1.32 R2 using a 4 parameter-fit standard curve to determine the results. To optimise the assay, dilution factors and concentrations of the initial antibody, standards and known Hp samples, as well as conjugated antibody were altered until an optimal value was achieved. The inter- and intra-assay coefficients of variation (CV) were determined from replicates of 23 samples and mean of CV of eleven repeats of control samples. Limit of detection (sensitivity) of the assay was determined from 4 blanks samples. The specificity of the assay was assessed by western immunoblots, nitrocellulose membrane incubation in conjugated Ab, followed by detection using enzyme substrate, of Hp containing milk samples and samples of commercial milk spiked with known concentrations of purified bovine Hp. One hundred and forty nine milk samples were collected from individual quarters of 41 dairy cows (Cochno Farm, University of Glasgow) into sterile 50ml tubes and stored at -20^{o}C until analysed. Composite milk samples were also obtained from each cow and all the samples were then analysed using the optimised sandwich ELISA. Somatic cell counts (SCC)from routine milk quality records were available for composite milk samples, Mann-Whitney test was performed to determine the difference in Hp concentration between subgroups of composite milk samples based on SCC (above and below 200 x10^3 cells/ml).

Results The optimised assay for Hp in milk was validated with specificity determined by western blot of spiked milk showing only one band at the Mr of the □-chain of Hp with spiked milk although milk with naturally elevated Hp showed additional higher Mr bands, possibly due to aggregation of Hp. Intra-assay CV was 2.5% (n=23), while inter-assay CV was 30.0 %(low control) and 28.5% (high control) and the limit of detection was determined as 0.2 µg/ml. Of the149 milk samples from individual quarters the median was 0.36 µg/ml with a range of <0.2 to 42 µg/ml. Using 2□g/ml as a cut-off value, 8% of samples had an elevated Hp concentration.

In the composite milk samples, there was no significant difference in Hp concentration between the subgroups with less or more than SCC of 200 x10^3 cells/ml. However a greater proportion of composite samples in the group with SCC > 200 x 10^3 cells/ml had elevated Hp (Figure 1) than in the group with SCC < 200 x10^3 cells/ml.

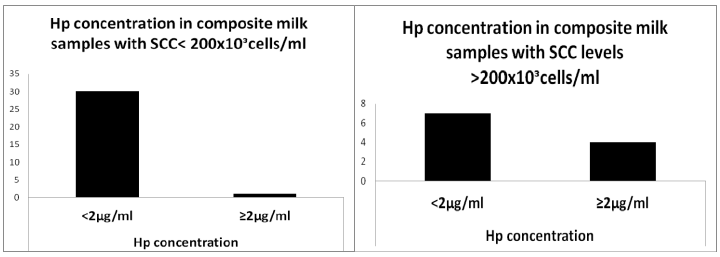

Figure 1 Hp in composite milk

Conclusion The assay developed to measure Hp concentrations in bovine milk was found to be sensitive, precise and specific. Determination of the concentration of Hp in milk from dairy cows on a West of Scotland farm showed that a wide range in concentration was present from <0.2 to 42 µg/ml and indicated 2 □g/ml as a possible cut-off point for elevated Hp. In composite milk samples the range was less, with a maximum of 5.5µg/ml, presumably due to the dilution effect of milk from non mastitis quarters on milk from quarters with mastitis present. This may also explain the observation of no significant difference between the Hp concentrations of the two different SCC subgroups although a higher percentage of the samples with high SCC also had elevated Hp concentrations. Selection of the cut-off point for the analytes may also explain this discrepancy. Further investigations are required to understand the reason for this divergence between these mediators of innate immunity in the bovine mammary gland.

Acknowledgement We acknowledge Mr Ian Cordner for helping with the milk sample collection.

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Mathematical modelling of the causal factors of keel bone fractures in free range laying hens using an *ex vivo* impact testing protocol

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Introduction The occurrence of keel bone fractures in laying hens is extremely high with up to 90% of birds in some flocks suffering breaks (Wilkins *et al.*, 2011). Recent studies have shown that two factors in particular – hazardous housing environments with many obstacles (e.g. perches, water lines, feeding troughs), and keel bone strength - synergized to determine the probability of fractures. It is thought that collisions with objects within the free range housing systems and the associated kinetic energy (KE) are the main cause of keel fractures. Low breast muscle mass in modern hybrid laying hens leaves the keel particularly vulnerable to impact (Fleming *et al.*, 2004).

Material and methods In this study 80 ex-vivo hens at ages 31 and 45 weeks, were exposed to impacts of defined energies using a drop-weight impact tester. Methods used in this study included palpation of the keel bone for old fractures followed by removal of the keel bone for visual inspection and grading of both the old and new impact fractures using a categorical measure of severity based on the extent and nature of damage; dual energy x-ray absorptiometry to assess bone mineral content (BMC) and bone mineral density (BMD); biomechanics to determine keel strength by 3-point bending and compression testing. Scores were related to bird factors and collision factors, to mathematically model (using MLwiN 2.15 (Rasbash *et al.*, 2009) a computer operated statistics programme) the causes of keel fractures in relation to key controlling elements and to facilitate prediction of their occurrence.

Results The results of this study indicate that as the potential energy (PE) (assumed to be equal to KE) increases the number and severity of the keel fractures also increases (Figure 1). The mathematical model generated two statistically significant factors that influence the probability of a fracture occurring – impact KE and keel surface BMD. This study revealed no statistical link between impact KE and fracture severity and also showed that bone strength within the normal range did not significantly influence fractures.

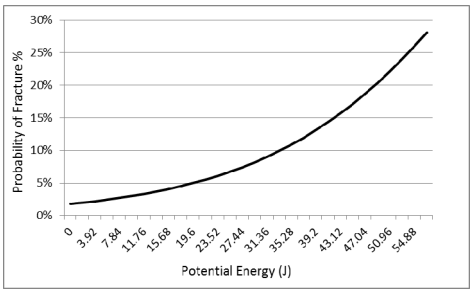

Figure 1 The relationship between calculated potential energy of the bird and the probability of a fracture.

Conclusion These studies conclude that modifications to housing systems may be a more effective intervention than improving keel bone strength in reducing fracture levels. The mathematical model could be used by the poultry industry to design a housing system in which a negligible percentage of the population suffer from keel bone fractures. Our study also provided means to assess influencing factors affecting susceptibility to fractures which can be used in future work.

Acknowledgements The authors wish to thank Noble Foods, Yew Tree Farm, The University of Bristol and the Wellcome Trust.

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A survey of attitudes and practices of UK veterinarians towards pain and the use of pain relieving drugs in breeding pigs

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Introduction During their lifetime breeding pigs may undergo pain as a consequence of diseases (such as mastitis or cystitis), injuries (from fighting or housing conditions) or during farrowing. Although a number of different anti-inflammatory drugs are now authorised in the UK for pigs for conditions involving pain and inflammation, their level of use by pig farmers and vets is not known. The aim of this survey study was to find out the level of analgesic use in breeding pigs by veterinarians and gauge their attitudes to pain in pigs.

Material and methods A list of 129 names and email addresses of vets in the UK listed as working with pigs were taken from the database of Pfizer Animal Health. A web-based survey was designed using Snap survey software (Snap Surveys Ltd), which was uploaded to Snap Webhost with a data-link function to automatically email an invitation with a link to the survey to the 129 vets, followed by a one week reminder. Three weeks later, the same email invitation with a link to the survey was sent to members of the Pig Veterinary Society. The survey consisted of 17 questions in five sections, including: 1) Type of veterinary work; 2) Pain relief drugs used or prescribed for pigs and for which conditions; 3) Scoring conditions for pain on a scale from zero (no pain) to ten (worst possible pain); 4) The top 3 most painful conditions in gilts and sows and the level of agreement with statements about pain; and 5) Demographic information. Survey responses were automatically stored in Snap Webhost and were downloaded and imported back into the Snap software. Results were then exported into Excel and descriptive statistics extracted using Genstat 11. Pain scores were analysed using a linear mixed model, with condition scored and gender as fixed effects.

Results A total of 37 completed questionnaires were received. Three respondents indicated that they did not treat pigs, leaving 34 (8 females, 26 males) complete survey responses. Time spent working with pigs ranged from 2 to 100 % and years of pig experience from 1 to 45 years. The most frequently used anti-inflammatory drug was meloxicam (used or prescribed by 88% of vets), followed by dexamethasone (68%), ketoprofen (53%) and flunixin (47%). Other drugs reported as being used as analgesics were azaperone, ketamine, butorphanol, buprenorphine and local anaesthetics.

Table 1 Descriptive statistics for pain scores given to conditions in gilts and sows

Painful condition	Mean±SEM	Median	Min	Max	Male mean±SEM	Female mean±SEM
Severe lameness (e.g. broken leg)	9.3±0.2	10	6	10	9.2±0.2	9.8±0.2
Infectious mastitis	7.2±0.2	7	4	9	7.2±0.3	7.3±0.5
Difficult farrowing	7.1±0.3	7	4	10	6.8±0.3	7.9±0.5
Lameness (minimal weight bearing)	7.1±0.3	8	2	10	7.5±0.3	5.8±0.8
Shoulder sore (open wound)	5.6±0.3	6	1	9	5.3±0.4	6.2±0.7
Respiratory disease	5.0±0.4	5	1	10	5.2±0.4	4.4±0.7
Gastrointestinal disease	4.4±0.3	5	1	10	4.3±0.4	4.5±0.5
Normal farrowing	4.3±0.34	5	0	8	3.9±0.4	5.6±0.8
Mean pain score	6.6±0.2	6	0	10	6.5±0.2	6.7±0.4

The pain scores given to specified conditions are listed in Table 1. The condition scored as most painful was severe lameness, where scores ranged from 6 to 10 and the least painful a normal farrowing, ranging from 0 to 8. Although there was no overall significant difference in pain score given by males and females, there was a significant condition x gender interaction ($p<0.001$). Males rated lameness and respiratory disease as being more painful compared to females; whilst females scored farrowing (normal or difficult) and shoulder sores higher than males. The majority of respondents (97%) either agreed or strongly agreed that pigs recover better with pain relief. Thirty two per cent agreed that it is difficult to recognise pain in pigs (50% disagreed and 18% neither agreed nor disagreed). For the statement asking respondents if they keep up-to-date with all the latest literature on pain relief for pigs, 56% agreed, 27% disagreed and 15% neither agreed nor disagreed. Vets were also asked if they thought pain relief drugs were too expensive for pig farmers to use regularly; 29% agreed, 41% disagreed and 24% neither agreed nor disagreed.

Conclusions Results show that veterinarians do use a variety of anti-inflammatory drugs to treat conditions involving pain and inflammation in gilts and sows and that there is almost complete agreement that pigs recover better when they are used. However, the level of pain assigned to different conditions varied considerably and with gender. This variation in perceptions of pain may limit the use of analgesics in breeding pigs. Other barriers to the increased use of pain relief may include recognising pain in pigs, keeping up-to-date with the latest literature on pain relief and the perception that these drugs are too expensive for farmers to use regularly. Understanding the attitudes and perceptions of pig vets may help target future education, training and research into pain in pigs.

Acknowledgements The authors are grateful to BBSRC and Pfizer for funding the study and the Pig Veterinary Society for sending out the survey to its members.

A study of survival times for cattle from 12 UK dairy herds that have been tested for *mycobacterium avium* subspecies *paratuberculosis*

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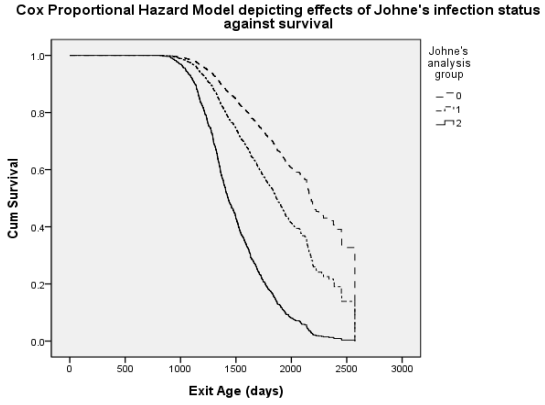
Introduction Johne's disease in cattle which is caused by infection of *mycobacterium avium* subspecies *paratuberculosis* (MAP) is of huge economic importance to the farming industry. Infection can lead to reductions in productivity and health of the animal leading to economic losses through decreased milk yield, lowered fertility and decreased carcass weight. Current testing methods do not have high enough specificity and selectivity to give a gold standard test and resulting in undetected infected cattle which can transmit infection within and between herds. Current literature states that early testing is not economically viable as few cattle test positive due to the long development process of the disease, and few reductions in productivity or survival have been noted. Literature agrees on adverse effects of the disease in the clinical phase, for example: lower milk yields, higher risk of lameness and mastitis, emaciation and chronic diarrhoea. This study set out to test whether an early positive Johne's test status using milk ELISA led to a shorter survival time for cattle (birth to exit date or last milk recording) and whether productivity levels were lower in the first lactation.

Material and methods A convenience sample of 3328 cattle from 12 herds was collated and anonymised from a data base at a milk testing laboratory in South West England, (Quality Milk Management Services, Wells, UK). A cow was deemed positive if it had two or more positive out of three consecutive test results, other animal disease statuses from test result combinations were negative, provisionally positive and uncertain. Specific data on confounding factors that could affect a cows survival time within a herd were collected. These factors were: clinical mastitis, lameness, average daily milk yield, subclinical mastitis (counts of over 200,000cells/ml and last somatic cell count. Cattle were sorted into three groups depending on their test history before three years old, i.e. tests were from the first lactation; the groups for analysis were: negative – all tests were negative, provisionally positive – tested positive only once, positive – tested positive at least twice within three consecutive tests. Both Kaplan-Meier and Cox Proportional Hazards Model were performed on the data to test if a positive Johne's status in the first lactation would lead to decreased production and a lowered survival time of these cattle within the herd. Factors that had a p value <0.005 were retained in the models and classed as significant.

Results The study found that 21.8% of the positive cattle in the data set were deemed positive for MAP infection in the first lactation. These positive cattle had a shorter mean survival time of 1310.1 days compared to the provisionally positive and negative cattle with respective survival times of 1388.9 and 1409.7 days. This pattern was observed for all of the 12 individual herds, further to this decrease in survival time, decreases in productivity were also recorded. Positive cattle on average gave around four litres less milk daily (25.594l) compared to the negative group (29.298l). Kaplan-Meier analysis confirmed the raw data findings about survival time reductions for the positive group. Cox Proportional Hazard Model found that all three Johne's test status groups ($p < 0.001$), mastitis ($p = 0.018$), average daily yield ($p < 0.001$) and Log of the last SCC ($p = 0.040$) were all significant when determining survival within the herd. Positive cattle were shown to be 5.027 and provisionally positive cattle 1.7687 times more likely to leave the herd early when compared to the negative group.



Cox Proportional Hazards Model with regards to MAP infection status (Johne's analysis group [0]negative, [1]provisionally positive and [2]positive). Exit age (days) is from birth to exit date for cattle which left the herd, and from birth to last milk recording date for those that were still alive at the end of the study.

Conclusion The study aimed to ascertain the effects of infection with MAP on a cow's survival time within a herd and on her productivity and health status. The study showed marked reductions in survival times of the positive cattle and that they were 5.027 times more likely to leave earlier than negative cattle. Mean survival times were found to be around 100 days lower for the positive animals compared to the negative and provisionally positive animals. Another important factor was the clear reduction in milk production within the first lactation between the different analysis groups. Positive cattle on average produced almost four litres less milk daily when compared to the negative cattle and approximately two litres less than the provisionally positive cattle. These factors show the importance and economic benefits of early testing for MAP infections in cattle. Early detection allows for better on farm management of infected cattle to reduce spread of the disease and reduce production losses on farms.

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Slaughterhouse sampling as part of Johne's Disease control in cattle in Scotland

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Introduction The PARABAN project is a Scotland-wide initiative to develop and deliver farm specific 'best practice' for the control of *Mycobacterium avium* ssp *paratuberculosis* (MAP) in cattle using Knowledge Exchange. There are a range of partners, including nine 'Champion Farms'. As part of this project scientists at the University of Glasgow are sampling all adult animals culled from the Champion Farms at slaughter or as fallen stock.

Material and methods The 'Champion Farms' each have a tailored monitoring and control programme devised with input from the farmer, their vet and PARABAN advisors, taking into account the history of the disease on the farm, the physical facilities available and farmer objectives. Culling decisions based on live animal test results were incorporated into each farm-specific programme to complement the programme already in place to maintain each herd.

With the co-operation of slaughterhouses throughout Scotland and the north of England a length of small intestine and a draining lymph node from near to the ileo-caeco-colic junction were collected from each animal, irrespective of whether a positive or negative result had been obtained from serum ELISA or faecal PCR in life. The tissues were then fixed in formalin and a single section from each was prepared for histopathology using Haematoxylin & Eosin (H&E) and Ziehl-Neelsen (ZN) stains. These were then examined for evidence of MAP infection.

Between October 2011 and June 2012 one hundred and sixty five animals were sampled in this fashion. These were provided by eight of the nine PARABAN farms. The disease pattern differed on each farm so the availability of animals suspected to be carrying MAP has been uneven across the farms.

Results The results were collated with those from live animal tests:

Table 1 Results summary

		Histopathology result			
		Positive	Suspicious	Negative	Total
Serum ELISA result	Positive	15	4	28	47
	Negative	2	7	109	118
	Total	17	11	137	165

A positive on histopathological examination was a sample where lesions typical of MAP infection were seen together with acid-fast organisms on the ZN section. Where there were no acid-fast organisms present but the H&E slide had evidence of MAP lesions the sample was described as 'suspicious'.

Conclusion At present the presence of MAP has been confirmed by histopathology on six of the eight PARABAN farms that have provided animals for sampling though Table 1 reveals the degree of imperfect agreement with serum ELISA results. This has indicated that despite extensive, and on some farms prolonged, efforts to control the disease at the level of the herd MAP is still a problem on these farms. As part of the PARABAN project this work is supporting and supplementing decision making on farm within a MAP control plan as it has influenced interpretation of the live animal test results relative to an expected decrease in MAP prevalence. Further samples have been collected in the 2012 – 2013 season.

Acknowledgements The authors would like to thank all the PARABAN partners for all assistance rendered, Richard Irvine and the staff of the post-mortem room and histopathology laboratory at the University of Glasgow, the slaughterhouse staff who have enabled sample collection and the Scottish Funding Council for funding the project.

Student self-assessment of their level of competence in generic skills and the contribution of the different final year components to the development of these

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Introduction In this study, we assessed students' opinion of their level of competence in generic skills and how much they thought the different components of their veterinary training contributed to those. We hypothesised that each of the different components would contribute differently to their self-assessed competence.

Material and methods This study surveyed 231 veterinary students after completion of their course before their final exam. Participation was voluntary and anonymous and ethical approval for the study was granted by the institution's Ethics and Welfare Committee; the response rate was 73%.We asked the participants to rate their competence in generic skills and their perception of the contributions that the different components of the final year made to those generic skills. This questionnaire was modified after the CETL-Zoology Research Skills Questionnaire (http://www.reading.ac.uk/cetl-aurs/).

Results The table below shows the self-assessed competency scores (mean ± SD, scale from 1 to 5, 1=not competent at all, 5= extremely competent) and self assessment score (mean ± SD) on 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)

General skills	Competence	Research project (RP)	Extramural studies (EMS)	Intramural studies (IMR)	Free study
Communication skills – writing	3.97 ± 0.63	3.36 ± 0.63	2.64 ± 0.75	3.37 ± 0.72	2.42 ± 0.71
Communication skills – oral	4.03 ± 0.66	2.50 ± 0.67	3.84 ± 0.47	3.82 ± 0.45	2.18 ± 0.50
Information gathering	3.78 ± 0.68	3.57 ± 0.57	2.90 ± 0.67	3.35 ± 0.68	3.05 ± 0.78
Information evaluation	3.52 ± 0.64	3.48 ± 0.64	2.82 ± 0.70	3.25 ± 0.71	2.89 ± 0.73
Statistics	2.46 ± 0.95	3.39 ± 0.80	2.05 ± 0.35	2.10 ± 0.43	2.13 ± 0.42
Teamwork	4.38 ± 0.68	2.22 ± 0.57	3.59 ± 0.59	3.83 ± 0.46	2.24 ± 0.53
Ability to work independently	4.24 ± 0.71	3.40 ± 0.72	3.10 ± 0.68	3.38 ± 0.67	3.40 ± 0.77
Management skills	3.45 ± 0.76	2.80 ± 0.81	2.88 ± 0.71	3.20 ± 0.76	2.66 ± 0.80
Time management skills	3.96 ± 0.78	3.24 ± 0.74	3.10 ± 0.76	3.65 ± 0.56	3.17 ± 0.81
Problem-solving	3.74 ± 059	2.82 ± 0.73	3.34 ± 0.68	3.66 ± 0.57	2.87 ± 0.72
Critical thinking	3.61 ± 0.64	3.05 ± 0.71	3.24 ± 0.70	3.53 ± 0.62	2.89 ± 0.69
Designing experiments	2.87 ± 0.93	3.32 ± 0.74	2.14 ± 0.49	2.15 ± 0.53	2.17 ± 0.49

Conclusions With the exception of time management the free study period in the final period appears to contribute the least to any of the competencies. The clinical component contribute the most to communication skills regardless of the location (university based intramural studies (IMR) or practice based extramural studies (EMS)), however IMR contributed more to any of the other skills compared to EMS. Students judged themselves the least competent in research-related skills, such as study design and statistics. This is somewhat worrying, since knowledge about these is essential for critically evaluating research reports, which in turn is essential for evidence based practice. The contribution of the RP in relation to the other components of the final part of their course was judged the greatest for these skills, together with information gathering and evaluation (also essential for evidence based practice). This supports the notion that students would benefit from a strengthened research component leading to enhancement of the skills linked to evidence generation and evaluation that are essential to reflective practitioners

Acknowledgements The authors would also like to thank all the participating students.
The authors would like to thank Dr Anne Crook and staff at the Centre for Excellence in Teaching and Learning in Applied Undergraduate Research Skills at the University of Reading, UK, for their permission to modify and use part of their Research Skills Questionnaire.

An evaluation of a bovine clinical case simulation software programme in veterinary education

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The current undergraduate veterinary course is being re-structured to increase vertical and horizontal integration of clinical content across the 5 year curriculum. As part of the re-structure there will be an increase in student centred learning activities to augment traditional didactic teaching. Student centred learning activities (practical classes, self-directed learning tasks, small group teaching etc) aim to provide a constructivist learning environment (Tynjälä, 1999). Appropriate E-learning tools must be utilised to maximise cost and staff time efficiency during this re-structure. 'Crook-Moo'® is a simulation clinical software program which allows students to practice the methodology of clinical examination, differential diagnosis and appropriate laboratory testing of sick bovine animals (Mansell and Beggs, 2012). Case material (textual and audio-visual components) can be entered by an expert user; each case can then be worked up by a student through free-text entry. When a student has completed a case, automated formative feedback is provided, highlighting areas of the clinical examination, laboratory testing or differential diagnoses that were inappropriate or missed.

The aim of the current study was to evaluate the potential use of the program as a component of the new clinical phase of the undergraduate veterinary medicine course at the University of Glasgow.

A pilot session with 25 4th year undergraduate students was undertaken, followed immediately by completion of a student questionnaire by each participant (Lewis and Whitlock, 2003). Four areas were specifically addressed (generation of constructivist learning environment, usefulness of automated feedback, perceived value and preferred method of access, use in a professional examination) followed by the opportunity for free text entry.
117/125 responses to statements assessing successful generation of a constructive learning environment were in agreement. 46/50 responses to statements on automated feedback agreed they were useful. All participants agreed that more access to more cases would be beneficial; 22/25 disagreed that restricted access during tutorials would be the best way to achieve this. 10/25 disagreed that the program could be used as part of a professional exam. Free text entries were consistent with these responses.

The student responses indicated that the program delivers a /high quality constructivist learning environment, that the automated feedback is useful but can be improved, that students would prefer open access, and that there were mixed feelings regarding potential use in a professional examination. Several specific areas of improvement of the program were identified.

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Learning from experts: towards a unified teaching approach for equine lameness examinations

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Introduction Lameness assessment is a day one skill expected from veterinary graduates (RCVS, 2010). This is not without reason: the first step to correctly diagnosing and treating the cause for lameness is to identify the affected limb. However, even experienced assessors show disagreement on the task (Keegan *et al.*, 2010). Part of the problem might be the absence of standardised procedures describing what precisely to look for in a lame horse; although assessment of head and hindquarters movement are popular, books often quote a vast amount of potential lameness pointers and only reflect the view of a single author.
The aim of the present study was to examine lameness pointers chosen by a large group of experts to investigate whether a general assessment strategy exists that can be taught to students.

Material and methods Gaze data of 26 equine lameness experts from 10 high-ranking UK veterinary institutions were recorded using an eyetracker (Tobii T60). Experts evaluated video recordings of horses during trot on a straight line and in a circle. For each participant, gaze data were manually mapped onto 16 body regions of each horse. Percentage viewing time was calculated for the different movement directions and data were averaged across all 14 horses, discriminated by the participant's classification as i) sound, ii) the most affected limb being a forelimb or iii) the most affected limb being a hind limb. Data distributions were examined across all participants and dendrograms were created to examine similarity in viewing approach.
Participants detailed the features they chose when assessing horses for lameness on straight and circle in a questionnaire. The number of participants naming each feature was counted and features ranked. For the popular assessment of head and hind quarters movement on the straight line, the exact feature specifying the lame leg was extracted from the answer sheets (where available) and again counted and ranked across participants.

Results Results from eyetracking showed that viewing times and areas of interest were largely consistent across experts during assessment on the straight, most time being spent looking at the head and upper pelvis. In contrast, viewing times and areas of interest differed widely between participants when assessing horses on the circle. Although dendrograms revealed five to six clusters of similar strategy for assessment on the circle, most experts showed a very unique strategy.
Results from questionnaires described 16 features on the straight and 17 features on the circle to identify forelimb lameness. For hind limb lameness, 24 features were described for assessment on the straight and 35 features on the circle. Descriptions how to identify the lame limb from head and hindquarters movement were inconsistent.

Conclusions While assessment of head and hind quarters will result in a strong foundation when teaching lameness examinations to students, at present it remains unclear which strategy to teach for lameness examination on the circle. Better understanding of subjective evaluation of horses is essential to progress in this area and to providing the foundations for the development of a more unified approach.

Acknowledgements We would like to thank the Animal Care Trust (ACT) for funding this study and Jon Ward (Acuity ETS) for generous support with the eyetracking equipment. We would like to thank all our expert participants for their time: Andy Bathe, Bruce Bladon, Dave Bolt, John Burford, Sue Dyson, Sarah Freeman, Mark Georgetti, Luise Harrison, Marcus Head, Benoit Henrickx, Fran James, Tim Mair, Rob Pilsworth, Sarah Powell, Chris Rea, Ceri Sherlock, Ellen Singer, Charlie Smith, Merry Smith, Roger Smith, Sarah Boys-Smith, Sarah Taylor, Mark Tunstill, Anna Turk, Martin Weaver and Tom Witte. Further thanks to Anna Liedtke, Vicky Robain, Emil Olsen and all horse owners involved in this study.

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Livestock science into practice: demonstrating the value of research to students

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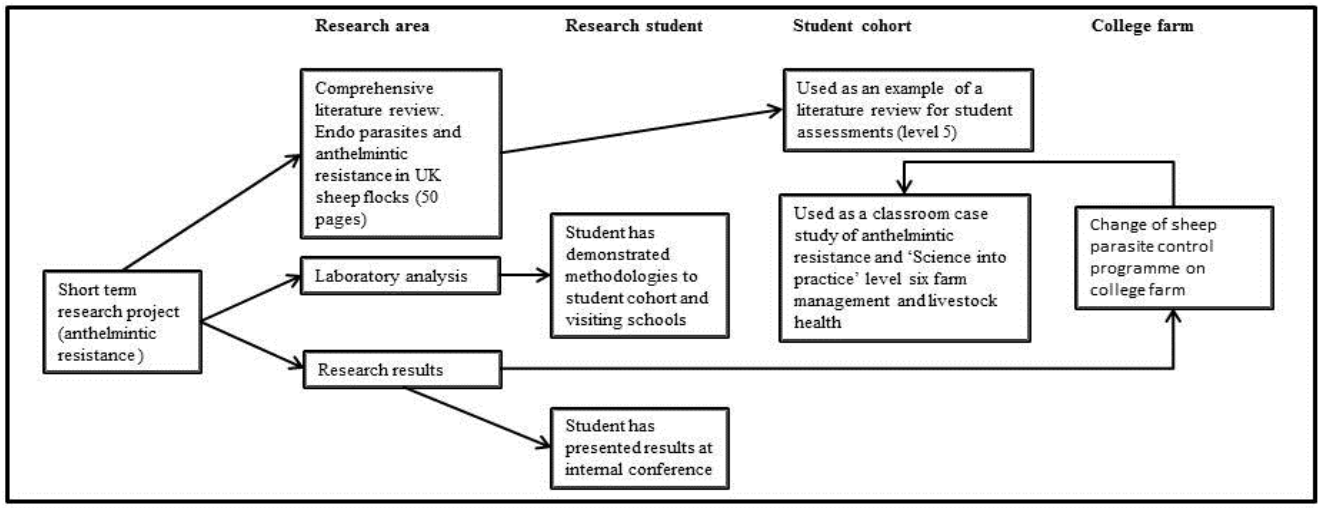
Introduction Many Higher Education practitioners are currently discussing the issues of integrating research into teaching (for examples see Johnson *et al.,* 2010, Cabral and Huet, 2011, and King *et al.*, 2011) in a variety of academic disciplines. Anthelmintic resistance in UK sheep flocks is well documented (Taylor *et al*, 2009) and the primary aim of the project was to gauge the level of resistance to the commonly used (for this farm) benzimidazole (BZ) and macrocyclic lactones (ML) drenches. This paper outlines a Biotechnology and Biological Sciences Research Council (BBSRC) funded student project that investigated sheep endo-parasites and anthelmintic resistance in the Writtle College sheep flock, and how the project outcome was linked into undergraduate teaching and learning and management of the college flock.

Material and methods Utilising industry standard methods (see Stubbings and Dodgson, 2012) for faecal sampling, faecal egg counts (FEC's) were calculated for the 2012 crop of 140 lambs, both before and after routine treatment for internal parasites. Stubbings and Dodgson (2012) suggest that anything under a 95% reduction suggests resistance is present.

Results Eggs and larvae were identified from four species of internal parasite, *Haemonchus*, *Nematodirus, Trichuris* and *Ostertagia,* high levels of coccidia oocysts were also observed. The reduction in FEC's for the BZ drench was 55.6% against a 77.8% reduction for the ML drench. The results were then combined with the literature review as a bound document for the college library and then integrated into level six livestock production modules. A schematic of this integration is illustrated in Figure 1.

Figure 1 Integration of student project into teaching and livestock management



Discussion BBSRC funded student projects are designed to maximise the student participants' research experience. The study of anthelmintic resistance in a college sheep flock presented opportunities for skills development in areas including experimental design, field data collection, use of industry standard techniques, literature searching, academic writing skills, basic data handling and analysis and microscopy alongside the more practical aspects of sheep husbandry. Anecdotal evidence from Course Scheme Review Committees and Staff Student Liaison meetings suggests that Agriculture students often do not see the relevance of research and how it is applicable at farm level. The successful use of a simple research project such as this demonstrating 'science into practice' resulting in changes to farm management has proven to be invaluable for both students and tutors alike. Further scientific issues regarding sheep production currently being investigated using the process outlined in Figure 1 include maternal bonding in ewes and lambs, colostrum quality and its influence on growth rates and linking morphometric measurements into carcase classification.

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"Research is a waste of time.....or maybe not? "- Clinical students' perceptions and research output of an embedded research project

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Introduction Research training as a part of clinical education serves two purposes: it has to equip the future clinician with the background to practise evidence-based veterinary medicine and the future researcher with the background to take up a research career path. "Background" in this context not only means research-related skills, but has to include a positive attitude towards research itself and its output in terms of clinical papers.
In this study, we assessed students' attitude towards and research output from an eight week research project embedded in the final year of the veterinary curriculum and the factors influencing these measures. We hypothesised that these measures would be associated with perceived quality of supervision, perceived difficulty of the project, student effort, career plans and their attitude to the project before it commenced.

Material and methods At the authors' institution all students enrolled in the clinical programme conduct an eight week research project (RP) in the final part of their course. The topic of the RP is largely student-determined. They are allocated an appropriate supervisor, who can be a full-time clinician, a full-time researcher or a clinician/researcher, who helps them turn their ideas into a methodologically sound and ethically acceptable individual investigation. At the end of their allocated research time, the students produce a 5000-word written report, including a literature review and a presentation of their findings, and at the end of the year they undergo 30 minute oral examination in order to defend their project in front of two examiners.
This study surveyed 231 students after completion of this RP. Participation was voluntary and anonymous and ethical approval for the study was granted by the institution's Ethics and Welfare Committee. A questionnaire was handed out to the students before the last lecture of their course, after a short oral presentation explaining the reason for the study; the response rate was 73%.
The questionnaire consisted of 30 questions, comprising ten demographic questions, eight questions in five point Likert-scale format, one ranking question and 11 categorical questions). Each question allowed for open comments. Students were asked about their attitude towards research before and after they have conducted their RP, the perceived difficulty, the perceived quality of supervision , their career plans and publications that have arisen from the RP. Data were analysed using regression models.

Results
Student attitude Almost half of the sampled students had a negative or indifferent attitude before they conducted their RP, whereas 75% had a positive attitude afterwards. Post-project attitude was moderately correlated to perceived relevance to the profession, perceived difficulty, perceived quality of supervision and perceived supervisor enthusiasm, but not supervisor-student contact time or e-mail response time. Students who were planning to pursue a career in referral level clinical work, research or education had significantly higher post-project attitude scores than students who were planning to work in first opinion practice in the long term. Multiple regression analyses with student attitude scores as the dependent variable, and perception of supervision, perceived difficulty and previous attitude score as factors, resulted in an adjusted R^2 value of 0.302, with standardised coefficients of B=0.3 for perceived difficulty, B=0.29 for quality of supervision and B=0.18 for previous attitude. These results indicate that 30% of the variation in student post-project attitude can be explained by these three factors.
Publication output Fifteen (8.9%) students had submitted an abstract to a conference at the time of the survey, ten (16.9%) students were planning to submit an abstract to a conference, four (2.4%) students had submitted a manuscript to a journal and 34 (20%) students were planning to submit a manuscript to a journal. There was a significant difference in perceived quality of supervision between students who had already submitted a publication/abstract or were planning to do so and the rest of the students. Students who had help from their supervisor with study design ($p=0.002$) and data collection ($p=0.05$), but not data analysis or write-up, were more likely to submit or plan to submit a paper/abstract. Students were more likely to submit or plan to submit a paper/abstract if their supervisor encouraged them ($p<0.001$), but not less likely to submit if their supervisor discouraged them ($p=0.23$). Role of the supervisor had no significant effect on publication, but there was a strong trend for students who had submitted or were planning to submit a paper/abstract to have a supervisor who worked as a combined clinician and researcher.

Conclusions Student attitude and experience with a compulsory, embedded research project were clearly influenced by multiple factors, the weightings of each factor varying between individuals: previous attitudes, perceived quality of supervision and perceived relevance to their professional future. To make a short-term research project a beneficial learning experience for the students and result in a worth-while publication output it has to be embedded within a research-strong curriculum, within a framework created by research enthusiastic faculty.

Acknowledgements The authors would also like to thank all the participating students.

Peer assisted learning: a novel teaching method for canine castration

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Introduction Opportunities for veterinary students to practise neutering procedures on EMS placements and rotations vary significantly but are considered essential 'Day One' competences for veterinary graduates (RCVS, 2006). Following student requests for additional neutering experience, a peer-assisted learning (PAL) programme was trialled, enabling 4th year veterinary students (peer tutors) to tutor 2nd year students (learners) on performing a castration on canine cadavers. The benefits of PAL in other scenarios have been widely published (Field *et al.*, 2007, Wadoodi and Crosby, 2002). The aim of this study was to investigate if PAL is perceived as a beneficial teaching method to increase peer tutor confidence and competence.

Material and methods Twenty-four 4th year peer tutors, randomly selected from a group of volunteers, attended a 2-hour workshop on basic teaching skills. A revision opportunity for the castration procedure was provided on cadaver specimens. The 24 peer tutors then trained 105 2nd year learners, during a 3-hour practical class of 4*45 min sessions, with an approximate 1:2 tutor-learner ratio. Peer tutors (24) and learners (105) completed pre and post activity questionnaires. Local ethical approval for the study and informed participant consent was obtained. A 100mm visual analogue scale (VAS) was used to measure responses to a series of statements, in addition to free-text and single response questions. Second year practical exam performance was assessed. Statistical analyses comprising paired T-test were performed in Minitab v16. Thematic analysis was used for free text comments.

Results Peer tutor perceived confidence and competence in canine castration, measured on a 100mm VAS, increased from a mean of 41.8 (4.1 S.E.M) to 78.4 (2.0 S.E.M) and 40.7 (4.1 S.E.M) to 73.4 (2.0 S.E.M) respectively ($p<0.005$), following participation in PAL. In addition to increased competence in performing castrations, communication and leadership skills as well as improved knowledge were considered important benefits of PAL. Peer learners appreciated their senior colleagues as peer tutors, rating them as extremely well informed (95.1 (0.7 S.E.M)) and gave the session a recommendation rating of 96.3 (0.6 S.E.M) to other students. Although peer tutors (21/24) and learners (52/105) mentioned 'lack of knowledge' and concerns over teaching the 'correct' technique as potential issues, learner performance in practical examinations was equivalent to other skills taught using more traditional teaching methods. Peer learners cited greater approachability (84/105), higher tutor-learner ratio (48/105) and increased empathy with their situation (51/105) as the main advantages of PAL over more traditional methods.

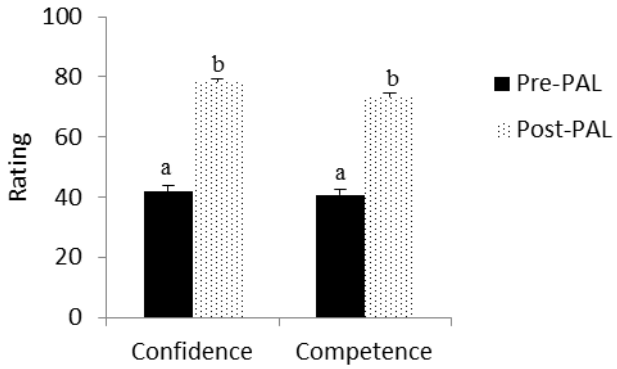

Figure 1 Mean rating of Peer Tutor confidence and competence in canine castration before (pre) and after (post) Peer Assisted Learning (PAL) programme (n=24)
Different subscripts denote statistical significance $p<0.005$

Conclusion This study supports the use of PAL to improve exposure and perceived confidence and competence in a 'Day One' skill for veterinary students. Improving confidence and competence in surgical skills may increase the chances for students to benefit from opportunities during EMS placements.

Acknowledgements The authors wish to thank the students who participated in this study as well as Dr Richard Lea and Erica Gummery for their logistical support.

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The effect of breed and herd size on the housing system in UK dairy farms

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Introduction Sustainable intensification of UK farming, driven by population, environmental and economic pressures requires an increase of efficiency within food production systems. Simultaneously, the phasing out of EU milk quotas brings opportunities for expansion to meet greater demands for dairy products (Foresight 2011). Annual UK statistics show that average yields and herd sizes continue to increase. Milking more cows per herd and attaining higher levels of production can increase efficiency and improve economies of scale. The productivity of high yielding cows may not be sustained by grass based systems alone. The aim of this work was to evaluate the prevalence of indoor feeding systems and assess relationships among breed, herd size, and housing system.

Material and methods A survey of UK dairy farmers was jointly conducted by Cattle Information Service and SRUC. A total of 828 responses were collated and clustered into breed, herd size, and housing systems groups based on responses. The groups that were generated were: 1) Those that farmed traditionally, grazing their cows for 24hrs in better weather (n=247), 2) Respondents that fed all milking cows inside each day, for part of the day during the summer (n=341) and, 3) Respondents that housed cows for 24hrs a day during the summer (n=62). Variables analysed were herd size, farm location and breed type by frequency of response using boxplot in Minitab (v16).

Results The survey represented 6% of dairy production holdings in the UK. The herds contained 139,467 cows which represented 8% of the 1.8 million adult dairy cows in the UK in 2011(DairyCo 2012). Holstein, Holstein-Fresian, Fresian, and Ayrshire breeds were found on 92% of farms and accounted for 94% of all cows within the survey. Of all respondents the average herd size was 174 (sd. =117.9) cows, above the UK figure of 117, whilst the mode for herd size was 130.

Over two thirds of the farmers that were involved in the survey have adopted some form of indoor summer feeding system and only 30% of all respondents farmed in a traditional manner, grazing their cows in better weather. Forty-one percent of all respondents indicated they were feeding all their milking cows inside during the summer for part of the day. Those that didn't feed all animals inside tended to indoor feed specific cows such as high yielders or those in early lactation. Approximately 7% of all respondents have adopted a system in which their cows are fed and housed inside all year.

Figure 1 illustrates that as herd size increases, farmers tend to move from a more traditional grazing system towards continuously housing their cows during the summer. This could indicate that increased herd sizes in low input systems are not as profitable as more intensive systems that begin to become economical at larger scales.

Results indicated that larger herds predominantly use Holstein and Holstein Fresian breeds. Of those respondents that fed and housed their cows inside during the summer, 98% milked Holstein or Holstein-Fresian cows. Results also highlighted the predominance of dairy farms in western and northerly regions of the UK.

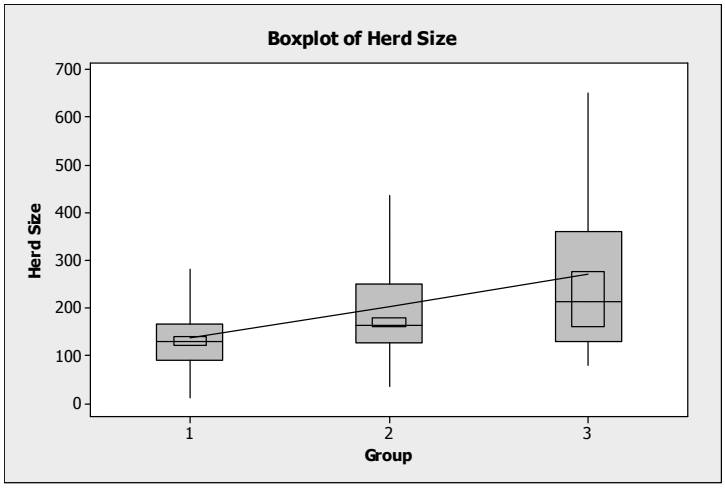

Figure 1 Boxplot of herd sizes by system group

Conclusions Traditional all summer grazing does not seem to be the predominant system in UK dairy farms. Other systems such as, all year round indoor feeding and continuous housing seem to be becoming more common. On average, herd sizes are larger in more intensive systems.

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Comparison of seasonal effects on the chemical composition of *Panicum maximum* and *Moringa oleifera* mixtures

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Introduction During the dry season, nutritive values of grasses decrease and may become scarce at this period, leading to negative effects on the animals. Under this condition, introduction of tree legume will be of great importance. Tree legumes are capable of yielding high protein forage during critical dry periods of the year when both quality and quantity of pasture grasses are limited (D'Mello, 1992). The objective of this study was to examine the effects of forage proportion and season on the chemical composition of *Panicum maximum* and *Moringa oleifera*.

Material and methods During season of the year (rainy and dry season), *Moringa oleifera* (Moringa) leaves were harvested from multipurpose tree garden of the Federal University of Agriculture, Abeokuta, Nigeria. *Panicum maximum* (Panicum) was also harvested 15cm above ground level at 6weeks after cut back. The two forages were subsequently mixed together at different proportion and season. Samples (300 g) were taken from each treatment and oven-dried at 65 ^{0}C to a constant weight. The dried foliage samples were hammer-milled through a 1mm sieve and used to analyse crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Crude protein and ash were analysed according to the standard methods of AOAC (2000). The NDF, ADF and ADL were determined according to Van Soest *et al.* (1991). The design of this study consisted of 2 × 5 factorial arrangement of 2 seasons (rainy and dry season) with 5 forage mixtures (100% Moringa, 100% Panicum, 50% Moringa + 50% Panicum, 70% Moringa + 30% Panicum, and 30% Moringa+70% Panicum). Each treatment was replicated four times. Data collected were subjected to the analysis of variance (ANOVA) using SAS (2002) package.

Results The dry matter content of 30% Moringa + 70% Panicum at rainy season was significantly higher ($P < 0\ 05$) than other treatments. There were significant differences ($P < 0.05$) in the CP contents of the forage proportion at different seasons with 50% Moringa + 50% Panicum at rainy season recording the highest ($P < 0.05$) value of 144.3 g/kg DM. The ash or inorganic components was higher in 100% Moringa at dry season than in other treatments. Panicum at 100% during dry season had highest ($P < 0.05$) NDF value (659.6 g/kg DM).

Table 1 Effect of interaction of forage proportion and season on the chemical composition of *Panicum* and *Moringa* (g/kg DM*)*.

Treatments	DM	CP	ASH	NDF	ADF	ADL
Rainy season						
100 % Moringa	925^{ab}	102.3^{bcd}	85.4^{cde}	406.8^{d}	252.0^{cd}	74.2^{bcd}
100 % Panicum	915^{ab}	84.10^{cd}	81.4^{de}	627.0^{ab}	374.7^{a}	71.8^{cd}
50 % Moringa + 50 % Panicum	910^{ab}	144.3^{a}	71.4^{e}	563.8^{b}	300.1^{bc}	61.1^{d}
70 % Moringa + 30 % Panicum	912^{ab}	119.3^{abc}	99.4^{abc}	628.7^{ab}	341.9^{ab}	129.0^{a}
30 % Moringa + 70 % Panicum	930^{a}	124.3^{ab}	78.3^{de}	585.3^{b}	241.6^{def}	69.7^{cd}
Dry season						
100 % Moringa	915^{ab}	114.8^{abc}	114.4^{a}	336.3^{e}	189.1^{f}	93.9^{abcd}
100 % Panicum	900^{bc}	78.4^{d}	94.4^{bcd}	659.6^{a}	267.2^{cd}	105.3^{abcd}
50 % Moringa + 50 % Panicum	885^{c}	110.9^{abcd}	79.2^{de}	493.7^{c}	237.8^{def}	119.9^{ab}
70 % Moringa + 30 % Panicum	910^{ab}	84.5^{cd}	77.4^{de}	570.4^{b}	252.0^{cde}	110.1^{abc}
30 % Moringa + 70 % Panicum	900^{bc}	132.3^{ab}	103.5^{ab}	428.9^{d}	195.9^{ef}	75.0^{bcd}
SEM	8.8	8.8	4.5	19.9	14.7	11.5

Conclusions The above results showed that the two forage species at different proportions, had better quality than the sole grass, 100% Panicum in terms of chemical composition for both rainy and dry season. This alluded to the fact that Moringa yielded higher protein forage than the grass, and it maintained its nutrients throughout the seasons of the year.

Acknowledgement The authors are grateful for the assistance rendered by the Mr. John Idehen during the laboratory analyses.

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Investigating image analysis techniques for predicting intramuscular fat percentage from computed tomography reference scanning (2 dimensional information) in Texel Lambs

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Introduction One of the main drivers influencing the decisions made at point of purchase regarding lamb is the level of visible fat, with lamb often being perceived as fatty. In different livestock species meat eating quality (MEQ) traits such as flavour, tenderness and juiciness are known to be linked to fat levels. This association of MEQ attributes and fat levels are largely due to the positive association with intramuscular fat (IMF) (Savell and Cross, 1988). Consumer preference for leaner meat, coupled with the meat processing industries preference for a reduction in carcass fat have led to continued intense selection for lean growth and reduced fatness. IMF and carcass fat are genetically positively correlated (r_g 0.62) (Lorentzen and Vangen, 2011). Given the genetic relationship between IMF and carcass fat and the possible impact on MEQ, it has been recognised that there is a need to have independent measures for carcass fat and IMF enabling selection against this positive genetic correlation. The objective of this study is to identify predictors for IMF based on muscle density measured by computer tomography (CT).

Material and methods Data from Pure-bred Texel lambs (n=449) of both sexes (female n=246 and entire males n=203) were produced over three years (2003, 2004 and 2009). The lambs were reared to weaning as singles (n=245), twins (n=177) or artificially hand reared (n=27). Age at slaughter ranged from 96d to 234d (mean 148.7d, sd 21.62), weight at slaughter ranged from 19.7kg to 52.2kg (mean 34.3kg, sd 5.5kg). All lambs were CT scanned at finishing. Two-dimensional (2D) cross-sectional scans were taken at 3 defined anatomical positions: through the top of the leg at the ischium bone (ISC), the loin at the fifth lumbar vertebra (LV5); and through the chest at the 8th thoracic vertebra (TV8). Image analyses were performed to separate carcass from non-carcass tissues (Glasbey and Young, 2002) and the density of each pixel within the carcass portion of each image was measured and allocated to fat, muscle or bone, according to pre-determined density thresholds using STAR software (Mann et al, 2003). Areas (mm^2) and average densities (HU) of each tissue were calculated, as well as standard deviations for the density values of all pixels allocated to each tissue. *M. Longissimus lumborum* samples were removed post-slaughter, IMF extracted using petroleum ether in a Soxhlet extraction, and results averaged. The CT reference scan data included average muscle density (MD), average fat density (FD), standard deviation of muscle density (MSD) and standard deviation of fat density (FSD) from the segmented carcass portions of the reference scans (ISC, LV5 and TV8). Average soft tissue density (combining pixels allocated as fat and muscle) (STD) and standard deviation of soft tissue density (STSD) were also included, taken from LV5 only in models which did not include information from the ISC or TV8 scans.

Results Chemically extracted IMF content ranged from 0.27% to 6.17% (mean 1.48%, sd 0.74). Muscle density (MD) alone accounted for a moderate amount of the variation in IMF when information from all three reference scans were included with less of the variation being explained when MD in the LV5 scan alone, was included in the model. The best overall model, using either the reference scan information or LV5 only, is model E (Table 1), which included both muscle and fat densities and standard deviation of densities. The novel STD measure showed no improvement in the accuracy of prediction and no improvement in accuracy when the STSD was included in the model. STD in the TV8 and ISC was not calculated and therefore no comparison between reference and LV5 performed.

Table 1 Linear regression models between IMF and CT tissue density parameters, with adjusted coefficient of determination (R^2) and residual mean square error (RMSE)

	Ref[1]		LV5[2]	
Model	R^2	RMSE	R^2	RMSE
A – MD	0.38	0.55	0.24	0.61
B – MD+MSD	0.55	0.47	0.51	0.49
C – FD	0.39	0.55	0.04	0.69
D – FD+FSD	0.45	0.52	0.13	0.65
E – MD+MSD+FD+FSD	0.63	0.43	0.55	0.47
F – STD	-	-	0.53	0.481
G – STD+STSD	-	-	0.53	0.479

[1]Data from 3 reference scans. [2]Data from the LV5 reference scan only

Conclusion Muscle density alone accounted for a moderate amount of the variation in IMF when information from all three reference scans were included, with less of the variation being explained when muscle density in the LV5 scan alone was included in the model.
The accuracy of prediction is also increased as more variables are included in the model, with the best overall model, using both the reference scan information or LV5 only, being model E, which included both muscle and fat densities and standard deviations of densities. Information from the ISC and TV8 improves the prediction when included in the models and would suggest that there is valuable information in these scans increasing the accuracy of prediction.

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The effects of processing and storage duration on the fibre composition of ear pod tree *Enterolobium Cyclocarpum* seeds

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Introduction The nutritional problems confronting livestock owners in Africa especially during the dry season cannot be overemphasized. In extreme cases, provision of supplementary feed to animals during this period is always a difficult task (Arigbede *et al.,* 2006). Multipurpose tree species have been found to be very useful as dry season feed resources for livestock especially at the end of the dry season when grass plants are scarce and its nutritional level is low. These tree species are actively producing new foliage and seeds (Strange, 1980) at this period of the year. Unconventional protein sources from browse trees, legumes and seeds of trees were reported to be sustainable for ruminants in the tropics (Bamikole *et al.,* 2004). Evaluation of indigenous multipurpose tree species (MPTS) is therefore one of the most significant interventions in addressing the seasonal shortages in the quality and quantity of forages from natural pasture. This study therefore investigates the effects of processing, storage method and duration of storage on the fibre composition of *Enterelobium cyclocarpum* as feed supplements.

Material and methods The *Enterolobium cyclocarpum* seeds were sourced from the seedlot of the Department of Pasture and Range Management. Three processing methods were adopted which include toasting, pelleting and unprocessed (control). 10kg of *E. cyclocarpum* seeds were divided into three equal parts of 3.33kg and each of the fractions was subjected to these processing methods. However, these seeds were completely dehydrated by drying before been processed. The experimental design was 3×3 factorial i.e. three processing methods (control, toasting and pelleting) and three storage duration (4, 8 and 12 weeks). Data collected were subjected to 2-way analysis of variance according to SAS 2002. Significant means was separated using Duncan and Multiple Range test (Duncan 1955).

Results Duration of storage at week four and twelve had highest level of ADF and NDF while the levels of hemicelluloses and cellulose were considerably high ($P<0.05$) at week four. The ADL content increased along with the duration of storage. Pelleted method of processing produced highest ($P<0.05$) ADF, ADL and cellulose contents as depicted in the table.

Table 1 Effects of storage duration and processing methods on fibre composition of *E. Cyclocarpum* seeds (g/kg)

Duration	ADF	NDF	ADL	HEM	CEL
4weeks	269.9^{a}	484.3^{a}	150.2^{c}	214.4^{a}	119.8^{a}
8weeks	277.7^{b}	421.8^{b}	176.5^{b}	144.1^{c}	101.2^{b}
12weeks	291.8^{a}	477.3^{a}	192.3^{a}	185.5^{b}	99.50^{b}
SEM	0.52	0.79	0.64	1.02	6.30
Methods					
Pelleted	296.7^{a}	461.5^{ab}	178.4^{a}	164.8^{b}	118.3^{a}
Toasted	267.9^{b}	468.4^{a}	178.5^{a}	200.5^{a}	89.4^{b}
Unprocessed	274.8^{b}	453.5^{b}	162.2^{b}	178.7^{b}	112.6^{a}
SEM	0.49	0.96	0.69	1.12	0.62

Conclusions These results show that storing *E. Cyclocarpum* for a long period did not change the fibre fractions to an unacceptable level to the detriment of the animals. The toasted form of processing had more values to the seeds in terms of the increase observed in the neutral detergent fibre produced.

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Preliminary assessment of the nutritive value of corn cob fermented with single and mixed fungi cultures *in vitro*

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Introduction Large quantities of agricultural and agro-industrial by-products (AIBPs) such as corn cob, rice bran, cassava peels, etc are produced and most of them are regarded as waste. However, these products considered as waste can potentially be converted into various value-added products including biofuels, chemicals, and substrates of fermentation to produce improved animal feed stuffs through lignocellulose biotechnology. This preliminary, *in vitro* study therefore assessed the nutritive value of corn cob fermented with single and mixed cultures of selected fungi species.

Material and methods Corn cob (CC) were collected from designated centres, sundried (≤ 90% DM) and then, 200g of milled (1.0 mm sieve) CC was measured into six different petri dishes each and replicated four times. The measured cowpea husks were moistened (15mls/10g) with distilled water (Adedire *et al*, 2012) and the spore solutions of respective fungi species were added at the rate of 20mls/100g 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. oligosporu*s (TRH) (Adedire *et a*l, 2012) and then mixed together thoroughly. The crop residues were allowed to ferment anaerobically for 72 hours. Triplicate samples of fermented and unfermented products were analysed to determine crude protein, ether extract, crude fibre, ash on dry mater basis according to A.O.A.C (2000) methods; neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) according to Goering and Van Soest (1970); hemicelluloses as the difference between NDF and ADF; cellulose as the difference between ADF and ADL and non fibrous carbohydrates (NFC) using the equation: NFC = 100 – (CP + EE + ASH + NDF) according to Calsamiglia *et a*l 1995. Data obtained were statistically analyzed with the one-way ANOVA using SPSS (15) and means were separated sing Tukey comparison test.

Results Fermentation generally increased the crude protein content and to a lesser extent the total ash of the corn cob samples. Fermentation also led to slight reduction in the crude fibre and ADL fractions, but the reduction of ADLwas appreciable when TRH was used to degrade. Hemicellulose content was also significantly increased by fermentation while NFC content was generally depleted when corn cob was fermented with the fungi species and their mixed cultures.

Table 1 Chemical composition and fibre fractions of fermented and unfermented corn cob (%)

	UCC	ASP	RHZ	TRI	ARH	ATR	TRH
Crude protein	2.47^{a}	7.42^{e}	7.02^{b}	8.40^{f}	7.96^{e}	7.79^{d}	8.63^{g}
Ether extract	0.60^{a}	0.92^{b}	0.96^{b}	0.92^{b}	0.92^{b}	0.94^{b}	0.94^{b}
Total ash	2.80^{a}	4.52^{c}	4.78^{f}	4.69^{e}	4.59^{d}	4.75^{f}	4.46^{b}
Crude fibre	37.40^{f}	34.42^{c}	35.01^{d}	34.38^{c}	34.38^{c}	34.26^{b}	32.52^{a}
NDF	36.43^{c}	35.73^{b}	35.61^{a}	37.55^{f}	37.53^{f}	37.50^{e}	37.01^{d}
ADF	36.38^{f}	35.20^{e}	35.02^{d}	33.45^{a}	33.94^{c}	35.00^{d}	33.63^{b}
ADL	8.92^{f}	7.02^{c}	7.04^{c}	5.48^{b}	7.17^{e}	7.10^{d}	5.08^{a}
Hemicellulose	0.05^{a}	0.53^{b}	0.59^{c}	4.10^{g}	3.59^{f}	2.50^{d}	3.38^{e}
Cellulose	27.46	28.18	27.98	27.97	26.77	27.90	28.55
NFC	58.42^{f}	51.41^{d}	51.63^{e}	48.44^{a}	49.00^{bc}	49.02^{c}	48.96^{b}

UCC – unfermented corn cob. Means within each role with different superscript are significantly different ($P<0.05$)

Conclusion The fungi species potentially improved the nutritive value of corn cob largely through enhanced protein content. But their ability to effect significant reductions in the indigestible fibre fractions (ADF, ADL, and Cellulose) was limited**.** The potental of corn cob as a feed stuff for herbivours or pseudoherbivours like rabbits should be further explore.

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Utilization of *Moringa oleifera* and *Moringa stenopetala* by weaner rabbits

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Introduction Tropical forage of good quality is available for limited period of the year (during and shortly after rainy season). *Moringa* is resistant to drought and this could serve as alternative feedstuff in animal diet during the dry season. Rabbit production is encouraged in Nigeria as a means of improving the daily protein intake of individuals (Ekpenyong and Biobaku, 1986) especially because of its short gestation period. Rabbit production is promising in Nigeria but grains are expensive and scarce but forages are cheap and abundant. The use of *Moringa oleifera* as feed for rabbits is limited and *Moringa stenopetala* has not been fed to rabbits in Nigeria. This study was therefore designed to investigate the utilization of *Moringa oleifera* (MO) and *Moringa stenopetala* (MS) by weaner rabbit.

Material and methods The experiment was carried out at the Rabbit Unit of Obafemi Awolowo University Teaching and Research Farm, Ile-Ife, Osun State, Nigeria. It is located at altitude of 240m above sea level, 7˚ 28□N and 4˚ 23□ E. Ile Ife ecologically typifies the hot and humid tropical forest zone. In a twelve week feeding trial, forty eight weaner rabbits of about five weeks old were allotted into three treatments with each treatment consisting of sixteen rabbits. Animals in T1 were fed 50% basal diet and 50% MO, T2 50% basal diet, 25% MO and 25% MS and T3 50% basal diet and 50% MS respectively. They were fed at 4% of their body weight. Parameters measured included; feed intake, weight gain, carcass and haematological characteristics. At the end of the experiment, five rabbits from each treatment were selected based on similarity in weight and were slaughtered for carcass and haematological evaluations. The data obtained were statistically analyzed with the General Linear Model of SAS (2008) and the Duncan New Multiple Range Test option of SAS (2008) was used to detect significant differences among means.

Results The results of this experiment showed that rabbits fed 50% basal diet and 50% *Moringa stenopetala* (T3) had the least performance characteristic. There was a significant increase ($p<0.05$) in feed intake and weight gain for animals in T1 while animals in T3 had the least performance characteristics. The carcass characteristics (Table 2) of the animals in different treatments were not significantly different ($p>0.05$). The haematological parameters measured in the experiment were in the range: PCV (34.0 – 35.0%), RBC (5.8 – 8.2x10^6 µl) and WBC (368 – 488x10^3/ml).

Table 1 Performance characteristics of weaner rabbits fed *Moringa spp.*

Parameter	T1	T2	T3	SEM	P
Dry matter intake (g/day)	265.32[a]	223.36[a]	179.04[b]	1.29	0.219
Initial weight gain (g)	475.63	471.25	474.38	27.48	0.998
Final weight gain (g)	1156.88[a]	1113.13[a]	893.13[b]	32.76	0.001
Total weight gain (g)	681.25[a]	641.88[a]	418.75[b]	27.40	0.000
Daily weight gain (g/day)	8.11[a]	7.64[a]	4.99[b]	0.33	0.000
Feed conversion ratio	16.36[b]	14.62[c]	17.94[a]	0.88	0.001

Mean within each row with different superscript are significantly different ($p<0.05$)

Table 2 Carcass characteristics of weaner rabbits fed *Moringa oleifera* and *Moringa stenopetala (%).*

Parameter (%)	T1	T2	T3	SEM	P
Dressing	45.78	45.87	46.51	0.82	0.93
Shrinkage	0.39	0.26	0.21	0.05	0.34
Liver	2.62	2.77	2.42	0.12	0.54
Skin	9.18	9.51	9.60	0.24	0.78
Heart	0.31	0.29	0.28	0.03	0.92
Lung	0.56	0.43	0.49	0.03	0.12
Head	12.37	13.36	12.54	0.63	0.08
Kidney	0.73	0.69	0.67	0.02	0.62
Stomach	1.81	1.86	1.93	0.05	0.66

Conclusions It can be concluded from the study that feeding *Moringa oleifera* and *Moringa stenopetala* does not have deleterious effect on carcass and heamatogical indices of weaner rabbits.

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Relationship between chlorophyll content (a+b) (SPAD value) and fatty acid composition of perennial ryegrass *Lolium perenne* – a preliminary study

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Introduction Although forage has a low total lipid content, ranging from 3-10% (Bauchart *et al*, 1984), it can nonetheless contribute to (1) energy provision to the animal and (2) the resultant fatty acid profile of animal products (i.e. meat and milk). The majority of forage fatty acids are contained within the thylakoid membranes of chloroplasts, and are thought to be an important structural feature in photosynthesis (Routaboul *et al*, 2000). The use of Soil Plant Analysis Development (*SPAD)* is a non-destructive method of measuring chlorophyll content of plants, which in theory should correlate to the chloroplast and consequently the lipid content. It determines chlorophyll content via optical density of the leaf blade. The aim of this study was to identify whether two varieties of perennial ryegrass (*Lolium perenne*), selected on the basis of differential *SPAD* values, actually differ in lipid content.

Materials and methods Twenty genotypes from two varieties of perennial ryegrass with 'low' *SPAD* and 'high' *SPAD* ranges were selected. *SPAD* readings were obtained prior to harvesting using a portable chlorophyll meter *SPAD* 502 (*Minolta*, Japan). Plants were harvested at ~2 months post-planting to a height of ~5cm with samples collected and freeze-dried. FAME extraction was carried out following the procedure of Sukhija and Palmquist (1988). FAME's were quantified with a gas chromatography system (CP-3800 with PAL Autosampler, Varian Inc, CA, USA) using a CP-select 100m x 0.25mm chemically bonded for FAME column (Agilent technologies UK Ltd, Berkshire, England, UK). Peaks were identified using a 37 FAME standard and quantified using C23:0 as internal standard. Data was analysed via GenStat 14 using one-way ANOVA, Spearman's Rank correlation and linear regression.

Results Overall mean *SPAD* value for the 'low' variety and the 'high' variety were 44.93 and 50.12, respectively, which are significantly different (P<0.01). Mean individual and total fatty acid (TFA) content of the two grass varieties are shown in Table 1. The 'high' variety was significantly higher for TFA (P<0.05), C16:0 (P<0.001), C18:2n-6 (P<0.01) and C18:3n-3 (P<0.001). No significant difference was observed between the two varieties in terms of C18:0 and C18:1 content. Significant correlations were found between *SPAD* value and C16:0 (P<0.05), 18:3n-3 (P<0.01) and TFA (P<0.05). Figure 1 illustrates the regression analysis for SPAD vs. TFA across both varieties.

Table 1 Comparison of mean SPAD value, individual and total fatty acid content (g kg-1 DM) of 'low' SPAD and 'high' SPAD perennial ryegrass varieties.

		Low	High	s.e.d	P
Palmitic	C16:0	4.81	5.94	0.326	***
Stearic	C18:0	0.40	0.46	0.031	ns
Oleic	C18:1	0.62	0.70	0.047	ns
Linoleic	C18:2n-6	4.07	4.98	0.305	**
Linolenic	C18:3n-3	18.84	24.79	1.636	***
Total	TFA	31.32	39.97	2.376	***

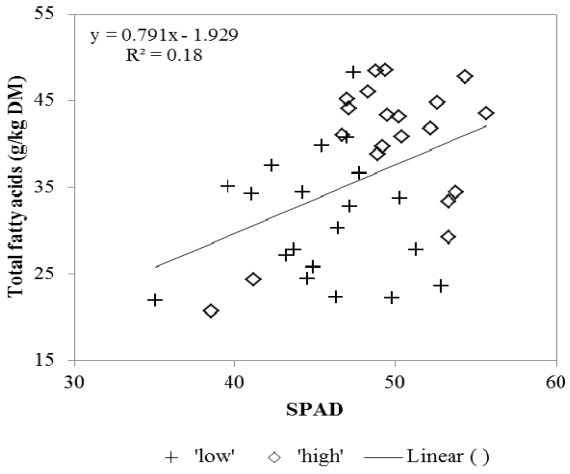

Figure 1 Relationship between SPAD and total fatty acids across two varieties of perennial ryegrass.

Conclusions These results show that chlorophyll (*a+b*) content, as determined by *SPAD* value, is associated with lipid content in perennial ryegrass. The increased total and individual fatty acid content in the 'high' *SPAD* variety, relative to the 'low' variety, suggests that *SPAD* has the potential to be used as an indication of lipid content and possibly as a breeding tool to select for increased lipid in forage. However, further investigation is needed to (1) confirm the relationship between SPAD and actual chlorophyll content, and thus direct chlorophyll content with fatty acid content, and (2) examine whether this relationship is similar across a wider range of varieties and species.

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Performance characteristics of growing snails *(Archachatina marginata)* fed diets containing differently processed Kenaf grain *(Hibiscus cannabinus)*

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Introduction The African giant land snail (*Archachatina marginata)* is a slow growing, hermaphroditic animal belonging to the phylum Mollusca. It is omnivorous but conventionally feeds on pawpaw leaf, water leaf and cocoyam tuber. Growth rate of snails have been greatly improved by supplementing their diets with concentrate feeds. (Omole, *et al.*, 2000). The ingredients used to compound concentrate feeds for snails have also increased competition for food between man and snails. Kenaf grain (*Hibiscus cannabinus)* is a non-conventional feed ingredient that can be used to compound snail feeds. However, one of the major limitations to the optimal use of Kenaf grain is the presence of anti-nutritional factors. Effects of anti- nutritional factors have been reduced by adequate processing of food. (Omoruyi, *et al.,* 2007). The aim of this study is to assess the effects of different processing methods of Kenaf grain on the performance characteristics of growing African giant land snails (*Archachatina marginata*).

Material and methods One hundred and twenty (120) healthy growing African giant land snails with live weight 74 ± 2.3g were used for this study which lasted for 12 weeks. Kenaf grains were processed by soaking in water for 24hours (SK), cooking in boiling water for 10minutes (CK) and roasting on fire for 10minutes (RK). Unprocessed Kenaf Grain (UK) served as the control. The experimental diets contained maize (22.00), brewer's dried grain (12.80), rice bran (15.00), fishmeal (4.00), ground nut cake (10.00), bone meal (2.15), oyster shell (9.80), vitamin – mineral premix (0.25) and 24.00g of differently processed kenaf grains in the different dietary treatment groups. The diets contained 24.44% crude protein and 2438.10kcal/kgME.
There were four dietary treatment groups with three replicates per treatment and ten Snails per replicate. The experimental design was Completely Randomised Design. The Snails were housed in a wooden cage with twelve compartments. Each compartment was filled with sandy loam soil to a depth of 7cm. Water and feed were offered *ad libitum.* Data on daily feed intake and weekly weight gain were taken using sensitive weighing balance. Shell length, shell width and shell thickness were taken on a weekly basis with the use of vernier callipers and micrometer screw gauge respectively. Feed conversion ratio was calculated and data obtained were subjected to analysis of variance (ANOVA) using SAS (1999) and treatment means of significant dependent variables were compared by Duncan option of SAS (1999).

Results Snails fed the control diet had the lowest ($p<0.05$) feed intake, total weight gain and final weight gain being 1173.63g, 259.08g and 333.91g respectively. However, Snails fed diets containing cooked, roasted and soaked kenaf grain had similar ($p>0.05$) but higher values than the control for all these parameters. Furthermore, snails fed the control diet had the highest ($p<0.05$) feed conversion ratio of 4.53. Similar ($p>0.05$) shell length, shell width and shell thickness (mm) were obtained for snails in the four treatment groups. Zero percent mortality was recorded across the treatment groups.

Table1 Performance of growing snails fed diets containing differently processed Kenaf Grain

Parameters	UK	RK	SK	CK	SEM
Initial Weight (g)	74.83	75.12	73.88	74.21	3.23
Final weight (g)	333.91[b]	364.37[a]	362.22[a]	362.32[a]	8.23
Total weight gain (g)	259.08[b]	289.25[a]	288.34[a]	288.11[a]	7.44
Total feed intake (g)	1173.63[b]	1188.80[a]	1187.96[a]	1184.11[a]	10.12
Feed conversion ratio	4.53[a]	4.11[b]	4.12[b]	4.11[b]	0.16
Shell length (mm)	12.09	12.12	12.11	12.13	1.13
Shell width (mm)	10.28	10.33	10.32	10.31	0.34
Shell thickness (mm)	0.12	0.13	0.13	0.13	0.01
Mortality (%)	0	0	0	0	

[ab] means along the same row with different superscripts differed significantly ($p<0.05$).

Conclusion The results showed that soaking, cooking and roasting of kenaf grain effectively enhanced the utilization of kenaf grain in the diet of growing snails. Inclusion of unprocessed kenaf grain in the diets of growing snails should be discouraged as it depressed feed intake, weight gain and negatively affected feed conversion ratio in snails.

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Methane emissions from grazing dairy herd replacements estimated using the sulphur hexafluoride technique

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Introduction Limited information is available on enteric methane (CH_4) emissions from grazing dairy herd replacements. Using the sulphur hexafluoride (SF^6) technique the objective of the present study was to quantify methane emissions of grazing dairy herd replacements at a range of developmental ages.

Material and methods Seventy two Holstein Friesian heifers were allocated onto one of two grazing seasons (early/mid, mid/late), with thirty six animals per season. Each season consisted of three measurement periods one month apart. Heifers sourced from the Institute dairy herd were allocated based on age into three developmental age groups of 12 animals. Calves were aged between 6-9 months, yearlings between 12-15 months and in-calf heifers between 18-21 months of age. Each group of 12 heifers were accompanied by 12 non experimental animals. No supplementary feeds were offered. Early/mid and mid/late grazing season commenced on 16 May and 15 August respectively. Pasture was predominantly perennial ryegrass. During early/mid heifers grazed in a leader-follower grazing system, whereas in mid/late they grazed in an independent rotational paddock system due to weather and soil conditions. The metabolisable energy content of the grass consumed by the calves, yearlings and in-calf heifers during the study averaged 11.5, 11.2 and11.1 MJ/kg DM respectively. Methane emissions were estimated using a modified SF^6 technique as described by Johnson *et al.*, 1994. Permeation tubes containing between 4-6 mg/d of SF^6 were orally administered to each heifer 7 days before the first CH_4 recording period commenced. Prior to this the permeation tubes were weighed weekly over a 6 week period and a permeation rate and expiry date of each tube was known. The release rates from permeation tubes were between 4-6mg/d. CH_4 production was recorded during two, four successive 24 hour periods and collected through a sampling tube on the nose piece (held on a head collar) connected to a sampling line to a pre- evacuated canister on the animals neck. Grass dry matter intakes (DMI) were estimated using the n-alkane technique (Smit *et al.*, 2005). Only data from the final 4 day recording period were used in the analysis. A two stage statistical analysis was performed using REML in GenStat 14 (VSN International Ltd, Hemel Hempstead, UK, 2011). The effect of Season, Age and their interaction on each variable was investigated firstly by fitting them as fixed effects – no random effects were fitted in this case. Probabilities of these effects were assessed by means of a Wald test. If any of these were significant ($P<0.05$) then pair wise differences between levels of treatment effects were assessed using Fisher's LSD test. Linear regression analysis was also performed between CH_4: LW and GEI: CH_4-E testing for differences between Age groups with Season fitted as a random effect.

Results Mean live weight (LW), dry matter intake (DMI), and methane emissions of calves, yearlings and in-calf heifers are shown in Table 1. LW, DMI and CH_4 emissions were significantly different for each of the developmental age groups resulting in significant differences in CH_4 emissions per unit of $LW^{0.75}$, DMI and unit of GEI. Overall CH_4 emissions per unit of GEI averaged 6.8%. Significant season and age group by season interactions were identified.

Table 1 Live weight, dry matter intake and methane emissions of calves, yearlings and in-calf heifers

	Developmental age group				Season			Significance		
	Calf	Yearling	In-calf	SED	Early/mid	Mid/late	SED	age	season	age.season
LW[1] (kg)	231[a]	407[b]	517[c]	15	378	392	12	***	NS	NS
DMI[2] (kg/d)	5.2[a]	8.0[c]	7.2[b]	0.31	6.2	7.3	0.26	***	***	NS
DLWG[4] (kg/d)	0.94[b]	0.85[b]	0.68[a]	0.059	1.05	0.59	0.048	***	***	***
CH_4 (g/d)	102[a]	170[b]	172[b]	5.5	153	143	4.5	***	*	***
CH_4 g/kg $LW^{0.75}$	1.7[b]	1.9[c]	1.6[a]	0.04	1.8	1.7	0.04	***	***	***
CH_4 g/kg DMI	20.5[a]	22.7[b]	25.0[c]	1.01	24.7	20.8	0.83	***	***	***
CH_4-E / GEI[3] (%)	6.0[a]	6.9[b]	7.6[c]	0.43	7.4	6.2	0.25	***	***	***

[1] Live weight [2]Dry matter intake [3]Gross energy intake [4] Daily live weight gain[abc] Means with different superscripts within rows are different ***P<0.001; ** P<0.01; *P<0.05

Conclusions Overall CH_4 emissions from growing dairy herd replacements were similar to IPCC (2006) guidelines at 6.5±1% of GEI however variation in emissions between heifer age groups and grazing season were observed. These differences in CH_4 emissions per unit of DMI, GEI and $LW^{0.75}$ between age groups may reflect differences in diet quality, heifer daily growth rates and the impacts of gestation but requires further investigation.

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Prediction of ultimate pH in beef *M. longissimus thoracis* using visible-near infrared spectroscopy

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Introduction The ultimate pH (pH_u) of beef *M. longissimus thoracis* (LT) normally ranges between 5.50 and 5.80, with values above this leading to meat that is darker in colour, has a poorer shelf-life, and can be tougher than meat with a normal pH_u (Kreikemeier *et al.* 1998). Meat in this condition is commonly termed dark, firm and dry (DFD) and can result from pre-slaughter stress experienced by the animal which depletes the muscle glycogen reserves. Meat processors often measure pH_u using a pH meter as part of their compliance requirements, but measurement is time consuming, and in commercial conditions, difficult to achieve on all carcases. A non-destructive, rapid method to measure pH_u would enable processors to identify carcases with meat in the DFD condition in a more efficient manner. Visible-near infrared (NIR) spectroscopy has shown some promise for measuring various meat quality parameters including tenderness in beef (Prieto *et al.* 2009a), but its application to measure pH_u in beef has not been widely investigated. The aim of this experiment was to determine the ability of NIR spectroscopy to identify beef LT with $pH_u \geq 5.80$ under abattoir conditions.

Material and methods 234 carcases (75 heifers, 118 steers and 41 young bulls) were quartered between the 10th and 11th ribs at 48 hours *post mortem*, a 2.54 cm section of steak containing the LT, was removed from the 11th rib section of each carcase at quartering. Ten replicate NIR spectra (350-1800 nm at 1 nm intervals) were collected using an ASD Qualityspec Pro (ASD Inc., Boulder Colorado) NIR spectrometer fitted with a 63.5 mm diameter active area scanning head by moving and rotating the scanning head on the LT surface as outlined by Prieto *et al.* (2009b). Spectra were collected after allowing the steak to bloom for two minutes (Shackelford *et al.* 2005). The NIR spectrometer was operated using a laptop computer running the Indico Pro program (ASD Inc.). The median absorbance value at each wave-length was calculated from the replicate spectra after screening to remove any poor scans using a principal component analysis ($\alpha = 0.25$). Extremes of the spectral range that contained excessive noise were also removed prior to analysis to form a working range of 495-1600 nm. Ultimate pH of LT was recorded on the steak at ambient temperature using a calibrated temperature compensating spear-type Testo 205 pH meter (Testo AG, Lenzkirch, Germany). One record was removed from the analysis due to poor quality median NIR spectra, the remaining 233 records were sorted in ascending order of pH_u and every fourth record was selected for inclusion in the validation dataset of 58 records. The remaining 175 records were used to develop a calibration model using type one partial least squares regression after the application of a standard normal variate and second derivative spectral pre-treatments to remove scatter effects as outlined by Esbensen *et al.* (2009). The model was applied to the calibration dataset using full cross-validation and to the validation set to determine final performance. The standard deviation of the pH_u in each dataset divided by the standard error of prediction (ratio performance deviation, RPD) gives an indication of the model performance relative to the variation in the dataset with higher values being better. All analysis was undertaken using the Unscrambler software version 10.1 (Camo software AS. Oslo, Norway).

Results Approximately one out of 10 replicate spectra was deemed to be sufficiently different from the others to warrant removal before calculating the median absorption at each wavelength. Both calibration and validation datasets had a similar mean, standard deviation and range (not shown). A strong calibration equation was developed, however performance reduced in the validation phases (Table 1). NIR was able to correctly identify all five samples in the validation dataset that had pH_u values ≥ 5.80 (Figure 1). One sample with a reference pH_u value of 5.56 was classified as having a pH_u value equal to 5.80 which was therefore misclassified.

Table1 Performance of NIR spectroscopy to predict pH_u in beef *M. longissimus thorcis*.

Calibration (n = 175)		Cross-validation			Validation (n = 58)		
R^2 (%)	RMSE	R^2 (%)	SE	RPD	R^2 (%)	SE	RPD
88.4	0.08	59.5	0.14	1.57	59.3	0.15	1.54

R^2 = coefficient of determination, RMSE = root mean square error and SE = standard error

Conclusions NIR spectroscopy may be a useful tool to measure pH_u under abattoir conditions and identify carcases with DFD meat, but there is a need to validate the results under a range of abattoir conditions.

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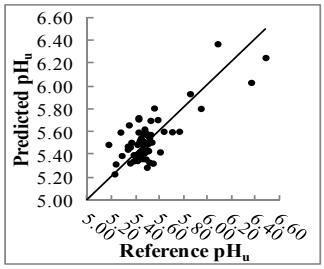

Figure 1 Prediction (validation) of pH_u in beef *M. longissimus thoracis* (n = 58) using NIR spectroscopy.

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Investigations into the effects of flax and echium oil supplementation of steer diets on lipid metabolism and the rumen microbiota reveal that Actinobacteria may play a role in ruminal conversion of 18:4 n-3 to 18:3 n-3.

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Introduction Red meat is high in human health detrimental saturated fatty acid (SFA) and low in human health beneficial polyunsaturated fatty acid (PUFA) due biohydrogenation of dietary PUFA to SFA by the rumen bacteria (Scollan *et al*., 2001; Huws *et al*., 2011). Flax oil supplementation of ruminant diets has been shown to beneficially inhibit biohydrogenation (Shingfield *et al*., 2011). PUFA, as well as being converted to SFA in the rumen, can also be converted to human health beneficial long chain PUFA (LCPUFA) in ruminant muscle. Nonetheless, the first part of this pathway (18:3n-3 PUFA to 18:4 n-3) is ineffective due to the poor conversion of 18:3n-3 PUFA to 18:4 n-3 in muscle. Echium oil is rich in 18:4n-3, thus if added to the steers diet, potentially circumvents the rate limiting step thereby ensuring more substrate for the production of LCPUFA in muscle. The aim of this study was to investigate the effects of supplementing flax and echium oil on rumen fatty acid metabolism and the microbiota using massive parallel next generation sequencing technology.

Material and methods 6 Freisian x Holstein steers were fed either grass silage, grass silage and flax oil (3.0% flax oil/kg silage DMI) or grass silage + echium oil (3.0% echium) oil/kg silage DMI) in a three period Latin square design. After each experimental period rumen samples were collected and fatty acid content monitored as described by Lee *et al.* (2005). PCR for 454 pyrosequencing was conducted in triplicate for each sample using V6-V8 primers (Huws *et al*., 2011) with added adapters and tags as suggested by Roche (Burgess Hill, UK). PCR cycling was as described by Huws *et al*. (2011) and amplicons were pooled and purified from agarose gels using the Qiagen (Sussex, UK) gel extraction kit. Amplicon purity and quantity were checked using the Agilent Bioanalyzer (Berkshire, UK) prior to emulsion PCR and sequencing on the Roche 454 FLX pyrosequencer (Burgess Hill, UK).

Results Flax oil caused a significant (P>0.05) increase in ruminal PUFA, CLA intermediates, 18:1 trans-11 and 18:0 compared to data from steers fed grass silage only (data not shown). Echium oil caused a significant (P>0.05) increase ruminal PUFA, CLA intermediates, 18:1 trans-11 and 18:0 concentrations (data not shown). Roche 454 pyrosequencing generated 726785 reads in total with an average length of 377 bp (±61.1), with reasonably equal representation for each treatment. A diverse array of rumen bacteria were identified within the rumen of the steers used in this study (Table 1). Bacterial Phylum Actinobacteria showed a significant increase in % abundance when echium oil was fed compared with grass silage alone (Fig. 2).

Table 1 Percentage of bacteria phylum present between each dietary treatment (ANOVA and Duncan's multiple range test conducted with a,b,c subscript letters being significantly different, at P<0.05.

Phylum	Echium	Flax	Grass	P-value	SEM
Actinobacteria	1.164 b	0.2485 a	0.4203 a	0.046	0.309
Bacteroidetes	0.04549 a	0.00143 a	0.01659 a	0.493	0.0358
Chloroflexi	0.03412 a	0.00000 a	0.00852 a	0.35	0.0288
Fibrobacteres	0.03777 a	0.00622 a	0.01860 a	0.714	0.0378
Firmicutes	33.2 a	11.69 a	22.92 a	0.161	9.82
Proteobacteria	0.2428 a	0.0548 a	0.1949 a	0.29	0.1133
Spirochaetes	0.00000 a	0.00014754 a	0.00007373 a	0.458	0.000112
Verrucomicrobia	0.09945 a	0.03783 a	0.09963 a	0.723	0.0864

Conclusions Flax oil caused changes in lipid metabolism similar to those reported previously (Shingfield *et al*., 2011) but little change in the rumen microbiota compared to those present in the absence of oil supplementation was seen. Echium oil increased 18:3n-3 suggesting that 18:4n-3 was converted to 18:3n-3 before entering the biohydrogenation pathway. In a parallel study we studied the effect of echium oil on steer muscle fatty acids and found no increase in 18:4n-3 or longer chain PUFA which may be explained by its biohydrogenation within the rumen. In the presence of echium oil bacterial members of the Phylum Actinobacteria were more enumerate compared to sequences obtained in the absent of oil supplementation. In summary, this study indicates that 18:4n-3 is readily converted to 18:3n-3 in the rumen potentially under the control of ruminal bacteria within the Phylum Actionobacteria.

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Effects of 27 days of treatment with growth promoting agents on adult pig *longissimus muscle* protease systems and *post-mortem* shear force after conditioning

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Introduction The inconsistent quality of pork meat is a major worry for the industry, and is mainly blamed on variable tenderness. Meat tenderness depends in part on the degree of alteration of the structural components of muscle and associated proteins post-mortem. Reduced activity of the calpain proteinase is associated with tough meat. The levels of calpastatin, a specific inhibitor protein that regulates the calpain proteinases, are strongly related to meat toughness (Kemp *et al.*, 2010). However it is known that both caspase and proteasome proteolytic enzyme systems are involved in skeletal muscle development and remodelling. Caspases are activated early in some pathological events, such as apoptosis (Kemp *et al.*, 2010), while proteasomes are a ubiquitin-dependent protein degradation system that cleaves regulatory, mis-folded and damaged proteins into small peptides, with the chymotrypsin-like variant being the most active (Strucksberg *et al.*, 2010). Their proteolytic attributes make these enzyme systems possible contributors to improved meat tenderness. The objective of this study was to evaluate the effects of a beta-agonist (BA - Ractopamine) and porcine growth hormone (GH - Reporcin) on the caspase3/7 and proteasome (chymotrypsin-like) activities, as well as calpastatin protein levels and tenderness of the longissimus muscle from adult gilts treated for 27 days.

Material and methods Forty five (45) White Duroc x (Landrance x Large White) gilts were sourced from PIC (Alpha Building, Nantwich, Cheshire), acclimated to the feed and environment for 5days, before being allocated to one of three treatment groups (Control, BA or GH n=15 per treatment). All pigs were then treated for 27 days. The Control group were fed a standard commercial diet *ad-libitum*, while the β-agonist (BA) group were also fed *ad-libitum* the standard commercial diet containing Ractopamine (20mg/kg) and the growth hormone (GH) group were fed the commercial diet *ad-libitum* and administered Reporcin (10mg) intramuscularly every other day until the day before slaughter. After slaughter, samples of *Longissimus dorsi* (LD) muscles were collected at 0hr post-mortem and snap frozen in liquid nitrogen, assayed for caspase 3/7 activity (Wagner *et al.*, 2003), proteasome chymotrypsin-like activity (Strucksberg *et al.*, 2010) as well as the quantity of calpastatin protein using western blotting (Sazili *et al.*, 2005). Chops from the same muscle were also collected, vacuum packed and aged for 5 or 8 days at 4^{o}C, then stored at -20^{o}C until analysis for Warner-Bratzler shear force. Data were analysed by ANOVA (using the Genstat statistical package), followed by a *Post Hoc* Dunnett's test.

Results No significant differences in Caspase 3/7 activities were observed between treatments (Table 1). There was a significant increase in chymotrypsin-like proteasome activity in the LD from BA treated pigs (p=0.041). Likewise there was trend for an increase in the level of calpastatin in pigs treated with BA (p=0.066). Chops from pigs treated with BA tended to have higher shear force values after 8 days of ageing, but this was not statistically significant (p=0.107).

Table 1 Protease Activities, calpastatin protein level and shear force of the LD from gilts treated with BA or GH for 27 days.

Measurement	Control (n=15)	BA (n=15)	GH (n=15)	SED[1]	P-value (ANOVA)
Proteolytic system					
Caspase 3/7 Activity (fluorescence/µg protein)	13.721	14.33	12.52	1.26	0.363
Chymotrypsin Activity (luminescence/µg protein)	18931	20978	17467	1327	0.041
Calpastatin (absorbance/□g protein)	2229	3401	2290	555	0.066
Warner-Bratzler Shear Force (Kg)					
5 days of Ageing	5.53	5.92	5.58	0.405	0.581
8 days of Ageing	4.93	5.48	5.21	0.257	0.107

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

Conclusions BA treated pigs tended to yield chops with higher shear force values and this was associated with significantly higher chymotrypsin activities and a trend for an increase in calpastatin levels.

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Effect of grape pomace supplementation on broiler meat quality characteristics

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Introduction Lipid oxidation is one of the primary causes for quality deterioration in meat. The antioxidant activity of wine by products (grape pomace powder and grape seed extract) in muscle foods has been reported in various studies (Goñi *et al*., 2007). However, use of grape pomace, the major residue of the wine industry, in animal nutrition as a natural antioxidant could be limited due to the low digestibility of polyphenols (Manach *et al*., 2004). In recent years, there is great social pressure for the reutilisation of agro-industrial co- and by products as well as for the production of "clean label" food products. Thus, current research has focused on natural antioxidants from co- and byproducts from raw materials to address consumers' concerns over the environment and food safety. However, inclusion of this type of ingredients in animal diets may not be an inexpensive option since novel and/or expensive biorefinery procedures are usually employed for their recovery and reutilisation. The objectives of the present study were to determine the effect of ground and dried grape pomace on the sensory characteristics of broiler meat during refrigerated storage.

Material and methods Three hundred twenty day old, Ross 308 broiler chickens were randomly allocated to one of four treatments (over 4 replicates). Birds were fed on a standard commercial diet containing either 0, 2.5, 5 or 10 g/kg feed ground and dried grape pomace denoted as CON, DGP 2.5, DGP 5 and DGP 10 respectively. Grape pomace consisted of peels, seeds and a small amount of stems from the Greek indigenous red grape variety Xinomavro. Diets in were provided *ad libitum* for 42 days before slaughter. Skinless breast (m. *pectoralis superficialis*) and thigh (m. *biceps femoris*) samples were air packed and stored at 4°C for 5 days. Meat colour was measured daily using CIELAB L*a*b* colour space. The oxidative stability was determined as thiobarbituric acid reacting substances (TBARS) on storage days 2 and 5. Analysis of variance (SPSS version 13.0, 2004) was used to analyse differences between treatments and within muscle type, and interactions between supplementation level and storage period. Linear and quadratic contrasts were performed to determine the response of the supplementation level on muscle lipid oxidation on storage days 2 and 5.

Results Inclusion of increasing amounts of grape pomace in the diets, did not change colour in breast and thigh muscle in overall and had no effect on limiting the extent of lipid oxidation (Table 1). Grape pomace products have been reported to affect muscle colour in *in vitro* studies (Carpenter *et al.,* 2007; Brannan, 2009). The antioxidant effect of grape pomace might have been demonstrated in case the storage period was extended as in the study of Goñi *et al*. (2007) that grape pomace delayed lipid oxidation in breast and thigh samples on the 7th day of storage. In contrasts, neither the linear nor the quadratic effects for lipid oxidation were significant for both tested periods. There were no significant interactions between supplementation level and storage period on both examined tissues.

Table 1 Shelf life parameters of breast and thigh muscle (n=6)

	Breast						Thigh					
Shelf life parameters	CON	DGP 2.5	DGP 5	DGP 10	s.e.d.	P	CON	DGP 2.5	DGP 5	DGP 10	s.e.d.	P
Day 2												
Lightness (L*)	57.29	58.47	56.65	58.09	1.031	ns	55.64	56.55	57.00	55.17	0.903	ns
Redness (a*)	13.21	12.59	13.15	12.13	0.412	ns	13.43	14.01	12.66	13.56	0.551	ns
Yellowness (b*)	0.39	1.21	1.03	0.87	0.490	ns	-1.44	-1.32	-1.11	-1.14	0.541	ns
TBARS[1]	0.309	0.339	0.337	0.300	0.026	ns	0.308	0.311	0.323	0.319	0.025	ns
Day 5												
Lightness (L*)	57.91	58.32	56.25	57.00	1.105	ns	54.24	54.90	54.80	55.79	0.915	ns
Redness (a*)	13.23^{b}	11.56^{a}	11.56^{a}	12.19^{abc}	0.505	*	13.98	13.96	13.94	12.86	0.736	ns
Yellowness (b*)	1.41	1.63	1.69	1.21	0.682	ns	-1.56	-2.23	-3.03	-1.86	0.750	ns
TBARS[1]	0.331	0.324	0.348	0.346	0.016	ns	0.340	0.337	0.353	0.346	0.013	ns

[1]mg malonaldehyde/kg muscle; ns not significant;* P<0.05; $^{a, b, c}$ Values with no common superscript are statistically different (P<0.05)

Conclusions The results are in agreement with previous studies where inclusion of grape pomace at levels considerably higher (30-60g/kg feed) than 10g/kg feed is required for effective inhibition of lipid oxidation in chill stored broiler meat. In practical terms, lipid oxidation was low in all treatments, not affecting the sensory characteristics of the meat. The effect of the simple processing procedure for the reutilization of grape pomace on its antioxidant function requires also further research to enable determination of optimum supplementation levels.

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Effects of chicory / perennial ryegrass swards compared with perennial ryegrass swards on the performance and carcass quality of grazing beef steers

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Introduction The use of chicory (*Cichorium intybus*) as a forage crop in the UK is increasing due to its high productivity, high feed value and mineral content (Li and Kemp, 2005), its ability to reduce internal parasites (Marley *et al*., 2003) and to increase carcase conformation in lambs (Houdijk *et al*., 2011). However, there has been no research into the effects of this forage when offered to beef cattle in the UK. The aim of the present study was to assess the effects of chicory / perennial ryegrass (*Lolium perenne*) swards compared with perennial ryegrass swards on the performance and carcass quality of grazing beef steers over a two year period.

Material and methods Triplicate field plots (2 ha) were established with either a chicory (cv. Puna II) / perennial ryegrass (cv. Premium) mix (7.4 kg ha^{-1} chicory / 22.2 kg ha^{-1} ryegrass) or a perennial ryegrass control (cv. Premium), sown at a rate of 29.6 kg ha^{-1}. Thirty-six Belgian Blue - cross steers (approx. 7 months old) were used, with 6 animals grazing each replicate plot. The experiment comprised of a standardisation and measurement period. During standardisation of 28 days, steers grazed a ryegrass/white clover permanent pasture as one group. Animals were allocated to their respective treatment on the basis of live weight and body condition score (BCS) determined 7 d prior to the measurement period. The measurement period started on 25 May 2010 and 12 April 2011 in year 1 and 2, respectively and continued until herbage availability dictated the end of grazing on 28 September 2010 and 11 October 2011 in year 1 and 2, respectively. During winter, animals were housed as one group and offered grass silage *ad libitum* mixed with 0.5 kg barley straw (fresh weight) per head per day. Liveweight data determined on day - 28, - 14 and day 0 was used to determine covariate growth rates. Individual steers were weighed and BCS on Day 0 and then every 14 d throughout, except during housing when animals were weighed every 28 d. In Year 2, steers was selected-out for slaughter as they were deemed as have reached a fat class of 3, with a target conformation of U, and the days to finish recorded. Carcasses were graded for conformation (EUROP classification) and fat class. Liveweight and cold carcass weight were used to determine killing-out percentages. Carcass slaughter grades were converted to a numeric score prior to analysis (Kempster *et al*., 1986). Treatments were compared by ANOVA using the standardisation period as a co-variate, using Genstat 11.1.

Results Data on steer performance comparing the first grazing season, winter housing period and then second grazing season showed that there were no differences in the daily liveweight gain of steers grazing chicory/ryegrass or ryegrass only swards in this experiment which averaged 1.04 kg /day, and this was reflected in the number of days to slaughter (Table 1). The conformation, fat grade and killing out proportion of beef steers grazing ryegrass or ryegrass/chicory swards were not found to differ in this study. Carcass conformation and fat scores in Table 1 are equivalent to R and 3 grades, respectively.

Table 1 Performance and carcase quality of beef steers grazing either chicory/ryegrass or ryegrass only swards

	Ryegrass	Chicory/ryegrass	sed	Prob
Live-weight gain (kg/day) Year 1	1.15	1.09	0.052	ns
Live-weight gain (kg/day) Winter	1.08	1.11	0.049	ns
Live-weight gain (kg/day) Year 2	1.07	1.00	0.135	ns
Days to slaughter [a]	137	136	12.7	ns
Conformation	85	93	8.7	ns
Fat grade	53	61	6.6	ns
Slaughter weight	638	632	12.3	ns
Killing out	0.55	0.56	0.004	ns

[a], number of days from turnout in Year 2 to slaughter

Conclusions In this study, there was no difference in the performance of beef steers grazing chicory / ryegrass swards or ryegrass only swards. In conclusion, the inclusion of chicory in the diet of grazing beef steers did not alter their performance or carcass characteristics when compared with beef steers grazing ryegrass only swards.

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Growth performance of crossbred lambs sired by high and low muscle density rams (as measured by computer tomography)

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Introduction Lamb meat quality may be improved upon via a permanent and cumulative method using genetic selection. *In-vivo* measures of meat quality are needed to aid breeding selection within a sample population. Muscle density as assessed by computer tomography (CT) has a negative genetic correlation with meat eating quality and associated intramuscular fat (Karamichou *et al.,* 2006). This study is part of a research programme investigating meat eating and nutritional quality of lambs produced, by selecting sires of extreme variations in muscle density. The aim was to evaluate the growth parameters 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. The selected rams were used in an artificial insemination mating programme to inseminate 230 North Country Mule ewes, which lambed over a 10 day period. Progeny lambs (n=395) had pedigree and relevant data recorded at lambing. Live weights were recorded at: birth; 8 weeks (mean age 58 d ± 0.09 se); 16 weeks (mean age 103 d ± 0.08 se); and immediately pre-slaughter (mean age 147 d ± 1.1 se). Average daily gain (ADG) was calculated from birth to each point of weighing. Slaughter lambs were selected on commercial criteria (target carcass weight: 18-21kg and fat class of 2-3L). Pre-slaughter live weight and ultrasonic back fat and muscle depth were recorded at the third lumbar vertebra. Lambs were reared on grass and weaning occurred at 22 weeks (mean age 154d ± 0.08 se). 79 lambs were not ready for slaughter and were not included in the slaughter data for this study. Data were analysed using GENSTAT 15 using a nested ANOVA design, with sire nested within high and low muscle density groups and a model fitting sex, dam age (2yr *v*. older), birth (single/twins/multiple)/rear type (single/twin/artificially reared) and slaughter batch, with age at measurement fitted as a covariate.

Results Table 1 Adjusted means summary for progeny five high and low muscle density rams for growth and slaughter traits.

Traits	n	Mean: Low muscle density sired lambs	Mean: High muscle density sired lambs	Average SED	P-Value: Muscle density group	P-Value: Sire nested with muscle density	CV%
Birth weight (kg)	395	5.7	5.7	0.17	ns	0.011	15.9
8 week weight (kg)	369	23.8	23.1	0.57	0.025	0.011	12.7
Birth - 8 week ADG (g/day)	369	310	300	0.01	ns	0.012	14.8
16 week weight (kg)	343	36.0	34.9	0.75	0.010	0.014	10.8
Birth - 16 week ADG (g/day)	343	291	283	0.01	0.040	0.008	12.1
Slaughter weight (kg)	264	42.8	42.7	0.58	ns	ns	5.5
Birth to slaughter ADG (g/day) (1)	264	259	251	0.01	0.043	0.024	13.5
Log average ultrasonic back fat depth (mm)	264	0.43 (2.67) (2)	0.40 (2.52) (2)	0.01	0.052	0.001	24.9
Ultrasonic muscle depth (mm)	264	26.1	26.4	1.7	ns	ns	3.8
Slaughter age (days) (1)	264	144	149	7.6	0.035	0.006	11.8

(1) No age covariate (2) Geometric mean

Conclusions Lambs sired by rams selected for low muscle density had higher average daily gains to 8 (P=0.012) and 16 weeks (P=0.008) and higher weights at 8 (P=0.011) and 16 weeks (P=0.014). Low muscle density sired lambs also took five fewer days to reach slaughter (P=0.035). Sire, as a fixed effect, had significant influence on all traits except for slaughter weight, as this had a fixed target, and muscle depth. There was also a tendency (though not significant P=0.052) that higher ultrasonic fat depth was observed in the low muscle density group. The reason for this is not clear, although selection for carcass lean content in Scottish Blackface resulted in correlated changes in muscle density (Karamichou *et al*., 2006). Further work on a sample of lambs produced by selecting sires of extreme variation in muscle density will investigate associations with key components of carcass and other meat quality traits.

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Effects of chicory / perennial ryegrass swards compared with perennial ryegrass swards on meat quality of grazing beef steers

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Introduction The use of chicory (*Cichorium intybus*) as a forage crop in the UK is increasing due to its high productivity, high feed quality, high mineral content (Li and Kemp, 2005) its ability to reduce internal parasites (Marley *et al*., 2003) and to increase carcase conformation in lambs without any detrimental effects on sensory meat quality (Houdijk *et al*., 2011). However, there has been no research into the effects of this forage on meat quality when offered to beef cattle. The aim of the present study was to assess the effects of chicory / perennial ryegrass (*Lolium perenne*) swards compared with perennial ryegrass swards on meat quality of grazing beef steers over a two year finishing period.

Material and methods Triplicate field plots (2 ha) were established with either a chicory (cv. Puna II) / perennial ryegrass (cv. Premium) mix (7.4 kg ha^{-1} chicory / 22.2 kg ha^{-1} ryegrass) or a perennial ryegrass control (cv. Premium), sown at a rate of 29.6 kg ha^{-1}. Thirty-six Belgian Blue - cross steers (approx. 7 months old) were selected, with 6 animals grazing each replicate plot. Animals were allocated to treatment on 25 May 2010 and 12 April 2011 and until 28 September 2010 and 11 October 2011 in year 1 and 2, respectively. During winter, animals were housed as one group and offered grass silage *ad libitum* mixed with 0.5 kg barley straw (fresh weight) per head per day. Steers was selected-out for slaughter as they were deemed as having reached a fat class of 3, with a target conformation of U. Muscle pH was checked at 2 and 48 h post-slaughter in the *M. longissimus* between the 10 and 11^{th} rib, using a Testo 230 pH direct probe. After dressing, the carcasses were chilled at 2 °C. A 250 mm-long section of the hindloin joint containing the *M. longissimus lumborum* muscle was removed from the left side of the carcass, posterior to the 10^{th} rib, and deboned. A 20 mm-thick steak was cut and the muscle dissected free of subcutaneous adipose tissue and used for analysis of individual fatty acids and vitamin E concentrations. The remaining section of loin was vacuum-packed and conditioned at 1ºC to 14 days from slaughter. After this, two 20 mm-thick steaks were cut and packed individually in modified atmosphere packs (MAP, O_2:CO_2; 75:25) and subjected to simulated retail display (4 ºC, 700 lux for 16 h out of 24 h). The remaining section of the conditioned loin was frozen and stored at 20 ºC prior to sensory analysis. Meat colour (L*a*b*) coordinates (CIE, 1986) were measured daily on the two MAP packed steaks on the surface of the steaks through the film lid using a Minolta Chromameter CR200 (Minolta Camera Company, Milton Keynes, UK). The chromameter was standardised against a white tile (L* = 97.78, a* = 0.19, b* = 1.84) covered in the MA top web film. Colour saturation (chroma), which is a measure of the intensity of the red colour, was calculated from the formula $[(a^*)^2 + (b^*)^2]^{0.5}$. Treatments were compared by analysis of variance using the replicate plots of each forage as a block effect using Genstat 11.1.

Results Measurements on pH and the colour of the meat showed there were no effects of including chicory in the diet of grazing beef steers (Table 1). There were also no differences found in the eating quality of the steaks, the fatty acid composition, vitamin E content or colour stability in simulated retail display from the two treatments (data not shown).

Table 1 *M. longissimus* pH, colour and stability after boning from beef steers grazing chicory/ryegrass or ryegrass only swards.

	Ryegrass	Chicory/ryegrass	sed[a]	Prob
pH at 2h	6.18	6.26	0.100	ns
pH at 48h	5.59	5.71	0.053	ns
Bloomed meat chroma at 48h	26.3	25.9	0.72	ns
Fat colour				
L* (Lightness)	67.1	67.3	1.05	ns
a* (Redness)	3.24	3.83	0.935	ns
b* (Yellowness)	17.2	20.7	2.99	ns

[a], sed values shown are $\log_{10}$ as data were transformed to normalise prior to statistical analysis.

Conclusions In conclusion, the results of this experiment showed that the inclusion of chicory in the diet of grazing beef steers did not alter meat quality, stability or meat sensory properties when compared with beef steers grazing ryegrass-only swards.

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Correlations between pre-slaughter morphometric characteristics and fat measurements in beef cattle

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Introduction Identifying when beef cattle are in prime condition for slaughter has always been an issue for the UK beef industry, resulting in significant financial losses due to animals not meeting premium classes and therefore not reaching maximum returns (Oliver *et al.,* 2010). Post-slaughter, carcases are valued on conformation and fat class. Farmers tend to assess when animals are ready for slaughter through visual inspection only and although this has been shown to be beneficial in pre-slaughter assessment, it is subjective by nature owing to possible human error. This currently results in less than fifty percent of all cattle sent for slaughter meeting UK specification (Brown and Powdrill, 2012); Therefore there may be a need for objective methods of live-animal assessment to aid farmers in pre-slaughter animal selection. This paper identifies correlations between different morphometric and fat measurements taken from beef cattle immediately prior to slaughter in order to inform future objective, practical and measurable live-animal assessment aids.

Material and methods Morphometric measurements were taken from 24 finished Belgian Blue-cross beef heifers 24 hours prior to being sent to the abattoir. All cattle were housed indoor during the winter months and allowed to graze through the summer. 15 different morphometric and fat measurements were taken on each animal: Weight (W); Anal Skin fold Thickness (AST); Brisket Skin fold Thickness (BST); P8 Fat Point Reading (P8FP); 10th Rib Fat Point Reading (10RFP); 12th Rib Fat Point Reading (12RFP); Height at Withers (WH); Height at Pelvis (PH); Width at Withers (WW); Width at Pelvis (PW); Width of Rump (RW); Length of Rump (RL); Length of Loin (LL); Heart Girth (HG) and Round Profile (RPR). Weights and measurements were taken using a standard weighing crush, tape measure, skin callipers height rule and an ultrasonic probe.

Results The strongest correlations are seen between height at withers (WH) and height at pelvis (PH); width at withers (WW) and width at pelvis (PW); width at withers (WW) and width of rump (RW); width at pelvis (PW) and width of rump (RW) ($P<0.01$). Unexpectedly, a strong correlation was also observed between heart girth (HG) and weight (W). Both anal skin fold thickness (AST) and brisket skin fold thickness (BST) displayed strong correlations with width at withers (WW) ($P<0.01$) and a slightly weaker (but still significant) correlation with width at pelvis (PW). No correlations were found when analysing the P8 fat point reading against all other measurements.

Table 1 Correlation matrix for 15 morphometric and fat measurements in Belgian blue heifers

	W	AST	BST	P8FP	10RFP	12RFP	WH	PH	WW	PW	RW	RL	LL	HG
AST	-0.015													
BST	0.324	0.221												
P8FP	-0.125	-0.263	0.345											
10RFP	0.079	0.549**	-0.035	-0.176										
12RFP	-0.241	0.179	0.068	0.034	0.034									
WH	0.618**	-0.163	0.119	-0.069	-0.164	0.528**								
PH	0.621**	-0.241	0.219	0.104	0.071	-0.399	0.748**							
WW	-0.087	0.561**	0.609**	-0.042	-0.207	-0.467*	0.135	0.109						
PW	0.092	-0.49*	-0.434*	-0.127	-0.307	0.545**	0.514*	0.278	0.796**					
RW	0.003	-0.299	-0.233	-0.051	-0.165	0.534**	0.218	0.065	0.767**	0.713**				
RL	0.581**	0.015	0.146	-0.022	0.216	-0.165	0.365	0.395	-0.19	-0.119	-0.16			
LL	0.688**	0.118	0.341	-0.016	0.345	-0.233	0.441*	0.524**	-0.06	0.017	0.157	0.63**		
HG	0.756**	0.105	0.448*	0.114	0.044	-0.092	0.438*	0.037	-0.3	-0.147	-0.022	0.528**	0.646**	
RPR	0.066	-0.225	0.2	0.267	-0.45*	0.065	0.096	0	-0.34	-0.224	-0.288	0.191	-0.084	0.229

*significant at $p<0.05$, ** significant at $p<0.01$

Conclusion The results indicate that certain morphometric measurements correlate significantly with each other. Heart girth has previously been used to predict weight in beef cattle and so the findings from this study concur with previous research linking heart girth to growth rates. Further research is on-going; and as sample size increases annually, combinations of pre-slaughter fat and morphometric measurements will be modelled utilising the final carcase classification as the dependent variable. The correlations show that there are significant relationships between certain fat and morphometric measurements, indicating that visual assessments of cattle could be misleading due to fatter animals appearing to have greater conformation over certain body points thus highlighting the need for a more objective approach.

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Nutritional comparison of three farmed fish species raised under the same conditions in Pakistan

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Introduction Freshwater fishes are not only a rich source of high quality protein, minerals and vitamins but they also contain nutritionally valuable lipids and fatty acids. *Cirrhinus (C) mrigala, Labeo* (*L*) *rohita* and *Catla* (*C*) *catla* are economically important freshwater fish species in Pakistan owing to their rapid growth, size, taste and nutritional quality. The healthy fish fat contains essential polyunsaturated fatty acids (PUFA) like arachidonic acid (ω 6), eicosapentaenoic acid (EPA= ω 3) and docosahexaenoic acid (DHA= ω 3) which are not synthesized in human body while they are essential for the growth and development. Therefore, these fatty acids (FA) must be supplied in the diets (Ismail, 2005). The knowledge of the proximate analysis and FA composition of these important carp fish species is desirable, due to recent dietary and medical emphasis on the role of PUFA in human health. Main objective of this study was to investigate proximate composition and FA in the muscles of three freshwater fish species being raised under the same conditions in Pakistan.

Material and methods Nine specimens of each of the *C. mrigala, L. rohita* and *C. catla* were caught from the Punjab University Fish Research Farm (PUFRF), Pakistan by following the approvals of the Punjab university ethics committee?. After recording the morphometric measurements, the specimen were dissected and muscle samples of each specimen were obtained and stored at -20 ºC. Frozen fish muscle samples were carried to the Newcastle University, UK by the prior authorisation from DEFRA in July, 2011. The muscles samples were stored at -20 °C on arrival but freeze dried to determine moisture contents and ground afterwards. The freeze dried muscle samples were analysed for their crude protein (Kjeldahl nitrogen x 6.25), ash by burning samples at 550°C and crude fat by using a Soxhlet apparatus. The fat samples were then analysed for fatty acids on a gas chromatograph by using a modified method of Sukhija and Palmquist (1988) as reported by Jabeen and Chaudhry (2011). Total carbohydrates were determined by subtracting the sum of crude protein, fat and ash from 100.. The data were statistically analysed using Minitab software to compare the effects of fish species on each of the nutrients. These effects were declared significant if $P<0.05$, very significant if $P<0.01$ and highly significant if $P<0.001$. Tukey's test was used if comparing more than two means for statistical difference at $P<0.05$.

Results The weights and lengths did not significantly differ ($P>0.05$) among the sampled fish specimens. However, the muscles of *L. rohita* were highest in crude protein (19.3 %) and fat (2.7 %) contents but lowest in carbohydrates (1.9 %) and moisture (75 %). Conversely, *C. catla* was highest in moisture (77.3 %) and ash (1.4 %) but lowest in crude protein (16.9 %) contents. While carbohydrates (2.8 %) were highest and ash contents (0.96 %) were lowest in *C. mrigala.* saturated fatty acids (SFA) and mono unsaturated fatty acids (MUFA) were equal in *C. mrigala*. However, muscle samples of *L. rohita* showed highest polyunsaturated fatty acids (PUFA) than the other fishes.

Table 1 Mean compositions (%) of three carp fish species netted from PUFRF, Pakistan

Composition	Fish species			SEM and significance
	C. mrigala	*L. rohita*	*C. catla*	
Total length (cm)	39.92^{a}	37.49^{a}	37.01^{a}	1.166
Total wet weight (g)	645.33^{a}	633.11^{a}	624.44^{a}	60.555
Condition factor (g/cm^3)	0.99^{b}	1.17^{a}	1.21^{a}	0.027^{***}
Crude protein	17.37^{b}	19.30^{a}	16.86^{b}	0.211^{***}
Crude fat	1.75^{b}	2.71^{a}	2.16^{b}	0.110^{**}
Total carbohydrates	2.82^{a}	1.85^{c}	2.36^{b}	0.096^{**}
Ash	0.96^{b}	1.17^{ab}	1.36^{a}	0.061^{*}
Moisture (% wet weight)	77.10^{a}	74.96^{b}	77.26^{a}	0.225^{**}
Saturated fatty acid (SFA)	44.17^{c}	46.03^{b}	48.09^{a}	0.199^{**}
Monounsaturated fatty acid (MUFA)	44.41^{a}	38.83^{b}	37.27^{c}	0.195^{***}
Polyunsaturated fatty acid (PUFA)	11.42^{b}	15.14^{a}	14.64^{a}	0.126^{***}

Means within the same row with the same letters did not differ significantly ($P>0.05$)

Conclusion In conclusion, *L. rohita* showed significantly higher muscle protein and PUFA contents than *C. catla* and *C. mrigala* and thus it is declared nutritionally better than the other two species when cultivated together.

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Roasted (dehulled) cowpea *(Vigna unguilata)* flour (RCF) as an extender in beef burgers

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Introduction Processed meat products are consumed by the masses. Made from less expensive meat cuts it is an irony that in most developing countries they are more expensive than the fresh cuts. To decrease the production cost and achieve the aim of meat processing, various non-meat protein products derived from plants and animal sources are being used as binders, fillers and extenders. Cowpea flour is used in the preparation of various plant protein diets including vegetarian dishes, fried foods and also used to boost the protein content of porridge for malnourished children. This work aimed to use cowpea flour as an extender in beef burgers by determining its cooking yield as well as cost of production and to also evaluate its sensory attributes.

Material and methods Cowpea (*Vigna unguilata*) was dehulled and milled using a hammer mill with a plate diameter of 1 mm. Each batch of 0.5 kg of the flour was roasted for 45 min. using a Wagtech oven set at 200 °C and allowed to cool for 20 min. The cooled roasted flour was packaged in polyethylene bags and stored in a freezer until required. Chilled lean beef trimmings, of shoulder, flank and thigh, were minced through a 13 mm diameter (Mado Super Wolf meat mincer). The cowpea flour, spices and water were weighed and mixed with the minced meat for the four treatments, with two replicates each. The treatments were T_1 (control product), T_2 (5 % inclusion), T_3 (10 % inclusion) and T_4 (15 % inclusion) of RCF. The mixture was minced through a 5 mm diameter plate and then molded with a 1007 burger mold into patties weighing 110 g each. The patties were stored at a temperature of -18 °C for 24 h, re-packaged after and kept at the same temperature till required. The patties were thawed overnight at 10 °C. The thawed beef burgers were grilled using Kenwood Health grill at a temperature of 180 °C to obtain a core temperature of 77 °C using a meat thermometer. Samples were taken from each treatment and weighed before cooking (broiling) and after cooking. The percentage cooking yield was obtained by dividing the cooked weight by the fresh weight and multiplying the answer by 100. The products were then sliced and kept in a microwave till they were served. A hedonic scale of 9 – 1 was used; where 9 means 'like extremely' and 1 means 'Dislike extremely'. Forty five untrained regular consumers of processed meat products were used as panelists from Kwame Nkrumah University of Science and Technology. At each sensory evaluation session, the coded samples of the different treatments were served following a completely randomized design, which ensured that no two panelists at any time were served in the same order. They were each served with water, disposable cups and napkins. Chemical analysis was carried out on the beef, CPF and the processed product for moisture, fat, protein and ash content using the procedures described by AOAC (1990). The data were analyzed using the one-way analyses of variance (ANOVA) in a Completely Randomized Design. Means and associated standard errors for measured parameters were computed using Genstat 2008 Version 7.2 DE for Windows and differences between means were detected using the Least Significant Difference (LSD) test.

Results The panelists detected significant ($P<0.05$) differences in taste, flavor, cooking yield and overall acceptability of the test samples (Table 1). No significant ($P>0.05$) differences were detected in appearance and texture. There was no significant ($P>0.05$) difference between T_1 (control product) and T_2 (5 % inclusion) for all the attributes (appearance, taste, flavor, texture and overall acceptance). The test products had significantly ($P<0.05$) higher cooking yields T_2 (80.8 %), T_3 (83.6 %) and T_4 (92.6 %) when compared to the control T_1 (67.83 %). This could be due to the moisture retention capacity of RCF. The reduction in production cost of the test products was due to lower cost of cowpea GH¢ 1.2 (US$ 0.60)/kg compared to that of minced beef GH¢ 8.00 (US$ 4/kg).

Table 1 Sensory attributes and cooking yield of beef burgers

Attributes	T_1	T_2	T_3	T_4	SEM
Appearance	6.5	6.8	6.9	6.8	0.21
Taste	7.1^{a}	6.6^{ab}	6.1^{b}	6.0^{b}	0.23
Flavour	6.8^{a}	6.5^{ab}	6.2^{b}	6.2^{b}	0.22
Texture	6.5	6.8	6.9	6.9	0.21
Overall acceptance	7.0^{a}	6.6^{ab}	6.2^{b}	6.3^{b}	0.22
Cooking Yield	67.8^{a}	80.8^{b}	83.5^{c}	92.6^{d}	0.55

Means with different superscripts within a row are significantly ($P<0.05$) different

Conclusions The study showed that dehulled roasted cowpea flour can be used as an extender in beef burger at an inclusion level of 5 % with no effect on appearance, taste, flavor, texture and overall acceptance. The cost of production can be reduced by GH¢ 0.30 (US $0.15)/kg of product. Thus making processed beef products more affordable to the consumer.

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Carcass characteristics and sensory evaluation of meat from extensively managed West African Dwarf and Red Sokoto goats in South Western Nigeria

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Introduction In Nigeria goat meat is preferred to beef in terms of palatability and delicacy. This preference is due to attributes such as high tenderness and juiciness (Simela *et al*, 2011). Also, there are no religious or traditional taboos against goat meat. Thus, this product is an important source of animal protein in rural areas. However, the productivity of indigenous goats in terms of meat yield is usually low, and this is mainly as a result of their low genetic potential and poor nutrition. . Goat meat evaluation has received little attention and as a result knowledge of yield and quality of goat meat is given little consideration when compared to mutton and beef and accurate records in the butchery(s) are not readily available. Therefore, this study was aimed at comparing the carcass characteristics and sensory qualities of West African Dwarf (WAD) and Red Sokoto (RS) goats managed extensively, which will in turn help in assessing the quality of goat meat that is available to the public and other related indices.

Material and methods Data were collected from reputable butcheries using the total number of 150 goats (100 RS and 50 WAD goats in Abeokuta and Ibadan metropolis respectively; both in South Western Nigeria). Prior to slaughtering, the animals were weighed to determine the live weight and the goats were slaughtered using recommended acceptable humane method (REF). . The weight at slaughter was taken and the gastrointestinal tract (GIT), the head, and feet were removed and weighed after eviceration. The animal was thereafter cut into two equal parts and weighed. Empty body weight (EBW), hot carcass weight (HCW) and dressing percentage were obtained (Fasae *et al.,* 2011)

Sensory qualities (colour, juiciness, flavour, tenderness, saltiness and overall acceptability) of the meat were also determined. Student T-test was used to separate the means of various measurements taken for RS and WAD goats (SAS 1999).

Results The mean live weight, slaughtered weight and empty body weight of WAD goats were significantly ($p < 0.05$) lower than those of RS goats. RS goats also had higher ($P < 0.05$) dressing percentage, hot carcass weight and dressed carcass weights than their WAD counterparts (Table1). Sensory evaluation (Table2) showed that WAD meat was superior in juiciness, flavour, tenderness and overall acceptability than the RS meat.

Table 1 Comparison of Carcass Characteristics of West African Dwarf and Red Sokoto Goats

Components (kg)	WS	EBW	HCW	DP	WG	WF
WAD	$9.17^{b}\pm0.54$	$6.54^{b}\pm0.42$	$5.27^{b}\pm2.36$	$51.1^{b}\pm0.99$	$2.54^{b}\pm0.13$	$0.55^{b}\pm0.22$
RS	$17.68^{a}\pm0.55$	$13.63^{ba}\pm0.41$	$11.01^{a}\pm0.40$	$57.90^{a}\pm0.91$	$3.92^{a}\pm0.14$	$1.19^{a}\pm0.22$

Table 2 Comparison of Sensory qualities of meat from West African Dwarf and Red Sokoto Goats

Parameters	Colour	Juiciness	Meaty Flavour	Tenderness	Saltiness	Overall Flavour	Overal Acceptability
WAD	6.78 ± 0.28	$6.33^{a}\pm0.53$	$6.78^{a}\pm0.52$	$6.56^{a}\pm0.63$	$4.67^{b}\pm0.69$	6.56 ± 0.38	$7.00^{a}\pm0.44$
RS	6.11 ± 0.68	$4.78^{b}\pm0.78$	$6.11^{b}\pm0.61$	$6.33^{b}\pm0.71$	$5.33^{a}\pm0.47$	6.67 ± 0.41	$6.44^{b}\pm1.67$

[a, b] Mean values in the same column with the different superscripts are significantly different ($p < 0.05$)
WS=Weight at Slaughter; EBW=Empty Body Weight; HCW-Hot Carcass Weight; DP=Dressing Percentage; WG=Weight of Gut; WF=Weight of Feet; WAD =West African Dwarf and RS =Red Sokoto

Conclusion RS goat had higher dressing percentage than WAD goat. The sensory evaluation showed that juiciness, meaty flavour, tenderness, saltiness and overall acceptability were affected by breeds and WAD goat possesses a higher value in the above parameters than RS goat. However, colour and overall flavour was not affected by breed. It is noteworthy that no WAD goat was slaughtered by butchers in Abeokuta metropolis.

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Evaluation of the combination of vitamin D_3 and papaya leaf on muscle antioxidant activity of spent chicken

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Introduction The poultry industry is faced with a large number of spent layer hens which are normally sold as old chickens and carry lower prices than the broiler chickens. Several approaches such as salt additives, calcium chloride marinating, infusion or injection have been applied to improve meat quality of carcass meat (Koohmaraie *et al*., 1988). Attempts to improve meat quality through improving tenderness through post-slaughter manipulations are either costly, labor intensive, need large storage areas or require longer storage time. This paper reports the effect of feeding vitamin D3 and papaya leaf meal on meat on the stability of chicken meat.

Material and methods The experiment was undertaken with 320 spent chickens, ISA-brown which were taken from layer farm of University Putra Malaysia after a period of laying of 80 weeks. Chickens were kept in individual cages under optimal condition. A 10 day adaptation was conducted and thereafter, the experiment lasted 21 days. The diets were fed individually in the feeders with a specified weight every day. Eight diets which were served in this research were basal diet, with or without vitamin D_3 which was supplemented with 0, 0.5, 1 and 2% of Papaya Leaf Meal (PLM). Papaya leaves were collected from local plants and separated from the stems, dried in a 65°C oven until constant weight. The dry leaves were ground, passed through a sieve of 1 mm and properly mixed the appropriate diet . The sample of PLM was analyzed for crude protein, crude fibre, fat, dry matter, ash and calcium content using atomic absorption spectrophotometer. At day 0, 7, 14 and 21, eighty the birds were slaughtered and left and right breast muscles of each bird were taken to assay antioxidant activity. Samples were kept in -80°C for later analysis. This experiment was a 2 (with and without vitamin D_3) x4 (four levels of PLM) factorial arrangement with a basis of Completely Randomized Design (CRD) with 10 replicates treatment.

Results and Discussion The interaction effects of vitamin D_3 and papaya leaf meal on antioxidant activity is shown in Table 1. For antioxidant activity, treatments contained 0.5, 1 and 2% PLM with vitamin D_3 had significant improvement ($p<0.01$) in antioxidant activity of meat compared to the group fed no vitamin D_3 at days 7, 14 and 21. For groups contained no vitamin D_3 as the level of PLM increased, the antioxidant activity decreased ($p<0.01$) over the experimental period. Interaction between vitamin D_3 and PLM was significant ($p<0.01$) at day 7, 14 and 21. Other research conducted to study Vitamin D_3 antioxidant activity and its mechanism that showed Vitamin D_3 has a membrane antioxidant activity which inhibits iron-dependent lipid peroxidation in liposomes compared to cholesterol, ergosterol and tamoxifen. On the other hand, studies on the papaya seed's antioxidant property demonstrated that total phenolic content of the seed extracts was found to have a positive linear correlation with the total antioxidant activity and chloroform-methanol extract of papaya seeds had and antioxidant activity due to a high phenolic content (Kothari *&* Seshadri, 2010).

Table 1 The interaction effect of papaya leaf meal and vitamin D_3 on antioxidant in spent layer hens

$VitD_3$	+				-				$VitD_3$	PLM	$VitD_3$ *PLM
PLM	0%	0.5%	1%	2%	0%	0.5%	1%	2%			
0day	0.16 ± 0.005^{d}	26 ± 0.003^{a}	0.24 ± 0.008^{c}	0.27 ± 0.004^{a}	0.29 ± 0.01^{a}	0.21 ± 0.001^{ab}	0.21 ± 0.001^{ab}	0.13 ± 0.005^{c}	***	***	***
7day	0.17 ± 0.003^{b}	0.27 ± 0.003^{a}	0.27 ± 0.004^{a}	0.28 ± 0.001^{a}	0.30 ± 0.002^{a}	0.22 ± 0.001^{b}	0.21 ± 0.004^{c}	0.14 ± 0.003^{d}	***	***	***
14day	0.17 ± 0.002^{b}	0.28 ± 0.003^{a}	0.28 ± 0.003^{a}	0.28 ± 0.001^{a}	0.31 ± 0.003^{a}	0.23 ± 0.002^{b}	0.22 ± 0.005^{c}	0.15 ± 0.002^{d}	***	***	***
21day	0.18 ± 0.002^{b}	0.29 ± 0.003^{a}	0.29 ± 0.003^{a}	0.29 ± 0.003^{a}	0.33 ± 0.004^{ab}	0.35 ± 0.103^{a}	0.23 ± 0.003^{ab}	0.16 ± 0.00^{2b}	***	***	***

The results are representative of ten spent chickens and are expressed as mean ± SEM.

Conclusion It could be resulted that the dietary supplementation papaya leaf up to 2% of dry matter in the diets included vitamin D_3 might have good effect on muscle antioxidant activity of spent chicken if this additive is supplemented with the diet a few weeks before slaughtering.

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UK Beef Genetic Evaluations – A Changing Landscape

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Introduction Genetic improvement has been shown to be permanent, cumulative and cost effective (Simm, 1998). Amer *et al.,* (2007) estimated the benefits of 10 years of genetic improvement, considering a 20 year time frame, was £4.9 million for terminal sire beef breeds, increasing to £18.2 million for dual purpose breeds. The UK pedigree beef industry has been performance recording since the 1970's with live weights being the main traits (Crump *et al*, 1997). Initially the genetic evaluations were within-herd, with the raw phenotypes adjusted for age and fixed effects. Across herd comparisons became possible with the introduction of Best Linear Unbiased Prediction (BLUP) Estimated Breeding Values (EBVs) in the late 1990's (Crump *et al*, 1997). All UK beef genetic evaluations were initially undertaken by Signet who was owned by the Meat and Livestock commission (MLC) and since devolution the successor to MLC in England, EBLEX. Around 2005, the Australian BREEDPLAN system entered the UK market. At the same time, Edinburgh Genetic Evaluations Services (EGENES) were contracted by Signet to provide the BeefBreeder genetic evaluations for UK cattle breeds (Coffey *et al.,* 2007*)*. This provided the ideal time to include maternal traits into the breeding goal (Roughsedge *et al.,* 2005) and streamline the computing system making use of the BASCO database – a beef and sheep database owned by three large UK breed societies – for performance recording and the provision of EBVs to the industry. There is currently a period of change occurring in UK beef genetic evaluations. Therefore, the aim of this paper is to document the genetic progress achieved by the BeefBreeder breeds to date and to outline the changes that are occurring in the UK beef genetic evaluations.

Genetic Improvement A summary of the genetic trends achieved for animals born in the ten year period 2001-2010 are given in Table 1. These were calculated based on final 2012 Signet BeefBreeder genetic evaluations. Only animals with an EBV accuracy of 50% or greater were considered when calculating the genetic trend. In the case of Salers and for age at first calf (AFC) this value was decreased to 40% to increase the number of animals contributing. The average genetic progress was the regression over the 10 year period. Although breeds have different breeding goals and emphasis on terminal traits, all breeds have increased in 400 day livtweight. Limousin, Stabiliser and the Lincoln Red breeds have made significant improvement with annual increases greater than 0.1 genetic standard deviations; this corresponds to over 2kg. The EBV for AFC is expressed as the proportion daughters calving earlier or later when given the opportunity to calve as a 3 year old. Very little progress was recorded for AFC when considering the EBV expressed as a proportion of early or late calvers. There were small negative trends when the trend was expressed as genetic standard deviations. While no significant improvement in AFC can be observed it is encouraging that AFC has held constant while the production traits have increased.

Table 1 The genetic improvement of live weight at 400 days (WT400) and age at first calf (AFC) during 2001 to 2010

Trait/Breed*	LIM	STA	BLO	SUS	LIN	RED	SAL
Number of animals recorded annually for WT400	4,600	1,270	260	330	120	80	100
Annual genetic progress for WT400							
(kg/year)	2.2	2.7	0.9	1.5	2.2	1.5	0.4
(genetic standard deviations/year)	0.13	0.15	0.04	0.09	0.12	0.09	0.03
Number of animals recorded annually for AFC	4,580	1,120	130	140	60	40	80
Annual genetic progress for AFC							
(proportion/year)	-0.002	-0.003	-0.001	0.008	-0.003	-0.001	-0.006
(genetic standard deviations/year)	-0.02	-0.06	-0.01	0.11	-0.06	-0.03	-0.08

* LIM=Limousin, STA=Stabiliser, BLO=Blonde d'Aquitaine, SUS=Sussex, LIN=Lincoln Red, RED=Red poll, SAL=Salers

The Changing Landscape Commencing April 2013, the British Limousin Cattle Society (BLCS) will provide performance recording for the UK Limousin breed. BLCS will utilise the BASCO database for their performance recording needs but have also developed the database so they can offer a paperless and web-based data capture system. The Limousin data analysis and genetic evaluations will continue to be provided by EGENES. The introduction of a third performance recording organisation in the UK is not the only change for UK beef evaluations. Research is currently being undertaken to develop genomic breeding values for Video Image Analysis carcase traits. This project is a collaboration between Anglo Beef Processors (ABP), BLCS and Scotland's Rural College (SRUC) and is partially funded by Technology Strategy Board and BBSRC. Due to be completed in 2014, one of the key deliverables will be the formation of a BLCS subsidiary company that will facilitate the collection of DNA samples for analysis and deliver genomic breeding values to UK Limousin animals positioning UK genetic evaluations amongst the world leaders.

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Using British Cattle Movement Service data to enhance pedigree construction and the benefits for genomic selection of carcase traits in UK Limousin cattle

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Introduction Video Image Analysis (VIA) of beef carcases can identify carcases with favourable characteristics (Pabiou *et al*. 2011). A 3 year project has recently begun to produce a SNP key so that UK Limousin animals can be genotyped and receive VIA carcase genomic breeding values. Central to the project is designing a Limousin reference population that possesses, ideally, low levels of relatedness between animals in the reference population and high relatedness to the selection candidates (Pszczola *et al*. 2012). To estimate relatedness it is important for the pedigree to be accurate and as complete as possible. The British Limousin Cattle Society has an extensive pedigree for UK purebred Limousin animals (stored on the BASCO database) which allows for an accurate representation of the pedigree sector but not the commercial sector. Since 1998 all UK animals are required to be registered with the British Cattle Movement Service (BCMS). Along with unique animal identification, the BCMS database contains information on dam and sire identities, date of birth and death, breed, sex and animal movements. With millions of records, BCMS data offers a unique and powerful opportunity to produce a rich pedigree incorporating data from other sectors of the bovine industry (e.g. dairy and commercial). This paper reports on the steps necessary to create a large across-sector super pedigree and estimate relationships for the wider Limousin population.

Material and methods The current Limousin reference population consists of 716 sires and dams (genotyped using the 800K Illumina BovineHD BeadChip). These animals were selected for their high impact on the pedigree Limousin population, accuracy of traditional Estimated Breeding Values and DNA availability. They represent historic Limousins born between 1980 and 2010, with the majority born in the late 1990's and early 2000's. A super pedigree of all bovines in the UK was created by merging all individual pedigree sources and stored in a designated SQL database. To avoid duplicates appearing in the super pedigree procedures were developed to cross reference and identify animals across the different databases, including matching identifications with formatting differences. Using the software CFC (Sargolzaei *et al*. 2006) levels of relationships were estimated within and between the following 3 Limousin groups: reference population, BASCO registered animals (pedigree) and BCMS registered animals (commercial).

Results The super pedigree was created by merging different databases: beef pedigree (BASCO n = 1.7 million), commercial (BCMS n = 48 million) and dairy milk recording databases (Holstein UK n = 9 million, National Milk Records n = 11 million). This resulted in a super pedigree of 65 million animal records. From the super pedigree a Limousin pedigree of 15,085,465 animal records were extracted for the relatedness analysis. However, due to computational limitations the Limousin pedigree had to be further reduced by implementing the following criteria (i) 716 Limousin genotypes, (ii) Limousin or Limousin cross animals born after year 2000, and (iii) going back 6 generations. The final Limousin pedigree had a total of 11,734,483 individuals: 716 in the reference population, 305,027 pedigree (BASCO) animals and 11,400,549 commercial (BCMS) animals. Table 1 presents estimates of average relationships within and between the groups. As expected with a large population, the average relationship between the reference population and BCMS is very low, as is between BASCO and BCMS. The average relationship within the reference population was 0.0849 and based on the values of simulated populations reported by Pszczola *et al*. (2012) could be considered to be medium to highly related.

Table 1 Average relationships within and between groups

Groups	Reference population	BASCO (pedigree)	BCMS (commercial)
Reference population	0.0849	0.0330	0.0023
BASCO (pedigree)		0.0175	0.0012
BCMS (commercial)			0.0001

Conclusion For the first time the wealth of information available in the BCMS data has been exploited through merging with other data sources to produce a super pedigree of all bovine animals in the UK. Linking the dairy and beef sectors could be invaluable given the influence of dairy on the beef industry. This powerful dataset will likely establish a foundation of broad spectrum use and cross-sector applications, demonstrating that the sum of the datasets is greater than the sum of the individual databases. One of the uses of this super pedigree has been to enable the estimation of relatedness between the pedigree and commercial sectors. This will enable more effective selection of animals for inclusion in the reference population. Estimation of the relationships within and between these groups will be an iterative process as we add more genotyped animals to the reference population and this initial estimate is a good starting point from which to improve the design of the Limousin reference population.

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Feasibility of using abattoir data and cattle movement records for carcass trait evaluations

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Introduction Genetic improvement of UK beef carcass traits currently uses predictor traits (weight and ultrasound measurements) taken on a relatively small number of live pedigree animals. In contrast, abattoir records are available in large quantities but mostly measured outside the pedigree sector. Returns from genetic improvement of beef cattle are expected to increase if the estimated breeding values (EBVs) were more closely associated with what farmers are paid for (carcass weight together with conformation and fat grades). A combination of data sources merged together including abattoir, national cattle tracing system and genetic evaluation data could contain sufficient information which could then be used to produce EBVs for carcass traits. This study produced a consolidated dataset of carcass traits and pedigree for beef and dairy cattle by combining all sources of information and examined the suitability of its use for future genetic evaluations.

Material and methods Abattoir data was collated from 2001 to May 2012 and an extract from British Cattle Movement Service (BCMS) data was provided in January 2012. Figure 1 shows the information available from the different data sources and these were joined by using the UK eartag. Carcass records were provided by 3 abattoir companies (2.9 m) and 82% (2.4 m) of these records were matched to BCMS data. The carcass traits available from abattoir records were net carcass weight (NCW), conformation (CONF) and fat class (FAT). A pedigree file was created based upon BCMS records and by matching to other national data sources to provide as much pedigree information as possible. This resulted in a pedigree of over 50 million animals (deepest pedigree = 13 generations). Editing of the dataset was carried out for genetic parameter estimation of NCW, CONF, and FAT for the animals with a Charolais sire, resulting in 17,125 animals. CONF and FAT were adjusted to a 15 point scale. The pedigree consisted of 6 generations with 43,069 animals. Genetic parameters were estimated in ASReml (Gilmour *et al.*, 2006), using an animal linear model.

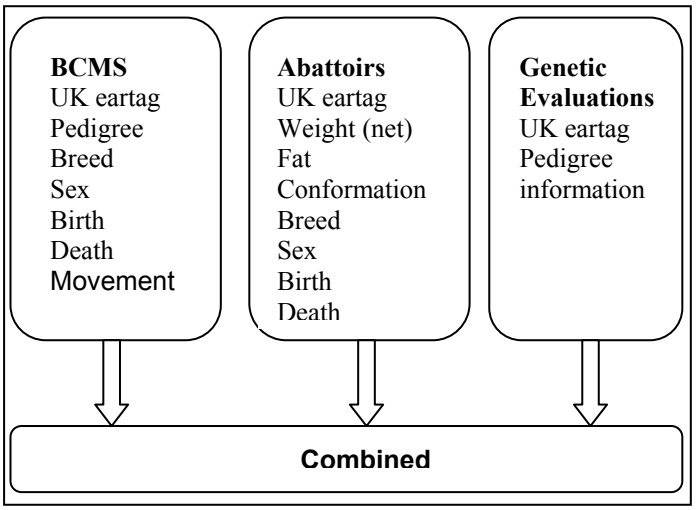

Figure 1 Compiling the data

Results The numerically largest breeds present in the slaughter population were Limousin, Aberdeen Angus, Holstein Friesian, Charolais, Hereford, Simmental, and Belgian Blue, and these accounted for 92% of the animals present in the matched abattoir/BCMS dataset. Holstein-Friesian was the most common dam breed, accounting for 46% of the slaughter population. Although it is not compulsory to record, sire was known for approximately 23% of animal records in BCMS, and this increased to 25% by using other pedigree sources. Across all breeds, the averages for the slaughter population aged from 3 to 36 months for NCW, days to slaughter, average net carcass weight daily gain, CONF and FAT were 323.7 kg, 743 days, 0.45 kg, -R, and +3 respectively. Genetic parameter estimates for NCW, CONF and FAT are shown in Table 1.

Table 1 Genetic parameters for carcass traits NCW, CONF and FAT. Heritabilities on the diagonal, genetic correlation above diagonal and residual correlations below diagonal (standard errors in parenthesis)

	NCW	CONF	FAT
NCW	0.29 (0.038)	0.38 (0.092)	-0.54 (0.117)
CONF (1-15 scale)	0.34 (0.027)	0.24 (0.036)	-0.67 (0.105)
FAT (1-15 scale)	0.20 (0.027)	0.04 (0.025)	0.14 (0.028)

Conclusion There is a wealth of data recorded in the UK, some of it being compulsory, which can have uses other than its original purpose (for instance BCMS), and when combined with other data sources, such as abattoir data, provide added value. The results of this feasibility study indicate that genetic analysis for carcass traits is realistic, particularly for breeds which make up a major part of the carcass population and have sufficient information on the sire. Encouraging the recording of sire identity by farmers in BCMS would further improve the usefulness of future data.

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Whole genome association study for carcass traits in Irish Holstein-Friesian cattle

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Introduction Carcass traits are critical to the biological and economical efficiency of cattle production and as such there is great interest in understanding the underlying genetic architecture influencing animal performance. Genome-wide association (GWA) approaches allow unbiased examination of a large number of genetic variants spread across the entire genome to detect regions associated to a trait of interest. The aim of the study was to identify regions of the genome associated to carcass performance using phenotypes of four economically important carcass traits; carcass weight (CWT), carcass fat (CFAT), carcass conformation (CONF) of progeny as well as cow carcass weight (CULL).

Material and methods Genotypes of 54,001 single nucleotide polymorphisms (SNPs) and predicted transmitting abilities, (i.e. measure of genetic merit), were available for 5,706 Holstein-Friesian sires from Ireland. These animals were representative of the genetic composition of Irish herds. Quality control was performed on both the genotypic and phenotypic data. Following quality control, 44,265 SNPs and data on 941, 768, 936 and 763 sires for CWT, CFAT, CONF and CULL, respectively, remained for analysis. Two statistical methods were used to quantify associations between SNP genotypes and phenotypes. The first method was a single SNP regression model (SSR) that associated each SNP individually with each phenotype in a linear mixed model while accounting for pedigree structure between animals. Association P-values were adjusted to correct for errors arising from multiple testing using the false discovery approach described by Storey and Tibshirani (2003). The resultant q-values (corrected p-value) were used to determine significance of each SNP (q-value < 0.05 (CWT, CFAT, CULL) and q < 0.005 (CONF)). The second statistical approach used was a Bayesian mixture model. This was a modified version of BayesB that allowed incorporation of prior knowledge such as the proportion of SNPs, π, assumed to have no effect on the trait of interest. SNP posterior probabilities (i.e. probability of association) (PP) for each trait were calculated using alternative values of π. SNPs with a PP>0.5 were considered high PP SNPs. The occurrence of each high PP SNP across all priors was estimated and used to get an average occurrence rate of SNPs for each prior. High PP SNPs in the prior with the highest average occurrence rate were deemed associated to a trait. All bovine genes (Btau4.0) within a 500kb region up and downstream of SNPs found significant using the Bayesian approach were identified. These genes were mapped to their human orthologs using the mapping available from hg19. The R package GOseq, without the correction for gene length bias, was used to identify significantly (P<0.05) over-represented KEGG pathways.

Results Significant associations using the SSR approach were detected on all autosomal chromosomes. A total of 2, 25, 27 and 48 SNPs were significantly associated to CWT, CFAT, CONF and CULL, respectively. A total of 44 unique SNPs were found significant using the Bayesian approach; 12, 6, 12, and 15 SNPs for CWT, CFAT, CONF and CULL, respectively. These SNPs explain approximately 6%, 3%, 3.8% and 8.8% of heritable variation for CWT, CFAT, CONF and CULL, respectively. However, validation in an independent population is required. From the pathway analysis, twenty-five different pathways were found significantly over-represented across all analyses. The most significantly over-represented pathways for each trait were the Jak-STAT signalling pathway (CWT), peroxisome proliferator-activated receptor (PPAR) (figure 1) signalling pathway (CFAT), Inositol phosphate metabolism (CONF) and Glutathione metabolism (CULL). The most significantly over-represented pathway was the PPAR signalling pathway ($p=9.58 \times 10^{-4}$). PPAR is a signalling pathway known to be involved in animal growth, in particular growth related to fat metabolism and proliferation.

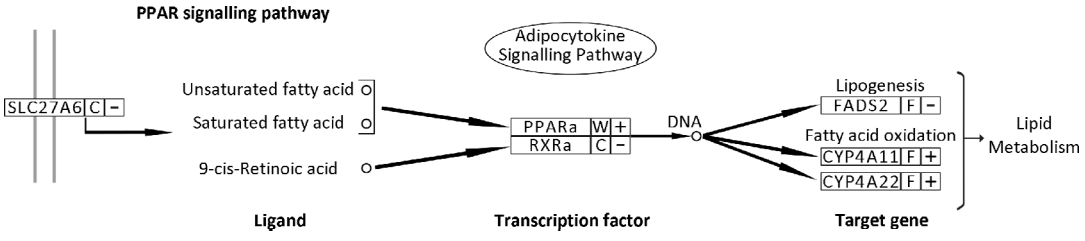

Figure 1 Genes in regions surrounding significant Bayesian SNPs found in the PPAR signalling pathway. Trait that SNP was significant for; W = carcass weight, C = carcass conformation, F = carcass fat. SNP effect; + = positive, − = negative.

Conclusion A number of regions across the genome were found to be associated with each trait using two different statistical methods. A number of genes and key molecular processes involved in growth and carcass traits have been identified. In particular genes associated with the PPAR signalling pathway are found in QTL both positively and negatively associated with several different carcass traits. Future research will involve exploiting high-throughput sequencing to identify causative mutations in these genes that may be responsible for variation in these phenotypes.

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Estimation of genetic parameters for milk yield across lactations in dairy goats

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Introduction Consumption of goats' milk is becoming increasingly popular in the United Kingdom. However, breeding value estimation for goat milk yield is not performed, and selection is based only on phenotypes. Several other studies have applied various methodologies to estimate breeding values for milk yield in dairy goats. These include models for total milk yield, cumulated milk yield (Valencia *et al.* 2005; Rupp *et al.* 2011) as well as for the repeatability of milk yield and random regression models using test day records (Zumbach *et al.* 2008). However, most of the previous analyses were based on relatively small datasets (Valencia *et al.* 2005; Zumbach *et al.* 2008), which may have affected the accuracy of the parameter estimates. The objective of this study was to estimate genetic parameters for milk yield in crossbred dairy goats in lactations one to six. This information will provide a basis for development of routine breeding value estimation for UK dairy goats.

Material and methods The research was based on data provided from two commercial goat farms in the UK. The dataset comprised 464,183 records on 13,591 crossbred dairy goats (Saanen, Alpine, Toggenburg). The pedigree file contained 28,184 individuals, out of which 2414 were founders. The dataset contained test day records of milk yield, lactation number (1 to 6), farm (2 farms), age at kidding (12 to 90 months), year (1987 to 2012) and season of kidding [summer (June to August), autumn (September to November), winter (December to February), and spring (March to May)]. Only goats with more than 3 test-day observations were used for analysis. Lactation length was restricted to 520 Days In Milk (DIM).
Covariance components were estimated with the average information REML algorithm in the ASReml package (Gilmour *et al.* 2009). The following random regression animal model for milk yield was used:

$$y_{ijklm} = \text{farm}_i + \text{yrseas}_j + \mathbf{L}'(DIM_k)\mathbf{b} + \mathbf{L}'(DIM_k)\mathbf{age}_l + \mathbf{L}'(DIM_k)\mathbf{a}_m + \mathbf{L}'(DIM_k)\mathbf{pe}_m + e_{ijklm}$$

where y_{ijklm} is test-day observation at DIM_k of cow m, kidding in year-season j, at age l and producing on farm i; $\mathbf{b}$ is a 1x5 vector of fixed regression coefficients on Legendre polynomials (fourth order) of DIM_k; $\mathbf{age}_l$ is a 1x5 vector of fixed regression coefficients (Legendre polynomials of fourth order) for the effect of age at kidding l; $\mathbf{a}_m$ is a 1x3 vector of random regression coefficients (Legendre polynomials of second order) for the effect of animal m; pe_m is the 1x3 vector of random regression coefficients (Legendre polynomials of second order) for the permanent environment effect of animal m; **L'**(DIM) is the row vector of Legendre polynomials for DIM_k (Kirkpatrick *et al.*, 1990) of size 5, 5, 3, 3 for the four regressions, respectively; e_{ijklm} is the random residual effect.
Standard errors for heritability and correlations were calculated for each estimate according to the methodology proposed by Fisher *et al.* (2004) and interpreted as Frigo *et al.* (2010).

Results Milk yield was the lowest in first lactation with a mean of 3.25±0.003 kg. In subsequent lactations (2nd to 6th lactation) yield increased up to 3.45±0.003 kg. Daily milk yield reached its peak value at around 80 and 60 days with 3.96 kg and 4.43 kg in first and second lactation, respectively. Subsequently after 150 and 80 days it started to decrease in the first and subsequent lactations, respectively. The decrease in the second and subsequent lactations was noticeably faster and yield after 200 days was lower in comparison to the first lactation. Heritability was the highest at 200 and 300 DIM reaching 0.47 and 0.30 in the first and subsequent lactations, respectively. After 300 DIM, the heritability started decreasing to 0.25 and 0.10 at 520 DIM in the first and subsequent lactations, respectively. This was caused by a large increase of permanent environmental variance between 300 and 500 DIM. It increased twofold in the first lactation, and threefold in the second lactation. There was a high genetic correlation between milk yield in the first and subsequent lactations, which reached between 0.66±0.402 and 0.83±0.123. Phenotypic correlation for the two traits was between 0.17±0.051 and 0.46±0.006.

Conclusions This study, using a random regression animal model, found that milk yield in first and subsequent lactations are highly correlated both on the genetic and phenotypic level. Estimates of heritability for milk yield were higher than most of the values reported in the literature (Zumbach *et al.* 2008; Rupp *et al.* 2011), though still in the range reported in this species (0.12 – 0.40). This data should facilitate genetic improvement of milk yield in goats as part of a broader multi-trait breeding programme.

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Lifetime maternal performance in hill sheep is influenced by body reserves

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Introduction Scottish Blackface ewes, much like the Australian Merino, are renowned for their ability to maintain production in environments characterised by extreme weather conditions and periods of severely restricted feed supplies. In Australia the ewe is required to recover from weaning a lamb during the tough summer months when feed supply is at its lowest. Lifetime wool guidelines based on the Australian Merino suggest that ewes with higher body condition entering the mating period will have higher fertility, and better survival during pregnancy with more of their lambs surviving to weaning at heavier weights (Young *et al.* 2011).
The feed availability from UK hill vegetation is typically low during the winter months (Armstrong *et al.* 1997) across the mating and gestation period. Profitability of hill based grazing systems is determined by the maternal performance of the Scottish Blackface ewe. It is hypothesised that high body condition at mating is genetically correlated to higher weaning rates and by selecting for increased body reserves as early as weaning the lifetime maternal productivity of the ewe will be improved.

Methods This analysis focuses on the genetic correlations (r_G) between ultrasound fat and muscle measurements with maternal performance traits. 21,049 Scottish Blackface lambs were ultra-sound scanned for fat and muscle depth at an average of 112 days of age. 6,347 ewe lambs were then managed across 4 reproductive cycles from 1988 to 2011, with first mating at 18 months of age. Ewes were grazed at two properties varying in topography, climate and vegetation, with one based in the Pentland hills, Midlothian and the other located in the Western Highlands (Conington *et al.* 2004; McLaren *et al.* 2012). Ewes were weighed and body condition scored (1 lean to 5 fat) prior to mating in November of each year, with the number of lambs weaned recorded at each cycle. Ultra sound measurements were analysed with an animal model containing the following fixed effects, farm + year + farm by year + sex + rear type (1,2+) + farm by marking grazing group + farm by weaning grazing group + weaning weight (covariate). 3-way interactions between farm, year and the grazing groups were fitted as random terms along with Dam as a permanent environmental effect.
The adult traits, mating weight (MateWT) and body condition score (MateBCS) and number of lambs weaned (NLW) were fitted with an animal model with the fixed effects, farm + year + farm by year + farm by grazing group + previous number of lambs weaned (0,1,2+)*, with the 3-way interaction between farm, year and the grazing group were fitted as random term.
*Previous number of lambs weaned was not fitted to NLW.

Results The body condition of the ewe has a positive influence on maternal performance. An increase in MateBCS by 1 score was associated with an increase in NLW in the corresponding cycle by 19 ± 3.4, 24 ± 3.0, 23 ± 3.3, 30 ± 4.0 lambs/100 ewes across the first four reproductive cycles respectively. Ewe body condition at the first mating was not genetically correlated to NLW in the first cycle (-0.03 ± 0.16). However, after the ewes had weaned a lamb the correlations between MateBC and NLW in the cycle were low to moderate and positive in the second third and fourth cycles with genetic correlations of 0.45 ± 0.18, 0.24 ± 0.21 and 0.41 ± 0.18 respectively.
Fat and muscle at weaning showed moderate positive genetic correlations with mating body condition (Table 1). Selection at weaning for increased fat and muscle will lead to increases in ewe MateBCS throughout the ewe's life. Selection on genetic fat alone will lead to increased condition at mating but also a smaller ewe (Table 1). Whilst fat and muscle in the lamb were moderately correlated with MateBCS the genetic correlations with NLW were weak.

Table 1 Genetic correlations between weaning ultra-sound fat depth, muscle depth and maternal performance traits across the first four reproductive cycles in Scottish Blackface ewes with standard errors presented in the parentheses.

		Weaning Fat			Weaning Muscle		
Reproductive Cycle	Ewes	MateWT	MateBCS	NLW	MateWT	MateBCS	NLW
First	5280	-0.28 (0.05)	0.41 (0.06)	-0.23 (0.11)	0.31 (0.04)	0.56 (0.05)	-0.13 (0.10)
Second	5030	-0.35 (0.05)	0.26 (0.07)	0.13 (0.10)	0.26 (0.05)	0.61 (0.06)	0.24 (0.12)
Third	4461	-0.35 (0.05)	0.37 (0.08)	0.05 (0.10)	0.18 (0.05)	0.56 (0.08)	0.10 (0.11)
Forth	3258	-0.31 (0.05)	0.40 (0.09)	0.14 (0.12)	0.15 (0.06)	0.46 (0.09)	0.23 (0.10)

Conclusion Ewes that have more condition at mating will wean more lambs. By selecting for increased fat and muscle reserves as early as weaning, improvements can be made in ewe body condition across its reproductive life. Improving the genetic merit for fat and muscle will breed a ewe that will recover from the previous reproductive cycle quicker and wean more lambs across its lifetime.

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Including lamb survival as a breeding goal in extensive sheep breeding programmes

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Introduction The survival of lambs in extensive sheep production systems is a major contributing factor to the economic efficiency of these farms and is an indicator of good animal welfare. Estimates of lamb pre-weaning mortality vary considerably between 10 and 30% and most of these mortalities occur within the first 3 days of postnatal life (Dwyer, 2007). Improving lamb survival through breeding is very relevant for extensively-managed flocks such as hill breeds, or for flocks where minimal intervention by man is practised. This is because it identifies individuals and families of sheep that differ in their inherent ability to lamb unaided, nurture and rear their young, as a well as identifying offspring that are less viable at birth and those that have either very low or very high birth weights (Conington *et al*., 2010; Dwyer and Lawrence, 2005, Dwyer 2007). Having lamb survival integrated into existing sheep breeding programmes facilitates the monitoring of it alongside that of other traits in the breeding objective. The objective of this project was to mine the current Signet-recorded Blackface data set to estimate the genetic properties of lamb survival, to define the trait to be used and appropriate model, and at the same time, to investigate the key factors influencing lamb survival.

Material and methods Data from Signet's Sheepbreeder performance recording scheme held on the BASCO database for the Blackface breed was used for this study. The final data set after editing to eliminate improbable records comprised 173,895 lamb records from 1976-2011 including 49,917 lamb birth weight records. A lamb survival (SURV01) trait was coded 0=dead if any lamb recorded at birth had no live weight recorded at either 8 weeks (early weight) or subsequent weighing occasions (scan weight), and 1=alive, if it survived at least until 8 weeks with a record of live weight. Lamb survival accounting for lambs known to be born dead (SURV12) from 27,111 lambs born 2007-2011, was coded 0=lambs known to be born dead, 1=born alive but no subsequent live weights (so assumed dead) and 2=lambs born with live weights. GENSTAT (Payne, 2009) Generalised Linear Mixed Model (GLMM) for binomial trait distribution was used to fit a logit transformation function to predict the probability of the effect of each factor for SURV01. All known factors significantly affecting lamb survival were included in the analyses. These were flock, year/season, age of dam, sex of lamb, birth type and birth weight (covariate). Year/season was fitted as a random effect together with dam of lamb for these analyses. The heritability (h^2) of SURV01 and SURV12 was estimated using an animal model with (+) and without (-) maternal heritability m^2 accounted for in ASREML (Gilmour *et al*., 2001) animal breeding software. For this, the data were restricted to 89,819 lamb records between 2000-2011 with 29,532 dams and 1943 sires from 29 flocks.

Results Lamb survival was 87.8% for the SURV01 and SURV12 traits, with 5.5% lambs identified as being born dead in the SURV12 2007+ data set. Compared to male lambs, female lambs have a 1.3 times better survival odds (Figure 1), a similar finding to that of Sawalha *et al*., (2007). Lambs from 3 year old ewes are 1.4 times more likely to survive compared to lambs from 2 year old ewes and have the highest survival odds compared to all other ewe ages (Figure 2). Twin lambs had higher survival odds compared to single (1.2 times) or triplet-born (1.8 times) lambs although this did not hold true when birth weight was omitted from the model, with twin lambs having lower survival odds (0.82) compared to single born lambs. The direct h^2(s.e) of SURV01(-) was 0.05(0.015) but dropped to 0.01(0.006) when m^2 was included in the model (0.08)(0.03) giving a total h^2 of 0.05 and 0.09 for SURV01(-) and SURV01(+) respectively. No significant differences were observed in parameter estimates between SURV01 and SURV12 (data not shown).

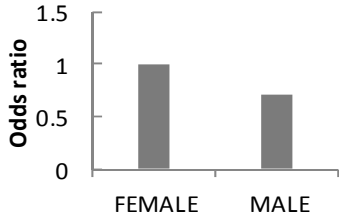

Figure 1 Survival odds of male vs female lambs

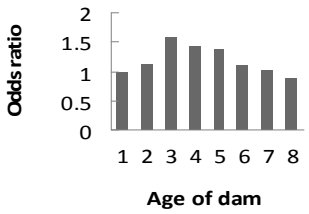

Figure 2 Survival odds acc. different dam ages

Conclusion Although variance components of lamb survival are small, sheep breeding programmes should include estimates of both direct and maternal lamb survival as the latter has a significant part to play in the genetic improvement of this trait. New management strategies could be deployed to manage male lambs separately from females in a similar way that twins are preferentially managed on hill farms, with quantifiable benefits for lamb survival.

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Effect of folic acid supplementation in the laying hen diet on the long chain n-3 fatty acid composition of eggs

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Introduction Enriching eggs with long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) is a viable means of increasing population intakes of these essential nutrients and can be achieved by the addition of fish oil or fishmeal to the hens' diet (van Elswyck, 1987), but this practice is expensive and may be unsustainable. Adding the precursor α-linolenic acid (LNA) to the hens' diet would be more cost effective and sustainable if nutritionally significant amounts of LC n-3 PUFA were then synthesised by the bird and deposited in the egg. However, supplementation of the hen diet with LNA resulted in only limited enrichment of eggs with LC n-3 PUFA (Antruejo *et al*., 2011). Factors controlling the conversion of LNA to LC n-3 PUFA in the hen are unclear, but lower folate status has been associated with altered patterns of DNA methylation and gene-specific dysregulation of transcription (Burdge *et al*., 2007). This may explain why folic acid supplementation was observed to increase the C22:6 (DHA) status of rats (Pita and Delgado, 2000). The objective of this experiment was therefore to determine the effect of folic acid (FA) supplementation of laying hen diets on the LC n-3 PUFA content of eggs.

Materials and methods Experimental details of the birds and diets are described in Hoey *et al*. (2009). Briefly, 48 hens (30-week old Hy-line brown laying hens, n=8 per treatment) were randomly allocated to one of six dietary treatments, consisting of a basal feed (commercial, crumbed pellet containing approximately 1 mg FA/kg feed) sprayed with incremental amounts of folic acid (0, 2, 4, 8, 16, 32 mg folic acid/kg feed). Whole eggs (minus shell) from six individual birds per treatment were then collected at 0, 6 and 12 weeks during the study and freeze dried. Fatty acid composition of dried egg samples was then analysed by one-step extraction and methylation (Sukhija and Palmquist, 1988), and the resulting fatty acid methyl esters were separated and identified by gas chromatography. Concentrations of individual fatty acids were calculated (mg fatty acid/ 50 g fresh egg weight) and the effect of dietary FA concentration, study week (W), and interaction between FAxW on the concentrations of individual fatty acids were then determined by analysis of variance. The relationship between the FA concentration of the diet and the DHA and LC n-3 PUFA content of the egg was determined by regression analysis.

Results There were no significant interactions between study week and dietary FA concentration. Egg LC n-3 PUFA contents declined ($P<0.001$) as the study proceeded. There was a significant ($P<0.001$) but inconsistent effect of FA addition on the fatty acid composition of eggs (Table 1). There was no linear relationship between diet FA concentration and either egg DHA ($R^2=0.03$) or LC n-3 PUFA ($R^2=0.032$) content.

Table 1 Effect of dietary folic acid concentration and study week on the fatty acid composition of eggs (mg fatty acid/50 shelled egg, fresh weight basis).

Fatty	Folic acid (FA) added to diet (mg/kg diet)						SEM	P	Study week (W)			SEM	P
acid	0	2	4	8	16	32			0	6	12		
C16:0	4486	4545	4391	4820	4881	4649	83.2	0.003	4487	4512	4688	57.0	0.027
C18:0	1448	1435	1320	1528	1394	1481	29.8	<0.001	1433	1373	1497	20.4	<0.001
C18:1 n-9	6653	6745	6460	6800	6609	6929	119.3	0.094	6385	6646	7066	83.8	<0.001
C18:2 n-6	2475	2571	2521	2683	2579	2681	62.3	0.064	2691	2475	2560	42.7	0.002
C18:3 n-3	152	151	149	164	148	157	3.8	0.016	160	146	154	2.6	<0.001
C20:4 n-6	302	300	282	321	301	298	7.4	0.010	308	291	302	5.1	0.056
C20:5 n-3	3.9	4.8	3.4	5.3	4.1	6.3	0.47	<0.001	5.3	3.9	4.6	0.32	0.013
C22:5 n-3	22.7	23.2	19.6	23.0	20.8	23.9	0.92	0.007	25.6	19.7	21.3	0.64	<0.001
C22:6 n-3	138	145	134	153	146	151	3.5	<0.001	156	134	143	2.4	<0.001
LC n-3 PUFA*	165	173	157	181	171	182	4.2	<0.001	187	158	169	2.8	<0.001

*Calculated as the sum of C20:5 n-3, C22:5 n-3 and C22:6 n-3.

Conclusions Supplementing hen diets with FA did not enrich the LC n-3 PUFA content of the egg.. However, the effect of increasing dietary LNA content (in combination with increased FA) on the conversion of LNA in the hen should be investigated.

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The effect of growth rate and feed composition on *Campylobacter* colonisation in chickens

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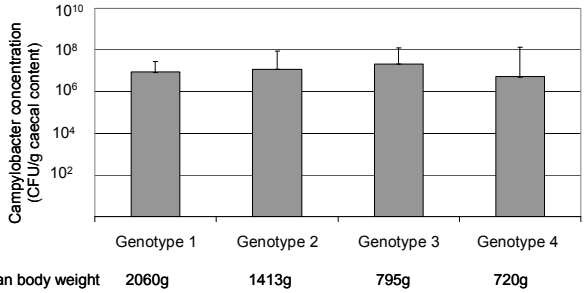
Introduction In the UK, the annual financial burden of Campylobacter enteritis in the human population is over £500 million. Through outbreak investigation, routine animal and food testing and source attribution studies there is general acceptance within the scientific community that a large number of human infections are linked to the chicken reservoir as a whole. Consequently, a great deal of effort has been and is currently being made throughout chicken production, along the whole food chain supply, to reduce the prevalence and concentration of Campylobacter within this reservoir. However interventions have only been successful in part; and clearly the dynamics controlling Campylobacter shedding in poultry have not been fully elucidated. As a result, a greater understanding of Campylobacter within this host is needed.
The aim of the current study was to examine two factors that may affect the colonisation of chickens with Campylobacter: 1) the role of growth rate of chickens, and 2) the role of feed composition and the interaction with different commercial broiler breeds.

Material and methods Two independent trials were carried out in duplicate on different trials farms (internal research facilities). Prior to bird placement, the farms were screened and shown to be positive for Campylobacter contamination and therefore presented a natural exposure. Trials were set up to address the above questions:
Trial 1 Thirty birds from each of four chicken genotypes with different mean growth rates (Genotype 1: 51 grams/day; Genotype 2: 34 grams/day, Genotype 3: 19 grams/day, Genotype 4: 18 grams /day) were penned at one day of age and were grown for six weeks under the same conditions. At 42 days of age, 15 birds per genotype were killed and caeca removed asceptically. Caecal contents were serially diluted, plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA) and Campylobacter colony forming units (CFU) per gram of caecal content calculated.
Trial 2 To explore effects of feed composition, eight different bird genotypes (denoted 1 to 8) were penned with each genotype exposed to two different feed compositions. Bird genotypes comprised birds of contrasting growth rate upto 35 days [from 32 grams/day (slow grow) to 71 grams/day (fast grow)] and all genotypes fed two types of diets: 100% balanced protein (wheat based) and 90% balanced protein (maize based). Feed differed in terms of cereal base as well as crude protein content. Each genotype was exposed to each feed treatment independently. After six weeks of growth, five birds per line, per feed group were killed (80 in total), caeca removed asceptically and Campylobacter enumerated as previously described.
Data from both trials were analysed in a REML variance components analysis (GenStat 14th Edition). CFU data were log transformed to improve normality. The model fitted included fixed effects of feed, genotype and interaction term, plus the covariate of body weight. Model interpretation was based on log transformed data but for ease of interpretation, non-transformed average CFU are presented.

Results *Trial 1.* Across genotypes 1-4, the mean Campylobacter concentrations were 8.9 x 10^6 CFU/g, 1.2 x 10^7 CFU/g, 2.2 x 10^7 CFU/g and 5.4 x 10^6 CFU/g, respectively (Figure 1). No significant differences between genotypes were observed ($P > 0.05$), despite significant differences in mean body weights between bird genotypes.
Trial 2 Campylobacter concentration did not significantly differ between bird genotypes ($P > 0.05$) (Figure 2). In addition, no significant interaction was observed between bird genotype and feed type.



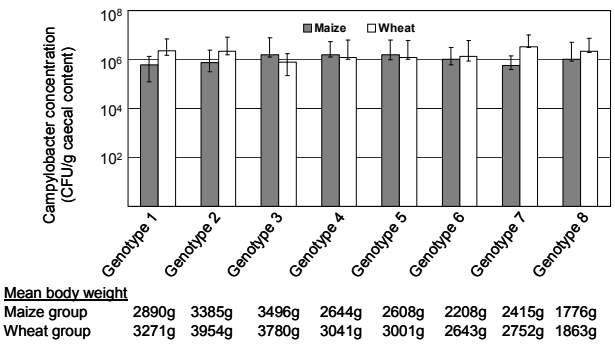


Figure 1 Mean Campylobacter levels by bird genotype. **Figure 2** Mean Campylobacter levels by genotype and feed type.

Conclusions These results demonstrate that in two independent repeated trials, following a natural exposure to the bacteria, the growth rate of chickens did not appear to play a role in the colonization and subsequent proliferation of Campylobacter within the caeca. Furthermore, in seeking to determine the differences in Campylobacter concentration after a natural challenge plus the impact of slowing growth rate through feed composition (and therefore rate of passage) we found no significant differences between chicken genotypes. The results indicate that other underlying reasons and mechanisms beyond growth rate and feed composition are likely to control and affect Campylobacter colonisation within the poultry host. Further studies to gain a better understating of colonisation dynamics and subsequent proliferation within poultry will aid the elucidation of why Campylobacter are a successful 'commensal' of poultry.

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Use of an implanted temperature-ID chip to estimate core body temperature in broiler chickens exposed to moderate heat stress

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Introduction Increased thermal loads imposed upon animals may induce heat stress leading to an increase in core body temperature (Swick, 1998). Thus core body temperature (measured either directly or indirectly) is an important index of the degree of heat stress and thus the welfare of the individual. Surgically implanted radio-telemetry data loggers have been used to give continuous, reliable measurement of core body temperature. The advent of temperature-ID chips injected intramuscularly into the animal offers a less painful, minimally invasive and rapid tool to estimate core body temperature which, if validated, might serve as a replacement for the use of data loggers or other devices involving more invasive procedures. The objective of this study was to evaluate the use of temperature-ID chips against surgically-implanted data loggers in estimating core body temperature of broiler chickens under conditions of moderate heat stress.

Material and methods Twelve female Ross broiler chickens (46 days old, average weight of 2.4kg) were used in a 2 × 2 factorial design experiment, with two levels of temperature (normal = 20°C and high = 30°C) and two levels of relative humidity (RH) (dry = 40% and humid = 70%) designed to impose heat stress for a period of 3 hours /day for three consecutive days using four identical climate chambers. Each bird was subject to surgery under surgical anaesthesia to implant a temperature logger device (Tiny tag, Gemini Talk 2, Omni instruments, UK) into the body cavity and a temperature-ID chip (Identichip®, Animalcare, York, UK) 3cm deep into the left breast muscle. Data were collected on core body temperature (CBT) at five phases of the heat stress protocol, namely pre heat stress (PrHS, conditions were 20°C/40% RH for all treatments), step up (ST, increase in temperature/RH over a 1-hour period), end of 3 hours of heat stress (3HS), step down (SD, decrease in temperature/RH over a 1-hour period) and post heat stress (PHS, 1 hour after the end of SD). The change in CBT was obtained by subtracting the CBT at PrHS from CBT at subsequent phases and this was analysed by repeated measures ANOVA. Correlation and regression analysis was also used. A Bland–Altman plot was used to validate the estimation of change in CBT from PrHS to 3HS measured by the two methods.

Results All temperature–ID chips functioned effectively while two loggers were faulty and data could not be retrieved from them. The change in CBT of the birds was highest (P<0.05) at the 3HS and SD phases (Figure 1). Although CBT-chip and CBT-logger were not correlated (P >0.05), a significant (P<0.05) positive correlation existed between change in CBT-chip and change in CBT-logger (R^2 = 0.71) which was accompanied by regression equations (P<0.05) namely change in CBT-logger = 0.171 + 0.749 change in CBT-chip. A Bland-Altman plot showed that the mean change in CBT-chip was 0.1°C less than that of the CBT-logger (Figure 2).

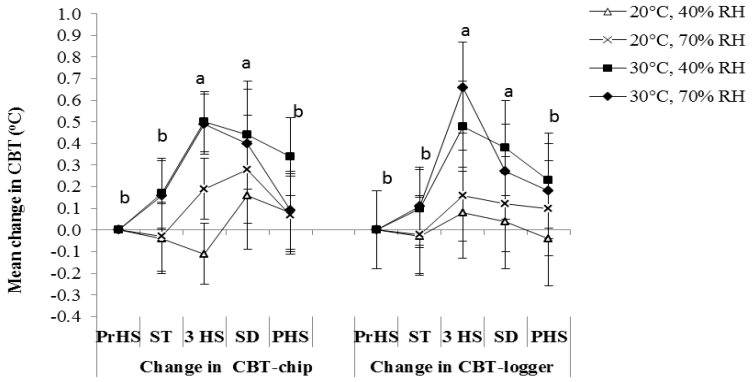

Figure 1 Change in core body temperature (mean ± 1 SEM) of broiler chickens at the different phases of the heat stress protocol as measured by the chip and data logger
Means with different letter are significantly different at P<0.05.

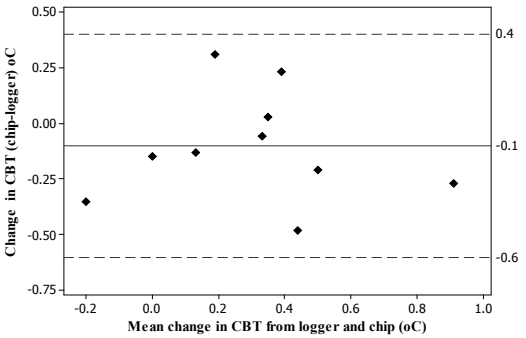

Figure 2 Bland-Altman plot of the change in core body temperature measured by the logger and chip (n=10)

Conclusions: Core body temperature measured from a data logger implanted deep inside the body cavity can be reliably predicted from the change in core body temperature measured from a temperature-ID chip so this method could be used to monitor sentinel birds under conditions of moderate heat stress.

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Comparisons of contrasting broiler hybrids on growth rate, feed efficiency and lean muscle deposition

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Introduction The modern broiler has undergone long term, balanced genetic selection for multiple welfare traits in addition to performance traits such as growth and FCR. There is growing global interest in the use of alternative slower growing broilers in particular for speciality or niche markets for which a broad gene pool is required. Fleming *et al.* (2007) showed that genetic selection of modern broiler pure lines has led to beneficial improvements in body composition resulting in a leaner carcase. The objective of this experiment was to compare a conventional broiler hybrid against slower growing alternative hybrids and to investigate the effect of growth rate on efficiency and carcase characteristics.

Material and methods Three modern broiler hybrids were categorised according to their growth potential: Standard; Intermediate; and Slow. Day old chicks were randomly allocated to one of 30 pens (10 replicates per hybrid). Birds were started at 80 birds per pen and stocking density throughout the experiment did not exceed 34 kg/m^2. Birds were offered *ad libitum* access to a maize based crumble diet chosen to represent a globally used broiler diet. A three phase feeding programme was used: Starter (12.6 MJ ME/kg; 205 g/kg CP) from 0 to 10 days, Grower (13.2 MJ ME/kg; 182 g/kg CP) from 10 to 25 days and Finisher (13.4 MJ ME/kg; 163 g/kg CP) from 25 to 53 days. Birds were reared in a conventional, temperature controlled house from day old to 53 days and no antimicrobials were used. Bodyweights and feed intake were recorded at 10, 25, 32, 39, 46 and 53 days. Standard broilers were grown to 46 days whereas Intermediate and Slow broilers were grown to 53 days to ensure that birds from all three hybrids were processed at similar liveweights. Ten birds per pen were selected at random for processing at 32 days (Standard), 46 days (Standard, Intermediate and Slow) and 53 days (Intermediate and Slow) to evaluate carcase characteristics. Birds were weighed individually at point of slaughter and eviscerated carcase, abdominal fat pad and breast muscle weights were recorded to each individual bird. Processing performance for Standard and Slow was corrected by interpolation to a common weight of 2kg. Data were analysed by ANOVA, unbalanced design in GenStat (14th Edition).

Results Farm and processing performance data are presented as hatched. Figure 1 shows the growth curves for each of the three broiler hybrids. The number of days to reach 2kg liveweight was 34.7, 37.9 and 47.0 for Standard, Intermediate and Slow respectively. Table 1 shows farm and processing performance at 46 days. Broilers of the Standard hybrid grew faster (P = 0.001) than those of the Intermediate and Slow hybrids. Both Standard and Intermediate utilised feed more efficiently (P = 0.001) and showed better (P = 0.001) eviscerated and breast meat yields than Slow. There was no effect of growth rate on liveability or abdominal fat pad yield at 46 days. However, when corrected to 2kg, abdominal fat pad yield was greater (P = 0.002) in Slow compared to Standard (2.43 vs. 1.95 %, SEM 0.088).

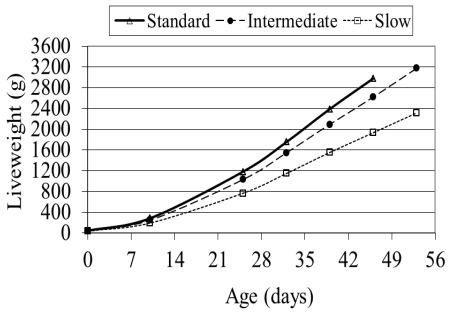

Figure 1 As hatched growth curves for standard, intermediate and slow growing broiler hybrids

Table 1 As hatched farm and processing performance of standard, intermediate and slow growing broiler hybrids at 46 days[1]

	Broiler hybrid				
Item	Standard	Intermediate	Slow	P - value	SEM
Liveweight (g)	2979^a	2621^b	1934^c	0.001	74.3
FCR	1.79^a	1.79^a	1.92^b	0.001	0.010
% Liveability at 2kg	97.9	98.9	98.4	0.530	0.61
% Eviscerated yield[2]	72.4^a	72.0^a	69.9^b	0.001	0.14
% Breast meat yield[2]	20.3^a	20.6^a	17.2^b	0.001	0.17
% Abdominal fat pad[2]	2.21	2.17	2.27	0.460	0.060

^{abc} Within a row, means without a common superscript differ (P < 0.05), [1]Values are least square means (n = 10), [2]Expressed as % of liveweight

Conclusions The Standard broiler hybrid grew faster, as expected, and was shown to be more efficient in utilising feed leading to improved deposition of lean muscle, such as breast meat, with lower levels of abdominal fat when compared to the Slow growing hybrid. The correlation of faster growth rate with lower abdominal fat yield observed in this experiment is in agreement with the observations of Fleming *et al.* (2007), who demonstrated that modern broiler pure lines grew faster and were leaner than their 1972 genetic control line counterparts, when compared under the same environmental conditions.

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Utilization of distiller's dried grains with soluble (DDGS) supplemented with enzymes on growth performance of broiler chickens

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Introduction Rano *et al*., (2012) have reported reduced feed intake and body weight gain in broilers consuming diets containing over 20% DDGS. This was attributable to poor feed digestibility and utilization, suggesting a role for enzymic improvement The inclusion of exogenous enzymes in wheat-based broiler diets is an increasingly common practice. Research has shown that supplementation of diets with enzymes capable of degrading the xylan improves the nutritive value of DDGS and wheat-based diets for broilers, resulting in improved performance (Annison, 1991). Therefore, this study aimed to determine the effects of feeding diets containing DDGS supplemented with enzymes on growth performance of broilers and the interaction between DDGS levels and enzyme supplementation.

Material and methods A completely randomized design with 3 X 2 factorial arrangements (3DDGS levels; 0, 15, 30 and 2 enzyme levels -, +) was used. One hundred and sixty eight day-old broiler chickens, vaccinated at day old at the hatchery against Infectious Bronchitis, were used for the experiment which lasted for 6 weeks. The chicks were individually weighed and assigned to 6 groups (treatments) of 28 chicks each. Each group was further divided into 4 sub-groups (replicates) of 7 birds. Birds were housed in floor pens containing wood shavings as litter material. Three isonitrogenous diets were formulated for the study by replacement of wheat and soya by DDGS, giving varying metabolizable energy and fibre content, and manufactured with or without enzyme inclusion (endo-1, 4-beta-xylanase 9200 U/g, alpha-amylase 1600 U/g and subtilisin (protease) 16000 U/g). All the diets were analysed for proximate composition. The diets and clean drinking water were provided *ad libitum* throughout the study. Feed intake, weight gain and feed conversion ratio were measured. All data were compared for significance at $P<0.05$ by GLM factorial analysis, using the Minitab 16 statistical package.

Results There were significant effects of DDGS inclusion level in all the parameters observed in the experiment (Table 1). A 30% inclusion, though not a 15% inclusion, reduced feed intake, live weight gain and feed efficiency. However, there was no significant main effect of enzyme inclusion on any of the parameters measured.

Table 1 Means for the main Effects of DDGS Levels & Enzyme on the performance of broilers fed experimental diets

	Diets								
	DDGS Levels (%)					Enzyme Inclusion			
Parameters	0	15	30	SEM	P value	No Enzyme	+Enzyme	SEM	P value
Feed Intake (g/b/d)	111.8^{a}	118.5^{a}	75.4^{b}	4.50	0.001	101.0	102.9	3.67	0.718
Weight Gain (g/b/d)	72.5^{a}	70.9^{a}	39.1^{b}	2.07	0.001	62.1	59.6	1.69	0.294
Feed Conversion Ratio	1.5^{b}	1.7^{b}	2.1^{a}	0.08	0.001	1.7	1.9	0.07	0.060

^{abc} Means bearing different letter superscripts within rows are significantly different ($P<0.05$), SEM=Standard Error of means

Table 2 Means for the DDGS Levels x Enzyme on the performance of broilers fed experimental diets

	Enzyme Inclusion							
	No Enzyme			+Enzyme				
	DDGS Levels (%)							
Parameters	0	15	30	0	15	30	SEM	P value
Feed Intake (g/b/d)	106.1	125.3	71.5	117.5	111.8	79.3	6.36	0.135
Weight Gain (g/b/d)	69.2^{ab}	78.9^{a}	38.3^{c}	75.9^{ab}	62.9^{b}	39.9^{c}	2.93	0.003
Feed Conversion Ratio	1.5	1.6	1.9	1.6	1.8	2.2	0.12	0.390

^{abc} Means bearing different superscripts within rows are significantly different ($P<0.05$). SEM=Standard Error of means

The DDGS x enzyme interaction was not significant for feed consumption and feed conversion ratio, meaning that the two variables acted independently. However, there were a significant interaction ($P<0.05$) on daily weight gain, where birds on 15% DDGS with no enzyme gained the highest weight of 78.9g/d while birds on 30% DDGS with no enzyme had the lowest gain of 38.3g/d.

Conclusions The inclusion of 15% wheat DDGS in the diet supports higher feed intake and body weight gain of broilers, but higher inclusion impairs performance. Addition of the enzyme used in this study at manufacturer's recommended level to wheat based DDGS diets especially at high level resulted in no significant improvement in performance of broiler chickens. However, the inclusion rates for this enzyme and other enzymes to high levels of wheat DDGS merit further investigation.

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An evaluation of performance and uric acid nitrogen on broilers given zinc supplementation

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Introduction Negative effects of manure from poultry houses such as odours and ammonia volatilization pose a nuisance and health risk to neighbours. Reduced performance of poultry can also result from high level of volatilization hence the proper handling of manure is of concern to environmentalists. The use of additives containing sulphates such as $ZnSO_4$ are potential producers of H_2S under anaerobic conditions (Smith *et al*., 2001) therefore considerations for the use of ZnO assume it to be a possible better additive in reducing NH_3 emissions and improving poultry performance arising from its non sulphate content. There is a dearth of information on the effects of ZnO supplementation on broiler performance and NH_3 volatilization hence the objectives of this study were to determine the effects of Zinc on the performance of broiler chickens and uric acid N in the manure of broiler chickens.

Material and methods Two hundred and Twenty Five, one week old chicks were allotted to six iso-nitrogenous dietary treatments containing 40, 50, 60, 70 and 80mg zinc per kg respectively. The treatments were replicated thrice with 15 chicks per replicate in a completely randomized design. The animals were fed *ad libitum* and necessary medications and routine vaccinations were administered as recommended by the Ahmadu Bello University Veterinary Hospital. Feed intake and body weights were obtained on a weekly basis using a top loading scale; leg deformity was recorded while feed conversion ratio and weight gains were calculated on a weekly basis. Manure was collected from each treatment at the fourth week and eighth week, properly mixed and then analysed for zinc. Faecal samples obtained at the eighth week of the experiment were also analyzed for nitrogen using the Kjeldahl method (Bremner and Mulvaney, 1982). Data were analysed using the GLM of Statistical Analysis System (2001) and significant differences between treatment means were separated with Duncan Multiple Range test.

Results Performance results as shown in Table 1 indicate that feed intake, final weight and weight gain of birds on the 40mg/kg Zinc and 50mg/kg zinc diets were lower ($P<0.05$) than those of 60mg/kg and 70mg/kg zinc diets. Birds on 70mg/kg zinc performed the best ($P<0.05$) while those on 80mg/kg zinc performed the least in terms of feed intake, final body weight, weight gain. Feed conversion ratio was also the best ($P<0.05$) among those on 70mg/kg zinc. The effect of zinc on manure uric acid nitrogen and manure zinc as displayed in Table 2 showed that uric acid nitrogen and zinc retained in the manure of birds fed 40, 50, 60 and 70mg/kg dietary zinc showed a progressive but non significant ($P>0.05$) increase as the levels of dietary zinc increased. The percentage nitrogen in manure from the birds on 80mg/kg zinc was significantly highest ($P<0.05$).

Table 1 Performance of broiler chickens fed varying levels of zinc

Parameters	40mg/kg Zn	50mg/kg Zn	60mg/kg Zn	70mg/kg Zn	80mg/kg Zn	SEM	P
Initial weight (g/bird)	100.00	100.00	100.00	100.00	100.00		
Final weight (g/bird)	1860^{c}	1850^{c}	1930^{b}	2040^{a}	1770^{d}	6.52	0.0001
Weight gain (g/bird)	1760^{c}	1750^{c}	1830^{b}	1940^{a}	1670^{d}	6.52	0.0001
Weight gain (g/bird/day)	31.43^{c}	31.25^{c}	32.68^{b}	34.64^{a}	29.82^{d}	0.12	0.0001
Feed intake (g/bird/day)	76.01^{b}	75.77^{b}	77.92^{ab}	79.76^{a}	72.38^{c}	0.01	0.0005
Feed Conversion Ratio	2.42^{b}	2.42^{b}	2.39^{b}	2.30^{a}	2.42^{b}	0.01	0.0289
Leg deformed (%)	0.00^{a}	0.00^{a}	2.22^{a}	2.22^{a}	44.40^{b}	0.02	0.0001

^{abcd} Means within row with different superscript differ significantly ($P<0.05$)

Table 2 Effect of Zinc on Uric-Acid Nitrogen and Manure Zinc

Parameters	40mg/kg Zn	50mg/kg Zn	60mg/kg Zn	70mg/kg Zn	80mg/kg Zn	SEM	P
Manure Nitrogen (%)	6.21^{b}	6.25^{b}	6.27^{b}	6.37^{b}	6.62^{a}	0.10	0.017
Manure Zinc (%)	0.05	0.07	0.06	0.08	0.08	0.00	0.91

^{ab} Means within row with different superscript differ significantly ($P<0.05$)

Conclusion The supplementation of Broiler diets with Zinc up to 70mg/kg is possible and enhances weight gain, feed conversion ratio and final weight. Manure nitrogen though better retained at 80mg/kg zinc inclusion is not recommended due to its associated leg challenge.

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Effects of dietary polyunsaturated n-3 and n-6 fatty acids on hatched egg weight loss of native broiler

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Introduction It is important to be able to measure the quality of broiler hatching eggshells in relation to their physiological functions. Various methods have been employed to determine eggshell quality. Incubational egg weight loss estimates eggshell porosity (Peebles *et al*., 1998). The cuticle of the eggshell has been shown to significantly influence the rate of water loss from eggs during incubation (Peebles and Brake, 1986) with subsequent effects on embryogenesis. Because the eggshell cuticle is composed largely of lipid, the fat content of the hen's diet may also modify the cuticle's impact on egg water loss and embryogenesis. Eggshell water vapour conductance is an accurate measure of the eggshell's functional ability to resist water vapour passage. Therefore, the objectives of this research were to determine the effects of eggshell cuticle removal and dietary fat source on egg water loss.

Material and methods A total of 72 Iranian native broiler breeder hens were fed a breeder diet from the 21st week of age. Breeder hens were fed 1 of 3 diets: commercial breeder diet with 15% extrusion flaxseed (ALA group), or 4% soybean oil (LNA group), or commercial breeder diet as control treatment (CON group). The determination of incubational egg weight loss was conducted using eggs collected from 27 to 32 week of age. Throughout the experimental period, 2106 eggs were collected for the determination of hatchability, embryonic mortality, and incubational egg weight loss. Of these eggs, 30 eggs per treatment (90 total eggs) were used for the measurement of incubational egg weight loss. After being allowed to reach room temperature, 50 % of the eggs, collected from each pen for the above determination, served as controls, while the others were washed to remove the eggshell cuticle. Cuticle removal was carried out by egg washing in a sodium hypochlorite solution according to the procedure of Peebles and Brake (1986). After washing, the eggs were rinsed, allowed to dry, and then weighed. Washed and unwashed eggs were set in a common incubator to investigate the egg weight loss. For measuring the egg weight loss, eggs were individually weighed before setting and weighed again On Days 6, 12, and 18 of incubation. Weight loss was calculated as a percentage of preset egg weight on a per day basis. The data were analyzed with a completely randomized design using the GLM procedure of SAS software (version 9.1, SAS Institute, 2003).

Results Our results indicated that the supplementation of native breeder hens with polyunsaturated fatty acid sources decreased incubational egg weight loss in unwashed eggs significantly ($P<0.01$), whereas no difference between dietary n-3 and n-6 fatty acid sources was observed. Dietary treatment did not have effects on incubational egg weight loss when eggshell cuticle was removed. As lipids are a major component of the eggshell cuticle, the effects of cuticle removal on eggs taken from hens fed different types of dietary fat would be expected to differ. However, in this study, the effects of cuticle treatment on incubational egg weight loss were influenced by dietary polyunsaturated fatty acid sources.

Table 1 the percentage of egg weight loss between Day 0 and Days 6, 12, and 18 of incubation.

	Egg weight loss					
Eggshell cuticle	Intact			Remove		
Day of incubation	6	12	18	6	12	18
ALA	3.85[b]	11.32[b]	14.98[b]	6.35	11.29	20.66
LNA	3.28[b]	10.17[b]	13.42[b]	4.38	11.69	15.24
CON	5.16[a]	14.11[a]	17.46[a]	4.59	11.86	15.06
SEM	0.293	0.669	0.888	0.66	1.86	1.62
P-value	0.0021	0.00038	0.0052	0.117	0.969	0.053

Conclusion The present study demonstrates that dietary polyunsaturated fatty acid sources modify the eggshell cuticle and decreased incubational egg weight loss in unwashed eggs.

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The effect of *Bacillus subtilis* on growth rates and body composition of broiler chickens

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Introduction The use of *Bacillus subtilis* as a feed supplement has been shown to improve feed efficiency and growth performance whilst reducing abdominal fat and cholesterol concentrations in the liver and carcass of broiler chickens (Santoso *et al*. 1995). *B. subtilis* strain KD1 isolated from broilers, when used as a feed supplement, was linked to the maintenance of a normal intestinal microflora with an increase in lactobacilli, known for their anti-inflammatory and anti-cancer properties, and a decrease in *Escherichia coli* (Wu *et al*. 2011). This study investigated the effect of the GRAS registered (Westers *et al*. 2004) *B. subtilis* (*Bacillus natto* Sawamura) as a probiotic feed supplement on: breast, leg, heart, liver and spleen weight and live weight gain in broiler chickens as well as the effect on pathogenic bacteria reported in a companion paper (Horton *et al*. 2013).

Material and methods Ross 308 broiler chicks (n = 200) were weighed and 10 euthanised as the day 0 initial slaughter group with organs dissected and weighed. The remaining chicks (*n*= 190) were divided into two groups and fed either commercial feed or feed supplemented with *B. subtilis* spores to provide 10^7 CFU/g as a starter, grower and finisher ration. These rations were supplied for 11, 15 and 10 days, respectively. The broiler facility consists of 4 self-contained berths, each of which were split into two pens. Treatment was allocated at random to each of these pens, with 22 – 25 chicks per pen. Temperature, lighting, humidity and ventilation were all controlled and set-up to simulate commercial broiler production. *Campylobacter* positive top-litter (100g) from 4 broiler farms was introduced to all pens. Appropriate feed and water was provided *ad libitum* via bell feeders and nipple drinkers with feed intake per pen monitored daily. On days: 14, 21, 28 and 36 five birds from each pen were weighed, euthanised and dissected. The whole breast without bone and skin, legs with feet and without skin above the hock, heart, and liver and spleen were removed and weighed to the nearest 0.01g. Live weights of all birds were recorded weekly on days: 0, 7, 14, 21, 28, and 36. Mean weight values, standard deviation of means and standard error of the means were calculated per diet per day (Excel). All growth data was statistically analysed using multivariate ANOVA with bird as the experimental unit, treatment as diet (Control vs. Intervention) * day of slaughter * pen and blocked according to berth (Genstat ®).

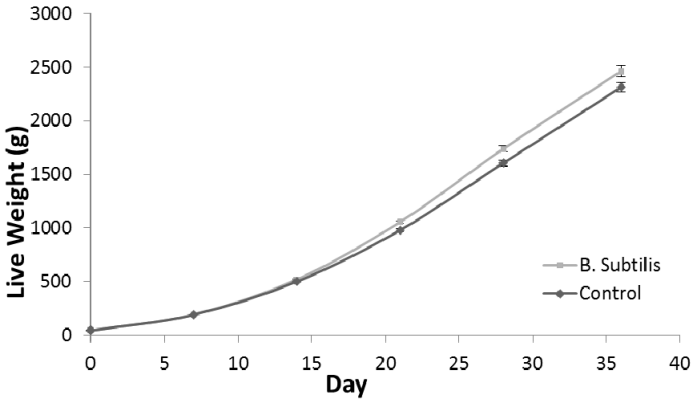

Figure 1 The effect of *B. subtilis* on average live weight gain

Results Birds fed commercial feed containing *Bacillus subtilis* spores (control) had significantly higher breast**, leg*, and live weights (*P*=0.009**) than those fed commercial feed alone, with the greatest difference seen on day 36 as shown in Table 1 and Figure 1. Combined liver and spleen (L&S) and heart weights were similar for birds from both groups. Mean feed intake per bird for the starter, grower and finisher ration periods was: 45.86g, 104.19g and 163.47g for the control group and 50.12g, 109.77g and 183.00g for the intervention group (Sd: 24.49, 37.06 and 26.28 for control and 22.66, 38.22, 22.90 for intervention).

Table 1 The effect of *B. subtilis* on organ weights of day 36 birds

Organ	Control	Intervention	s.e.d	P
Breast (g)	535.4	579.7	6.63	0.004
Legs (g)	547.6	589.9	7.69	0.028

Conclusions The results indicate that *Bacillus subtilis*, when used as a food supplement, improves breast and leg yield at 36 days of age. Birds fed probiotic were also considerably larger at 28 and 36 days of age. This correlates with birds fed probiotic having a higher feed intake and supports previous work that suggests broilers fed a *B. subtilis*-supplemented commercial diet have improved growth performance (Santoso *et al*. 1995). The increased weight gain by birds fed probiotic could be due to the *B. subtilis* adhering to the gut cell walls, rapidly consuming oxygen, and reducing pH, which favours growth of lactobacilli and suppresses growth of opportunistic pathogenic bacteria such as *E. coli*, leading to a healthier intestinal microflora.

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An *in vitro* study of the potential of diatomaceous earth to reduce *Campylobacter spp.* infections in poultry

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Introduction Pathogenic microorganisms such as *Campylobacter spp.* are one of the most common causes of food borne infections in developed countries. Consuming Campylobacter infected meat can cause diarrhoea, periodontitis, cramps, fever and pain, and costs European Union around £583M per year (EFSA, 2008). The food industry is searching for alternative methods to suppress bacterial growth in order to improve meat quality without the use of medicinal antibiotics. We tested Diatomaceous Earth (DE) which is a natural, soft siliceous, sedimentary rock that consist remains of fossilised sea-algae. DE is easily crumbled into a fine white powder and has abrasive structure. The aims of this *in vitro* study were to evaluate the action of DE, as a natural antibacterial product, in reducing Campylobacter survival, and secondly to find the most efficacious dose of DE for *Campylobacter* suppression for future *in vivo* investigations (El-Husseiny, 2008).

Material and methods The experiment was designed to test five different levels of DE concentrations (0%, 0.5%, 1%, 2% and 5%) in the presence of Campylobacter organisms. Firstly, *Campylobacter* positive organisms were cultured on mCCDA agar plates at 39°C in microaerophilic conditions. A single colony (<0.1g) was resuspended in 9ml of Maximum Recovery Diluent (MRD) and vortexed. This was then then further diluted by pipetting 1ml of sample into a fresh 9ml MRD tube. Secondly, five test tubes with 9ml MRD were prepared, containing autoclaved DE powder at: 0% - 0g, 0.5% - 0.05g, 1% - 0.1g, 2% - 0.2g, 5% - 0.5g. Subsequently, each tube containing 9ml MRD with DE received 1ml of previously prepared bacterial dilution. Finally 100µl from each final dilution mixture, was replicated onto twelve mCCDA agar plates (Table 1), spread and incubated for 2 days at 39°C in microaerophilic conditions. After this time bacterial colonies were counted and data was statistically analysed with use of ANOVA (GenStat 14.1).

Results Colony counts were converted to Log10 CFU/g. Campylobacter cells spread on plates in the presence of 0.5%, 1%, 1.5%, 2% of DE had lower colony counts (P<0.001) compared with the 0% control. The lowest CFU/g was observed at 2% DE plates (Graph 1). Higher amounts of bacterial colonies were observed with 0 and 5% of DE than the other treatments (P<0.001). In the case of 0% DE there was no antimicrobial product on the plate and the colony count for this treatment describes the original amount of bacteria in the initial sample. However in the case of 5% DE it is noticeable that bacterial count was similar to the original sample. There is a significant correlation between amount of used DE and bacterial growth on plates with the exception of 5%. Standard error was calculated for each sample and has been included on Grapg1.

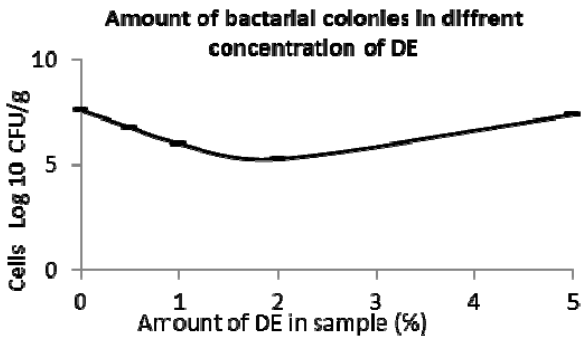

Figure 1 The effect of different amounts of DE on *Campylobacter* organisms

Table 1 Results of ANOVA analysis with use of GenStat program.

Average result of Log10 CFU/g for each treatment

DE	0%	0.50%	1%	2%	5%
Log 10 CFU/g	7.603	7.389	6.011	5.264	7.261
Replicates	12	12	12	12	12

Conclusions: These results show that DE has an impact on *in vitro* growth of *Campylobacter spp.* Small amounts of product reduced levels of bacterial colonies on mCCDA plates. However, excess of DE seems to have a protective effect for the bacteria. As previously reported DE has a strong capacity to absorb to bacterial cells on the surfaces due to their high specific surface area which in turn cause dehydration of the microorganism (Wang, 2012). However, high quantity of DE provides a kind of microenvironment around the bacteria especially in high pH, in which the local pH is less aggressive and thus bacteria can remain intact (Wang, 2012).

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The effect different levels of energy and protein with constant ratio on performance and carcass characteristics and blood parameters in broiler chickens

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Introduction A major concern of modern poultry enterprise is to reduce feed cost for optimal economic returns because feed constitutes approximately 70% of total production cost (Gerieifithes, *et al*, 1977). One way to reducing the feed cost is through improvement in the feed efficiency of birds. While formulating a broiler's diet, the main emphasis is placed protein and energy, because they are one of the major cost components of poultry diets and also they are two important components of food that generates a lot of interest and challenges to nutriationists (Kamran *et al*, 2008). The aim of this study Investigation The effect different levels of energy and protein with constant ratio on performance and carcass characteristics and blood parameters in broiler chickens

Material and methods Four hundred one-day-old ROSS 308 strain broiler chickens were randomly assigned to 3 treatments with 4 replicate containing 10 birds in each pens. The whole experimental period was divided in to three phases. Starter , grower and finisher according ROSS 308 (2005) management guide. The feed ingredients used for the formulation of experimental diets were analysed with AOAC method. Three experimental diets were formulated to have 3 levels of CP and ME, respectively in each phase: 23, 22 and 21 % CP with 3031.5, 2899.6 and 2768 kcal/kg in the starter phase. 22, 21and 20 % CP with 3174, 3030 and 2886 and kcal/kg in the grower phase and 20,19 and 18% CP with 3224, 3063 and 2902 kcal/kg in the finisher. The ratio between CP and ME was maintained at 131.81, 144.33 and 161.2 in the starter, grower and finisher diets, respectively. Feed intake and weight gain were recorded at the end of each phase. And feed conversion ratio (FCR) was calculated with using this data. At the end of experiment, 3 birds from each pen were randomly selected and slaughtered, and data on carcass yield, breast meat yield, thigh yield, abdominal fat, liver, heart and gizzard were recorded. Blood samples were collected from the wing vein of the birds by disposable syringes. At the end of the experiment, two birds from each replicate of treatments were slaughtered for separation of carcasses and bloodlet was taken to determine blood parameter's glucose, triglycerides, cholesterol, HDL, LDL and VLDL concentrations. Data were analyzed by the SAS software. Significant differences among treatments were identified at 5% level by Duncan's multiple range test.

Results results of the analysis of variance are presented in table 3. The results shown that there were significant differences ($P<0.05$) in weight gain, feed intake, and FCR between treatments during grower, finisher, and overall experimental periods. Weight gain was linearly decreased, however feed intake and FCR were increased ($p<0.05$) linearly with the reduction in dietary CP and ME levels. But these parameter not affected during starter period. It seems that there is a physical limitation for feed intake in very young chicks PER and EER were decreased linearly ($P<0.05$) in grower, finisher and overall of experiment, whereas no different was observed in starter period. Similarly, carcass characteristics such as carcass weight (CW), percentage of carcass, breast and thigh and other parameters of carcass like liver, heart weight and abdominal fat had no significant effect ($p>0.05$), Different levels of energy and protein with constant ratio caused no significant influence in blood concentration of glucose, cholesterol, LDL and VLDL ($p>0.05$). But there was significant influence on triglycerides and HDL concentrations (Table 1).

Total	FI(gl)	WG (g)	FCR	PER	EER	Glucose	Cholesterol	TG	LDL	VLDL	HDL
Control	3226.46^{c}	2248.37^{a}	1.89^{c}	2.93^{a}	0.26^{a}	248.56	160.35	128.63	103.58	48.66	52.46
T1	3244.20^{c}	2234.19^{a}	1.93^{c}	2.74^{a}	0.25^{a}	242.68	156.01	162.617	97.60	45.44	128.08
T2	4500.30^{b}	2209.8^{b}	2.27^{b}	2.67^{b}	0.20^{b}	238.88	145.05	177.71	86.23	38.53	97.40
SEM	25.47	12.320	0.014	0.081	0.178	0.875	0.284	0.647	0.099	0.375	0.126

FI: Feed Intake, WG: Weight Gain, FCR: Feed Conversation Ratio, PER: Protein Efficiency Ratio, EER: Energy Efficiency Ratio

Conclusion Feeding broiler chickens low ME and low CP with constant ME:CP ratio has adversely affected the growth performance, but carcass parameter not affected without any increase in liver weights and abdominal fat content. Different levels of energy and protein with constant ratio caused no significant influence in blood concentration of glucose, cholesterol, LDL and VLDL ($p>0.05$). But there was significant influence on triglycerides and HDL concentrations.

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Carcass characteristics of broiler chickens fed wheat-based diets supplemented with exogenous enzyme

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Introduction Inclusion of feed ingredients such as wheat and barley in diets of poultry is limited due to high amounts of non-starch polysaccharides (NSPs) present in them (Mehri *et al*., 2010). The predominant NSP in wheat for example is arabinoxylan for which poultry birds do not have the enzyme complement to digest. Non-starch polysaccharides have been reported to reduce digestibility of several nutrients in poultry (Chesson, 2001). Nevertheless, when wheat is included in broiler diets as a source of energy, its digestion can further be improved by supplementation with exogenous carbohydrase enzyme. Exogenous enzymes have also been reported to improve energy utilisation in broilers (Douglas *et al*., 2000). It was therefore the aim of this study to determine the effect of supplementing wheat-based diets with Roxazyme G2G (a cocktail of carbohydrase enzymes) on carcass quality in 56-d-old broilers.

Material and methods Seven diets consisting of a control diet with no wheat and 6 others containing 10, 20, and 30% wheat meal without or with Roxazyme G2G (added at the rate of 0.025%) were formulated with addition of other feed ingredients at constant levels. The diets met the NRC (1994) nutrient requirements for broilers. Four hundred and twenty one-day-old broiler chicken (Arbor Acre Strain) were wing-branded, and brooded for 7 days on a deep litter in a well illuminated and ventilated standard poultry house. At d 7 they were weighed and randomly allotted to the 7 diets on weight basis with 6 replicates of 10 birds each. At day 56, birds were weighed and slaughtered by cervical dislocation. The weight of the carcass primal cuts and visceral organs were recorded. The proximate composition of the wheat meal and diets was determined by the method of AOAC (2000; Method 982.30). Data obtained were analysed using ANOVA ($P<0.05$) (SAS, 2006). Mean differences were separated using Duncan Multiple Range Test.

Results Carcass characteristics of birds fed wheat-based diets supplemented with Roxazyme G2G are shown in Table 1. Birds on 30% wheat meal diet supplemented with enzyme had the highest live weight (2437g/b), which was significantly ($P < 0.05$) higher than the live weights of birds on the other diets. The lowest live weight (2074g/b) was however observed in birds on 30% wheat meal without enzyme. The weights of breast, back weight, thighs, drumsticks and wings of birds on 30% wheat meal with enzyme were significantly ($P<0.05$) higher than weights of these carcass part in birds on the other diets. There were significant ($P<0.05$) differences observed in the relative organ weights of the birds on the experimental diets. The weights of spleen, gizzard and heart of birds on the diets with enzyme were similar to those on the control diet. However, lower values were observed for spleen and gizzard of birds on the diets without enzyme supplementation compared to the control or enzyme supplemented diets. Inclusion of wheat meal in the diets without enzyme supplementation had a significant ($P<0.05$) reduction on the weights of thigh and gizzard. Supplementation of the wheat meal-based diets with enzyme significantly ($P<0.05$) increased the live weight, dressed weight, back weight, weights of thigh, drumstick, spleen and gizzard.

Table 1 Carcass characteristics and organs weight of birds fed wheat based diets (grams)

	Without Enzyme				With Enzyme			
Parameter	0	10	20	30	10	20	30	SEM
Live weight	2226^{b}	2191^{b}	2177^{b}	2074^{b}	2232^{b}	2144^{b}	2437^{a}	108.1
Dressed weight	1900^{b}	1836^{bc}	1807^{bcd}	1714^{d}	1913^{b}	1836^{bc}	2088^{a}	99.3
Breast	476^{b}	442^{c}	450^{bc}	437^{c}	460^{bc}	432^{c}	509^{a}	29.1
Back	347^{bc}	316^{d}	328^{d}	297^{e}	366^{ab}	339^{cd}	380^{a}	23.8
Thigh	233^{b}	239^{ab}	210^{c}	228^{b}	239^{ab}	226^{b}	250^{a}	14.9
Drumstick	226^{c}	227^{c}	228^{c}	214^{c}	243^{b}	221^{c}	257^{a}	14.3
Wings	187^{b}	178^{bc}	175^{c}	176^{c}	173^{c}	170^{c}	198^{a}	8.8
*Liver	2.29^{a}	2.26^{b}	2.19^{d}	2.22^{c}	2.20^{cd}	2.14^{e}	2.12^{e}	0.02
*Spleen	0.24^{a}	0.09^{b}	0.09^{b}	0.10^{b}	0.24^{a}	0.18^{ab}	0.14^{ab}	0.10
*Gizzard	2.02^{a}	1.87^{c}	1.85^{c}	1.93^{bc}	2.01^{ab}	2.03^{a}	2.02^{a}	0.13
*Heart	0.40^{ab}	0.42^{a}	0.37^{c}	0.36^{c}	0.40^{ab}	0.38^{bc}	0.38^{bc}	0.03

*Means on the same row with different superscripts are significantly ($P<0.05$) different; * expressed as % of live weight.

Conclusions The weight of carcass parts and relative organ weights showed that birds fed wheat-based diets supplemented with Roxazyme G2G were better than those without enzyme supplementation.

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Health status of turkeys fed supplemented *Moringa oleifera* leaf meal as an antioxidant in the diets

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Introduction Cells are under constant attack by free radicals, many of which are formed as a natural consequence of normal metabolic activity and as part of the immune system strategy for destroying invading microorganisms. As a result of the climate change, turkeys are most of the time reared under conditions of oxidative stress, extensive preventive medication and the effects on feed ingredients in terms of mycotoxin exposure. Therefore, there is need for antioxidant supplement to prevent damage to major organs and systems. With the increase in awareness for healthy products at affordable prices, the study was aimed at providing healthy and economically justified turkeys for consumption.

Materials and methods A total of ninety six unsexed, day old British United Turkeys (BUT) were randomly allotted to four dietary treatments with three replicates of eight poults per pen. Four dietary treatments were formulated according to NRC,1994 consisting of Moringa oleifera leaf meal at 0, 0.5,1.0 and 1.5 g/kg in a complete randommised design. Six pens, each of 6.2 m^2 were used. Turkeys were fed with ground (5mm mesh size) experimental diets *ad libitum.* Turkey poults were maintained at a brooding temperature of 35^oC for the first two days after which it was reduced by 2^oC every week. At the end of the experiment (16 weeks), blood samples were collected from one male turkey per replicate making a total of 12 turkeys to determine aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and uric acid. AST and ALT were carried out by the method of Reitman and Frankel (1957), creatinine was determined using enzymatic colorimetric method of Henry (1974) while uric acid was determined using the method by Fossati *et al*. (1980). Data obtained were subjected to the general linear model procedure of SAS (2000) and significant means were separated using Duncan's multiple range test (Duncan, 1955).

Results The values obtained indicated that creatinine level of turkeys in the various treatments were not significantly different, while uric acid, AST, ALT were significantly ($P<0.05$) reduced by the dietary supplement when compared to the control. Low levels of AST are normally found in the blood, when body tissue or an organ id diseased or damaged, additional AST is released into the blood stream.

Table 1 Effect of *Moringa oleifera* leaf meal on the health status of turkeys (0-16 weeks)

	Inclusion of *M. Oleifera* leaf meal (g/kg)				
Parameters	0	0.5	1.0	1.5	SEM
Creatinine (mg/dl)	0.9	0.8	0.8	0.9	0.00
Uric acid (mg/dl)	15.57^a	13.43^b	11.47^d	12.24^c	0.03
Aspartate aminotransferase (iu/l)	94.02^a	64.93^b	60.37^d	62.10^c	0.25
Alanine aminotransferase (iu/l)	14.67^a	14.00^b	12.13^c	12.10^c	0.05

Conclusions The results above showed that *M. Oleifera* to be effective in lowering most especially AST and ALT in the blood of the turkeys which is an indication of low toxic level in the blood. Since the liver is the first organ to be affected by any toxic substances and the amount of AST in the blood is directly related to the extent of tissue damage.

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Replacement value of 48-hours fermented Taro cocoyam meal (*Colocasia Esculenta var.esculenta*) for maize on performance of laying quails (*Coturnix coturnix japonica*)

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IntroductionIn Nigeria qualitative and quantitative feed provision for the developing poultry industry remains a challenge which has contributed to the rising animal protein deficit. The high cost of conventional energy sources like maize which is a major staple, is strangulating poultry production with attendant high cost of poultry products. The challenge of feed provision and high cost of conventional poultry products has led to the search for alternatives that could reduce the animal protein deficit. Taro cocoyam (*Colocasia esculenta var.esculenta*) is an affordable energy source whose potential as feed for poultry has not been extensively evaluated. A ten- week study was conducted to determine the effect of replacing maize with fermented taro cocoyam on the performance of laying quails (*Coturnix coturnix* japonica).

Material and methods Five dietary treatments were formulated in which 48-hours fermented taro cocoyam meal (*Colocasia esculenta var.esculenta*) was used to replace maize at 0, 25, 50, 75 and 100 percent. Two hundred and twenty five Japanese quails (*Coturnix coturnix* japonica) were randomly allotted to the five dietary treatments, of 36 hens and 9 cockerels each, replicated thrice with 12 hens and 3 cockerels per replicate, in a completely randomised block design. Feed intake was measured daily, eggs collected daily and the quails were weighed once a week. Data obtained on feed intake, weight gain, egg production and percentage hen day production were subjected to analysis of variance using the randomized complete block design as described by Steel and Torrie (1980). Least significant difference method was used to separate means that differed significantly (Steel and Torrie, 1980).

Results Quails on 75% and 100% diets consumed significantly (P-0.05) higher feed than quails on other diets. There was no significant (P>0.05) difference in the weekly weight gain of quails. Highest feed efficiency values were recorded with quails on diets 75% and 100%. Quails on diets 0%and 25% recorded highest body weights, daily egg production and percentage hen-day production. Delay in lay was observed with quails fed 75%and 100% taro cocoyam meal (*Colocasia esculenta var.esculenta*) diets.

Table1 Effect of replacement of maize with Taro cocoyam on laying characteristics of quails

Parameters	Treatments 0%	25%	50%	75%	100%
Ave. Feedintake (g/bird/Week)	128.34± 6.33^{c}	134.84±6.83^{b}	141.27±7.30^{b}	149.12±15.77^{a}	149.53±8.07^{a}
Feed efficiency	1.54±0.64^{c}	1.13±0.27^{c}	7.30±5.11^{b}	11.58±6.13^{a}	11.97±3.49^{a}
Ave.final body weight/quails(g)	187.61±3.61	185.44±3.78	169.48±2.20	160.12±1.84	155.25±2.79
Production/day/treatment	20.37±2.45^{a}	21.31±2.45^{a}	8.94±1.53^{b}	4.62±1.18^{b}	0.16±0.39^{c}
Hen-day production(%)	47.72±6.02^{a}	48.43±5.50^{a}	20.51±3.54^{b}	10.92±2.56^{c}	3.71±0.85^{c}
Time of lay	42 days of age(at the 6th week)	39 days of age(end of the 5th week)	46 days of age(end of 6th week)	51days of age(at the 7th week)	54 days of age(at the 8th week)

Different superscripts(a,b,c) within the same row indicates significant(P<0.05)differences

Conclusions The results indicated that 48-hours fermented taro cocoyam meal could replace maize favourably at 25%level in the diet of laying Japanese quails at 5% level of significance. It was equally observed that treatments influenced time of lay with delayed lay as level of fermented taro cocoyam meal (*Colocasia esculenta var.esculenta*) increased in the diet. Further investigations would be required to increase the percentage of fermented taro cocoyam meal that can replace maize beyond 25% in the diets of laying quails with no compromise on performance.

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Effect of feeding ginger by-product meal supplemented with palm oil on performance of starter broiler chickens

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Introduction In developing countries, competition between man and livestock for grains such as maize has led to the search for alternative ingredients that are cheaper. This includes industrial ingredients such as Ginger by- product meal (GBM). This study was conducted to determine the effect of feeding GBM supplemented with palm oil on the growth of broiler chickens.

Material and methods Four hundred and five Anak broiler chicks aged three days were procured and randomly allocated to the nine experimental diets. Each treatment comprised three replicates with fifteen birds each. The ginger by- product meal (GBM) is an industrial by-product obtained after the extraction of juice from ginger rhizomes washed and crushed in a local grinding machine. The proximate analysis carried out according to AOAC (1990) showed that GBM had an NFE value of 81.45. Nine experimental diets were formulated with three levels of GBM (0, 75 and 150g/kg) and three levels of palm oil (0, 40 and 60g/kg). The changes in live weight of the broiler chickens were taken weekly throughout the experimental period. Values for feed consumption, weight gain, feed to gain ratio and mortality were calculated and recorded. The design used for the experiment is completely randomized design in a 2 x 3 factorial arrangement. All data obtained from the trial were subjected to analysis of variance using the SAS (2002) general linear model and treatment means were compared for significance using Tukey's studentized range test.

Results Table 1 shows no significant ($p>0.05$) difference in the feed intake of birds for all the GBM diets. The final weight and weight gain of birds fed 75g/kg GBM diets were significantly ($p<0.05$) greater those of birds fed 150g/kg GBM diets but similar to those of birds fed 0g GBM diet. There was also no significant ($p<0.05$) difference in final weight and weight gain of birds fed 0 and 150g/kg GBM diet. Feed to gain ratio of birds fed 0 and 75g/kg GBM diets was significantly ($p<0.05$) better than that of birds fed 150g/kg GBM diets. Feed cost per kg gain was significantly ($p<0.05$) better for 0 and 75g/kg GBM diets than 150g/kg GBM diets. Mortality rate was statistically ($p>0.05$) similar for all levels of GBM. Post mortem result showed that the mortality reported was not due to the experimental diets. Final weights, weight gains, feed to gain ratios and mortality rate of birds fed 0 and 40g/kg palm oil diets were statistically similar ($p>0.05$) and significantly ($p<0.05$) better than those of birds fed 60g/kg palm oil diet. Feed cost per kg gain was significantly ($p<0.05$) better for 0 and 40g/kg palm oil diets than 60g/kg palm oil diet. Mortality rate was significantly ($p<0.05$) greater for birds fed 60g/kg palm oil diet compared to birds fed 0 and 40g/kg palm oil diets. There was no interaction between GBM and palm oil for intake, feed to gain ratio and feed cost per kg gain. Significant interactions were observed for final weight and weight gain with inclusion of 75g/kg GBM and 40g/kg palm oil in the birds' diet being superior to the other treatments (Table 2).

Table 1 Effect of Dietary Levels Ginger By-product meal / Palm Oil on Performance at Broiler Starter Phase

	GBM Level (g/kg)					Palm oil Level (g/kg)				
	0	75	150	SEM	P	0	40	60	SEM	P
IW (g)	60.00	60.00	60.00	0.00	0.68	60.00	60.00	60.00	0.00	0.84
FI (g)	880.00	940.00	940.00	20.00	0.08	940.00	930.00	880.00	20.00	0.13
FW (g)	570.00^{ab}	610.00^{a}	530.00^{b}	10.00	0.003	580.00^{a}	600.00^{a}	520.00^{b}	10.00	0.001
WG (g)	510.00^{ab}	550.00^{a}	470.00^{b}	10.00	0.003	530.00^{a}	540.00^{a}	460.00^{b}	10.00	0.001
F/ G	1.73^{a}	1.73^{a}	2.02^{b}	0.05	0.0003	1.79^{ab}	1.74^{a}	1.95^{b}	0.05	0.012
FC/Kg gain	120.00^{a}	117.60^{a}	134.35^{b}	3.09	0.002	108.22^{a}	120.57^{a}	143.16^{b}	3.09	0.0001
Mortality	0.75	0.75	0.48	0.02	0.58	0.48^{a}	0.20^{a}	1.02^{b}	0.02	0.02

^{ab} Means within rows with different superscript are significantly different ($P<0.05$)
IW.=Initial Weight; FI = Feed Intake; FW = Final Weight; WG = Weight Gain; F/G = Feed to Gain ratio; FC/Kg gain = Feed Cost/Kg gain

Table 2 Effect of Dietary Levels of GBM and Palm Oil on Performance of Broiler Chickens during Starter Phase

	Level of Ginger By-product meal (g/kg)										
	0			75			150				
	Level of Palm Oil (g/kg)										
	0	40	60	0	40	60	0	40	60	SEM	P
FW (g)	580.00^{b}	560.00^{bc}	560.00^{bc}	610.00^{b}	690.00^{a}	520.00^{cd}	570.00^{bc}	560.00^{bc}	460.00^{d}	25.00	0.02
WG (g)	520.00^{b}	500.00^{bc}	500.00^{bc}	550.00^{b}	630.00^{a}	470.00^{cd}	510.00^{bc}	500.00^{bc}	410.00^{d}	25.00	0.02

^{ab} Means within rows with different superscript are significantly different ($P<0.05$)
FW = Final Weight; WG = Weight Gain

Conclusion Birds fed 75g/Kg GBM and 40g/kg palm oil diet had the highest weight gain hence it is recommended for inclusion in broiler starter diets.

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The use of infra-red thermography to measure flank temperatures of dairy cows fed wheat- or maize-based diets

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Introduction Heat load and heat stress of animals is becoming of increasing concern, particularly in light of predictions of higher climatic temperatures. Dairy cattle can be particularly affected due to the high metabolic pressure of lactation adding to heat load. Whilst effective management can mitigate some heat load (for example provision of shading), other strategies may become increasingly important in allowing production variables, such as milk yield, to remain unaffected. Dietary manipulation may be one such strategy, particular as changes in climate may also affect feed availability. The temperature of an animal can be measured using infra-red thermography, and presents a means of remotely analysing animal temperature. Montanholi *et al* (2008) noted that the left flank of the animal represented the heat load of the rumen whilst the right flank represented body temperature – the difference between the flanks represents the heat of fermentation within the rumen. Reducing the heat of fermentation would clearly lower heat load and hence potential heat stress in adverse climates.

Material and methods As part of a trial reported by Moate *et al* (2012), infra red thermal images of a sub-set of cows were captured to analyse flank temperatures. Cows were offered either basal diet of 10.2 kg DM/day of lucerne hay and 12.2 kg DM/day of a concentrate mix containing16.3% cold pressed canola, 1.0% molasses powder, 1.0% minerals and 81.7 % of either crushed wheat or crushed maize (Wheat-fed vs. Maize-fed cows - full details of the dietary treatments used are described by Moate *et al* (2012).

Cows were randomly allocated to diets and one of two open circuit respiration chambers. Full details regarding the use of the Ellinbank open circuit respiration chambers for measurement of CH_4 emissions by dairy cows were described by Grainger *et al*. (2007).

A FLIR T640 camera body, coupled to a FLIR IR lens (f=13.1mm) was mounted in one of the two respiration chambers, using a Camzilla CZ1 mount. Thermal images were taken every 5 minutes over the 48h period the cows were in the chamber, using FLIR Research IR version 3.Max software, connected to the camera via a hard wire (USB cable). Temperature measurements were then derived from the images collected using the same software, using an average value from a pre-defined 10cm^2 area on the top of each flank.

Data for a total of 4 wheat-fed cows and 2 maize-fed cows was available for analysis. Data was analysed using ANOVA to look for temperature differences between flanks and between dietary treatments.

Results Wheat-fed cows showed a significantly higher flank temperature than maize-fed cows ($p<0.01$), on both the left and right flanks and had a significantly higher mean difference ($p<0.01$) (Table 1). This was evident over an entire 24 hour period and was even more pronounced over the postprandial period (up to 100 minutes after feeding).

Table 1 Mean flank temperature (°C) of cows on wheat-or maize-based diets, measured using infra red thermography (±SE).

		Wheat-fed cows	Maize-fed cows
Over 24h period	Left hand flank	31.5^a (0.07)	29.9^b (0.06)
	Right hand flank	30.3^a (0.08)	29.1^b (0.05)
	Mean difference	1.1^a (0.04)	0.7^b (0.03)
Postprandial period (up to 100 minutes after feeding).	Left hand flank	32.8^a (0.13)	31.3^b (0.21)
	Right hand flank	32.3^a (0.17)	30.6^b (0.18)
	Mean difference	1.4^a (0.15)	0.7^b (0.12)

Rows with different superscript numbers differ significantly $P<0.01$

Conclusions These results indicate that dietary composition may play an important role in either increasing or ameliorating the heat load of dairy cattle. Infra-red thermography may provide a useful tool for monitoring heat load and animal temperature. Whilst it may not be possible to avoid using feeds with high starch availability, the use of compounds to bind starch and prevent rapid fermentation may provide a way of reducing the heat increment of feed.

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Performance of heat stressed dairy cows in response to *Saccharomyces cervicae* and *Mannanoligosaccharids* feed additives under Egyptian conditions

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Introduction In Egypt, the increase of temperature- humidity index (THI) above the ambient level was associated with heat stress (HS) during at least 4 to 6 months each year. The HS condition influencing not only the physiological functions of dairy cows but also it is affecting the efficacy and profitability of dairy enterprises. Many researches investigated different strategies to reduce HS for dairy cattle including dietary modifications in regard to the hot environment. However, the problems concerning with the decrease of dairy cattle performance especially milk yield and fertility are still exist in many hot regions such as Egypt. An experiment was conducted aiming at alleviating the negative effects of HS on productive and reproductive performances of multiparous Holstein dairy cows (transition to early lactation periods) during hot Egyptian summer season using different sources of Direct Fed Microbial (DFM) feed additives .

Material and **methods** A total of 36 cows was randomly assigned into 4 dietary treatments (n= 9), the control group was fed on the basal diet (BD), the 2nd group was fed on BD + *Saccharomyces cervicae* (SC 10g/h/d), the 3rd group was fed BD + *Mannanoligosaccharid*s (MOS 10g/h/d), and the 4th group was fed on BD plus a combination of SC+MOS. The treatments were adopted at 4 weeks before the calving date of up to 8 weeks post-calving. The BD was total mixed ration (TMR) which was formulated to meet or exceed the predicted requirements (NRC, 2001). Atmospheric temperature (^{0} C), relative humidity (RH), THI, body surface temperature indices, rectal temperature and respiration rate were recorded throughout the experimental period. Moreover, body weight changes, BCS, milk yield, milk composition (fat, protein & lactose), milk urea nitrogen, and milk somatic cell count were also determined. Blood biochemical parameters (glucose, NEFA, cortisol, SOD, and progesterone) as well as some reproductive parameters were determined at different relevant weeks throughout the experimental period. Rumen samples for determination of pH and rumen ammonia concentration were collected on the 10th wk post-calving, Rumen fluid was obtained via a stomach tube 3 h after morning feeding, and pH of the squeezed fluid was immediately determined with a portable pH meter (HI8314, Hanna Instruments, Cluj-Napoca, Romania); 10 ml of fluid was preserved with 1 ml of 5% sulphuric acid for later analysis of ammonia nitrogen. Ammonia nitrogen was determined by the colorimetric phenol-hypochlorite method of Broderick and Kang (1980). All data were subjected to statistical analyses of variance (F-test) "one way ANOVA". It is a procedure used for testing the differences among the means of two or more treatments. It was noted that if means of subgroups are greatly different, the variance of the combined groups is much larger than the variance of the separate groups (Armitage, 1971).

Results Generally, the environmental data results (Atmospheric temperature, RH and TH) as well as, the body surface temperature indices confirmed the heat stress condition during the Egyptian summer season (June-September). The HS showed a trend to increase body surface temperature indices at all points (head, shoulder, rump& tail), rectal temperature, respiration rate, milk somatic cell count and to decrease milk yield, FCM, milk fat, milk protein, blood glucose and reproductive parameters traits. The SC supplementation significantly ($P<0.05$) decreased rectal temperature values, sustained better milk yield, FCM, and milk somatic cell count values and increased significantly ($P<0.05$) rumen PH, blood glucose and decreased rumen ammonia nitrogen, blood NEFA and SOD.

Conclusions In Egypt, the THI values recorded during the summer season confirmed a heat stress condition. HS lead to the increase of all animal body surface indices and rectal temperature and to the decrease milk yield, FCM, milk fat and milk protein. The SC dietary supplement to the ration of high producing dairy cows ameliorated the adverse effects of heat stress on the productive and reproductive performances as well as the overall net revenue under the Egyptian summer conditions.

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The effects of silage digestibility on the performance of finishing beef cattle, lactating dairy cows, pregnant ewes and finishing lambs offered diets differing in forage:concentrate ratio

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Introduction Grass silage forms the basal forage for the majority of finishing beef cattle, lactating dairy cows, pregnant ewes and finishing lambs during the winter indoor feeding period. Digestibility is the most important factor influencing the feed value of grass silage (Keady *et al* 2000). The effects of silage digestibility on concentrate requirements to maintain performance of lactating dairy cows, finishing beef cattle, pregnant ewes and finishing lambs is presented by Keady and Hanrahan (2013). The aims of this study were to determine the effects of silage digestibility on the performance of finishing beef cattle, lactating dairy cows, finishing lambs and pregnant ewes offered diets differing in forage:concentrate ratio.

Material and methods Data from 76 comparisons (34, 23, 10 and 9 comparisons involving finishing beef cattle, lactating dairy cows, finishing lambs and pregnant ewes respectively) in which grass silages differing in digestibility were offered as the sole forage, supplemented with differing levels of concentrate were used in the current study. Where not available, the digestible organic matter digestibility (D-value) of the grass silages was determined from dry matter digestibility using the equations of Keady *et al* (2001). For finishing beef cattle silage D-value, concentrate intake and carcass gain varied from 555 to 743 g/kg DM; 0.0 to 8.2 kg DM/head/day; and 0.08 to 1.17 kg/head/day, respectively. For lactating dairy cows silage D-value, concentrate intake and milk yield varied from 551 to 748 g/kg DM; 3.6 to 9.9 kg DM/cow/day; and 15.5 to 33.1 kg/cow/day, respectively. For finishing lambs silage D-value, concentrate intake and carcass gain varied from 664 to 732 g/kg DM; 0.00 to 0.94 kg DM/head/day; and -40 to 124 g/head/day, respectively. For pregnant ewes silage D-value, concentrate intake in late pregnancy and lamb birth weight varied from 630 to 762 g/kg DM; 15 to 25 kg fresh weight/ewe; and 3.48 to 5.53 kg, respectively. The data were categorised according to source and least squares procedures were used to fit a model with source as a fixed effect and the proportion of the forage in the diet as a covariate; the linearity of the effect of forage proportion was tested in all cases by fitting a quadratic term but this was not significant in any case.

Results The effects of silage digestibility on food intake and performance of lactating dairy cows, finishing beef cattle and finishing lambs are presented in Table 1. The mean response to an increase of 10 g/kg in D-value was 0.33 kg/day, 22.8 g/day and 9.3 g/day of milk yield of dairy cows, carcass gain of beef cattle and carcass gain of finishing lambs, respectively. The response to silage D-value varied significantly with forage:concentrate ratio of the diet. Whilst the response to silage D-value declines as concentrate feed level increased, it was still statistically significant when concentrate accounted for 60% of total DM intake. In studies involving pregnant ewes, the silages were offered *ad libitum* for up to 14 weeks of mid and late pregnancy. On average the pregnant ewes received 16.6 kg concentrate DM during the last 6 weeks of pregnancy. The mean response to each 10 g/kg increase in silage D-value was an increase in ewe weight post lambing of 1.3 ± 0.08 kg and an extra 52.3 ± 11.41 g in lamb birth weight. When the 9 comparisons involving pregnant ewes were analysed for the effect of concentrate input in late pregnancy (as a proxy for the proportion of forage in the diet) there was no evidence of any association (P> 0.05) between silage D-value and forage:concentrate ratio of the diet.

Table 1 Responses in animal performance to a change of 10 g/kg in silage D-value at various forage:concentrate ratios

Animal type	Performance trait	Forage: concentrate ratio			
		100:0	80:20	60:40	40:60
Lactating dairy cows	Milk yield (kg/day)	-	**0.58** ± 0.144	**0.37** ± 0.050	0.16 ± 0.100
	Fat (g/kg)	-	-0.01±0.220	-0.07 ± 0.076	-0.13 ± 0.152
	Protein (g/kg)	-	0.14 ± 0.093	0.06 ± 0.032	**0.26** ± 0.065
	Fat +Protein yield (kg)	-	**0.037**±0.0101	**0.026**±0.0035	**0.015**±0.0070
	DM intake (kg/day)	-	0.33 ± 0.277	0.20‡ ± 0.096	**0.07** ± 0.192
Finishing beef cattle	Live-weight gain (kg/day)	**44** ± 4.0	**27** ± 2.4	**11** ± 3.0	-6 ± 5.0
	Carcass gain (g/day)	**33** ± 2.3	**24** ± 1.4	**16** ± 1.7	**8** ± 2.8
	DM intake (kg/day)	**0.12** ± 0.010	**0.09** ± 0.006	**0.06** ± 0.007	**0.03** ± 0.012
Finishing lambs	Carcass gain (g/day)	**16** ± 2.3	**13** ± 1.3	**9** ± 0.9	**6** ± 1.5
	DM intake (kg/day)	**0.08** ± 0.007	**0.07** ± 0.004	**0.05** ± 0.003	**0.03** ± 0.005

Responses in bold are different from zero (P<0.05), ‡ P=0.057

Conclusion Each 10 g/kg in silage D-value, in diets 80:20 forage concentrate ratio, increases carcass gain of finishing beef cattle and lambs, and milk yield of dairy cows by 24 g/d, 13 g/d and 0.58 kg/d, respectively. Each 10 g/kg in silage D-value offered to pregnant ewes increases lamb birth weight by 52.3g. Whilst the response to silage digestibility declines as the proportion of the concentrate in the diet increases, the response to silage digestibility is still significant when concentrate accounts for 60% of feed DM intake.

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A comparison of two pasture-based dairy production systems for a wetland drumlin soil in the Border Midlands West Region of Ireland

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Introduction The objective of Irish production systems in the absence of milk quotas will be to increase milk solids (MS; fat plus protein) production by maximising grass utilisation and milk productivity per unit of available feed (McCarthy *et al*., 2007). Recent studies have reported significant milk productivity gains when both stocking rate (SR) and supplementation rate are increased simultaneously within the grazing farm system (Coleman *et al*. 2010). Such a strategy may be particularly useful on dairy farms with fragmented land holdings with limited capacity for expansion from grazing alone or where grazing efficiency is reduced due to a shorter grass-growing season or impeded land drainage. The objective of this experiment was to investigate the impact of two pasture based systems differing in overall stocking rate and feed supplementation level on grass production and utilisation on a wetland drumlin soil in the Border Midlands West region of Ireland.

Material and methods Physical performance data were obtained from 120 animals (60 per feed system,) on a 4 year (2008-2011) systems comparison study at Ballyhaise College, Co. Cavan. Animals were randomly allocated to one of two feed systems (FS) based on calving date, genetic potential (Economic Breeding Index; EBI) and parity. Once randomised, all animals remained on the same FS for the duration of the experiment. The two feed systems being evaluated were: a low cost enclosed system (HG FS) and a high pasture utilisation open system (HI FS). The HG FS had a stocking rate of 3.1 cows per ha and a concentrate input of 551kg per cow. The HI FS system had a stocking rate of 4.5 cows per ha and a concentrate input of 872kg per cow. Cows were turned out to pasture in early February with SR treatments managed separately. The available pre-grazing herbage mass (HM) was estimated above 35 mm on each paddock before grazing or silage harvest based on the average yield of five quadrant samples. The post grazing height was also measured after each grazing or silage cut using a folding pasture plate meter. Grazing efficiency was calculated on each paddock in each rotation as the proportion of the available herbage mass which was removed by grazing or silage harvest. Total annual pasture production for each farmlet was also calculated using the methodology of O'Donovan (2000). Animal performance and herbage production data for the 4 year measurement period were analysed using Proc MIXED of SAS (SAS, 2006). Feed system, year and parity were included as fixed effects in the final animal model. Grass production and utilisation were analysed using mixed models with block and block*FS included as random effects.

Results FS had a significant effect on milk solids yield per cow and per hectare. The higher total lactation MS yield per cow and per hectare achieved with the HI FS is expected, given the large increase in energy supply within this system due to increased concentrate and silage supplementation at grazing. FS had no significant effect on total average herbage production over the four years of the study (P=0.394). Over the entire grazing season pre-grazing yield was higher (P-value 0.014) for the HG FS due to the higher pre-grazing herbage masses achieved with this FS during autumn when the lower overall feed demand facilitated an increase in overall farm grass cover to extend the grazing season. Post grazing sward height (38.6mm) and grazing efficiency (95%) were similar for both FS. There was no significant FS effect on total herbage (P = 0.49) or grazed herbage (P=0.22) utilisation. However, FS had a significant effect on herbage utilized as silage with significantly more herbage conserved within the HG FS (1,704 kg DM/ha/yr) compared to HI FS (644 kg DM/ha/yr). Neither FS produced sufficient winter feed resulting in a feed deficit of 53% and 90% for the HG and HI systems, respectively.

Table 1 Effect of feed system on milk production, feed inputs, herbage production and utilisation

System	HG	HI	s.e.d	P	System	HG	HI	s.e.d	P
Milk solids (kg/cow)	377	390	4.3	0.017	Grass growth (kg DM/ha)	13,558	12,893	542.1	0.394
Milk solids (kg/ha)	1,153	1,786	17.2	0.001	Pre-grazing HM (kg DM/ha)	1,422	1,356	19.1	0.014
Concentrate (kg /cow)	551	872	9.1	0.001	Grass utilised (kg DM/ha)	8,445	9,139	390.2	0.220
Silage (kg DM/cow)	1,168	1,431	19.4	0.001	Silage utilised (kg DM/ha)	1,704	644	171.9	0.001
					Total utilised (kg DM/ha)	10,155	9,788	366.8	0.490

Conclusion Increasing SR and feed supplementation can significantly increase milk productivity per hectare on Irish dairy farms post EU milk quotas. However, such systems will not lead to increases in home grown forage production or utilisation and consequently reduce winter feed production. These results indicate that further significant increases in herbage production are required to justify higher SR systems.

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Factors influencing inseminations per pregnancy in high and low forage dairy feeding systems

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Introduction The modern high producing dairy cow has been associated with declining fertility, which has been attributed to the antagonistic genetic relationship between fertility and milk yield (Lovendahl *et al*. 2009) as well as differences in production systems and regions. This study was carried out to determine the influence of genetic merit, feeding system, body energy content and milk yield on the number of inseminations per pregnancy in cows under high and low forage feeding systems.

Material and methods The work was undertaken with a Holstein Friesian herd that comprised high (select) and average (control) genetic merit cows based on fat and protein predicted transmitting ability. The feeding system was either low or high forage diets with a target dry matter (DM) from forage of 50 and 75%, respectively. The low and high forage diets had metabolizable energy (ME) of 12.3 and 11.5MJ and crude protein (CP) of 185 and 180 g per kg DM, respectively. The genetic merit and feeding system formed 4 treatments - low forage select (LFS), low forage control (LFC), high forage select (HFS) and high forage control (HFC). A total of 1179 records from 383 cows between their first and fourth lactations and which calved between September 2003 and December 2010 were grouped according to the number of inseminations to achieve pregnancy. The groups were pregnancy with 1, 2, 3 and >3 inseminations representing 35, 23, 16, and 26% of the population, respectively. A general linear mixed model (GLIMMIX procedure) of SAS 9.2 at 5% probability was used to determine factors that influence the outcome of inseminations. Modeling of binary (pregnancy diagnosis results) data was done for all lactations with the cow as a random factor. The variable pregnancy diagnosis result was assigned a value of 1 if a cow was pregnant with the first three inseminations and 0 if the pregnancy occurred after more than three inseminations. The regression factors included genetic merit, feeding system, lactation number, days to first recorded heat (DFH), milk yield at service, calving and service body energy content.

Results The herd had more cows (48%) in their first lactation than cows in their second (28%), third and fourth lactation (24%). Cows that became pregnant after >3 inseminations had significantly ($p<0.05$) higher calving and service weight, service body energy content and condition score. There were no significant differences in DFH as well as days to first and last service between lactations. Genetic merit, lactation number, DFH, service body energy content and milk yield had significant effect ($p<0.05$) on pregnancy outcome with the first 3 inseminations. There was also significant interaction between genetic merit and feeding and hence these were combined and presented as a production system (Table 1). The odds ratio estimates in Table 1 show that LFC cows and cows in their first lactation have the highest chance of getting pregnant with the first three inseminations. LFC cows are 3.5 times more likely to get pregnant than HFS cows suggesting lower fertility in high genetic merit cows.

Table 1 Odds ratio estimates for pregnancy with the first 3 inseminations in cows under different production systems

Factor	*Production system			Lactation number		Days to 1st recorded heat	Service milk yield (litres)	Service body energy content (MJ)
	LFC HFS	LFS HFS	HFC HFS	1 3	2 3	67 66	32 31	4498 4497
Odds ratio estimate	3.5	2.8	1.4	3.5	1.6	1.0	1.1	1.0
Lower CL**	1.857	1.607	0.777	2.063	0.966	1.001	1.046	0.999
Upper CL	6.711	4.899	2.599	6.088	2.622	1.015	1.110	0.999

*HFS=high forage select; LFS=low forage select; HFC=high forage control; LFC=low forage control **CL = 95% confidence limit

Pollot and Coffey (2008) associated genetic selection for high fat and protein levels in milk with increasing the time taken to start luteal activity post-partum. However, LFS cows had higher odds of getting pregnant than HFC cows. This could be attributed to differences in ME and CP available in the rations. Cows on low forage diet had higher CP and ME which might have contributed to higher odds of getting pregnant. A unit change in days to first heat, milk yield and service body energy content does not seem to have much effect on an insemination outcome.

Conclusion The results show that the feeding system plays an important role in determining the outcome of an insemination in dairy cattle. Cows fed rations with relatively lower CP and ME have a lower chance of pregnancy with the first three inseminations. Genetic merit, days to first heat, service milk yield and body energy content seem important in determining a chance of getting pregnant with the first three inseminations and these could probably be used to develop models for predicting pregnancy for each insemination.

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Differential gene expression and alternative transcription in endometrial tissue of a lactating cow model of fertility

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Introduction A lactating Holstein cow model of fertility has demonstrated significant phenotypic variation between animals with good (Fert+) and poor (Fert-) genetic merit for fertility traits. Fert+ cows have shorter calving to conception interval, fewer services per conception, greater body condition score (BCS), and greater circulating concentrations of circulating insulin-like growth factor 1 (throughout lactation) and progesterone (during the luteal phase) (Cummins *et al*. 2012a,b). Using RNA sequencing data, our aims were to examine the endometrial transcriptome of Fert+ and Fert- animals on day 7 after a synchronised oestrus in non-pregnant animals and to compare gene expression and alternative transcription between the two groups. Typically day 7 marks the arrival of the pre-embryo into the uterus in pregnant animals. The embryo does not begin signalling its presence in the uterus until ~day 17. The null hypothesis of no difference in uterine gene expression between animals with divergent genetic merit for fertility is tested.

Material and methods Thirteen animals (6 Fert+, 7 Fert-) were biopsied for inter-caruncular endometrial tissue on day 7 after synchronized oestrus. All animals were identified as being in oestrus by subsequent progesterone profiling. RNA was extracted using a Trizol-based method and cDNA libraries were prepared for sequencing. Sequence data was aligned to the UMD3.1 reference genome for Bos taurus. Count data normalised using a trimmed mean of M-values (TMM) were used in the analysis of differential gene expression (DGE) between Fert+ and Fert- conditions. Using the Bioconductor package edgeR, the biological coefficient of variation was estimated and the data modelled as negatively binomially (over-dispersed Poisson) distributed. Differential expression was determined using an exact test adapted for over-dispersed data which calculated Benjamini-Hochberg false discovery rate (FDR) adjusted P-values at a level of 10% ($P < 0.1$). Analysis of physiological pathways over-represented in the set of significantly differentially expressed genes, again using FDR correction with adjusted $P < 0.1$, was conducted using KEGG pathways and GOseq, which accounts for gene-length biases. Signatures of alternative transcription were detected using a subset of six animals (3 Fert+, 3 Fert-) with software written in-house. SpliceGrapher was used to visualise and further analyse the genes found to produce alternative transcripts.

Results and Discussion We have found that: (i) 467 genes were significantly differentially expressed (adjusted $P < 0.1$) in the endometrium between Fert+ and Fert- cows on day 7 of the oestrous cycle; (ii) of these 467 genes, 49 (10%) were found in pathways by over-represent analysis, the top three pathways of which are: neuroactive ligand-receptor pathway (15 genes), calcium signalling pathway (12) and focal adhesion pathway (10); (iii) alternative transcription events occurred in 10 genes, all of which are differentially expressed. Multiple genes already implicated in fertility that show significant DGE are found. Genes involved in cytoskeleton and extracellular membrane, potentially functional in uterine remodelling and supporting the focal adhesion KEGG pathway include: actins *ACTA2, ACTB, ACTC1*; actinins *ACTN1, ACTN2, PDLIM3*; myosins *MYH11, MYLK*; calponin *CNN1*; filamins *FLNA, FLNB*; fibrinogens *FGA, FGB, FNBP1*; thrombospondin *THBS4*; R-spondins *RSPO1, RSPO3*; ponsin *SORBS1*; and tropomyosins *TPM1, TPM2*. Several DEG may function in the uterine lumen as histotroph to maintain the pre-embryo: serpins *SERPINA1, SERPINA3-7, SERPINA12, SERPINB12*; aquaporin *AQP5*; osteopontin *SPP1*; mucins *MUC1, MUC16, MUC20*; and vitronectin *VTN*. Ion and specifically calcium signalling genes appear frequently: *ACCN1, ACCN2*; *ATP2B2, ATP2B3, ATP2B4*; *CADPS, CAP2, CAPS, CAPSL*; gaba receptors *GABRB3, GABARD*; junctophilins *JPH2, JPH3*; purinergic receptors *P2RX1, P2RX2*; 16 potassium voltage-gated channels and 5 solute carriers. These three broad physiological groupings may result in dynamic endometrium function at both the structural and signalling levels. Alternative transcription at genes involved in the above pathways (*TPM4, CNN1, MYH11, MYLK, ACTB, PDLIM3*) lends support to the divergence at the gene level between the Fert+ and Fert- groups.

Conclusion The null hypothesis of no difference in endometrial gene expression between Fert+ and Fert- animals is rejected. Divergence between the groups manifests itself as intrinsic physiological differences and differentially expressed genes. These indicate a set of genes and biological processes that may underlie the phenotypic differences observed between Fert+ and Fert- cows and could be targets of future research into improving the fertility of Irish dairy cattle.

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Potential for use of high-dose oxytocin to improve diagnosis of mastitis in dairy cows and beef suckler cows

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Background In dairy cows, detection of clinical mastitis is routinely achieved either manually by a skilled herdsman or automatically in automated milking systems by in-line measurement of somatic cell count (SCC), lactate dehydrogenase or electrical conductivity. Detection of subclinical mastitis in dairy cows is typically achieved using repeated measurement of SCC, a value in excess of 200K cells/ml being regarded as indicative of infection. In beef suckler cows relatively little attention is paid to mastitis, perhaps because it is assumed to occur at low frequency. A recent study of 125 cows from 12 Swedish beef suckler herds revealed 14% non-lactating quarters, recoverable mastitis pathogens in 41% of cows, intra-mammary infection in 13% of quarters and SCC >200K in 18% of quarters (Persson Waller *et al* 2012). It is not safe to assume that beef suckler cows have a low frequency of mastitis infection. Since diagnosis is difficult, a significant welfare problem may exist, especially in relation to chronic sub-clinical mastitis.

Aim Our objective was to investigate a possible physiological basis for the development of a single-visit milk-based mastitis diagnostic test suitable for use in beef suckler cows. We hypothesised that high-dose oxytocin would create leaky mammary tight junctions, increased SCC and shedding of pathogen into milk, and thereby provide a milk sample with enhanced diagnostic potential.

Material and methods A total of 14 cows of the Swedish red and white breed were studied, 10 selected on the basis of current or previous high SCC (HIGH) and 4 on the basis of current and previous SCC less than 50K (LOW). Milking was twice daily. Milk samples were collected aseptically from each quarter at 5 consecutive milkings ending with a morning milking. At this fifth milking 100iu of oxytocin was administered by intramuscular injection. Sampling then continued as before for the next three milkings, and after a gap of 5 days for a further 4 milkings. The sample taken in the afternoon approximately 8h after the oxytocin injection was designated the test sample. Samples were analysed for mastitis pathogens, SCC and gross composition by standard methodologies.

Results Observable clinical signs of mastitis (clots) were seen in milk from 5 quarters and there was no specific association with the test sample. Clinical signs of some sort (clots and/or presence of a recognized mastitis pathogen) were seen in 14 quarters, and once again there was no association with the test sample. Milk fat was significantly depressed and milk protein was significantly elevated in the test sample compared to other samples. In this abstract we will focus on SCC. SCC was significantly elevated in test samples compared to all other samples (909±184 vs 312±49, mean±s.e., P>0.001 Anovar). There was no difference in this respect between HIGH and LOW. Within cows, the ranking of individual quarter SCC values was the same in the test sample set as in most other sample sets, but the discrimination between high SCC quarters and low SCC quarters was increased. There was no significant difference between SCC in the four sample sets collected at the end of the experiment and those collected before oxytocin administration. On the basis of an average cell count greater than 200K cells/ml in the five sample sets taken prior to oxytocin, 10 cows (15 quarters) were classified with subclinical mastitis. Of these 15 quarters, 6 quarters (40%) exhibited cell count less than 200K in at least one of these samples, and would have been misclassified on the basis of that single sample. Clinical mastitis signs of some sort were detected in a total of 72 of the pre-oxytocin samples, and of these, 25 samples (35%) had SCC less than 200K cells/ml, once again a source of misclassification. When considering the test sample alone and assuming a revised classification of cell count greater than 700K cells ml indicative of subclinical mastitis (fitted by simple visual analysis of all plots) 5 of these 6 quarters and 8 of the 9 other quarters would have been correctly classified. Of the 14 quarters with clinical signs of some sort at that sampling, 13 were correctly classified by the test sample alone. Of 19 quarters with clinical signs of some sort at any time, 14 (75%) were correctly classified by the test sample, and those that were not were all in cows in which at least one other quarter was correctly classified. A total of 18 quarters were classified as mastitic on the basis of test sample SCC >700K cells/ml, of which 3 (17%) were false positives on the basis that clinical signs had never been detected in these quarters (although one had an average cell count >200K cells/ml in the 5 pre-oxytocin samples). All were in cows that had at least one other quarter correctly classified as mastitic.

Conclusions The hypothesis that shedding of pathogenic bacteria would be increased by high-dose oxytocin was not supported. On the other hand, measurement of SCC in the single sample collected approximately 8h after oxytocin administration was shown to have much better diagnostic potential than other single samples. There were no deleterious effects of the single high dose of oxytocin. High-dose oxytocin could be a useful mastitis diagnostic tool in beef suckler cows, and in dairy cows where frequent sampling is not possible.

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Hepatic expression of genes of the somatotropic axis during dietary restriction and compensatory growth in Holstein Friesian bulls

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Introduction Compensatory growth is commonly exploited by cattle producers to reduce overwintering costs of beef cattle (Keane and Drennan, 1994). The somatotropic axis is of critical importance in the control of nutrient utilisation and partitioning for growth and development in cattle (Bauman, 1992) however there is a dearth of information on the molecular control of the somatotropic axis during compensatory growth. The objective of this study was to examine the effect of a period of restrictive feeding followed by a period of compensatory growth on the expression of key genes controlling the somatotropic axis in the liver, the main organ of IGF1 synthesis, in Holstein Friesian bulls.

Material and methods This study utilised tissue collected as part of the study of Keogh *et al*. (2012). Briefly, in that study, 60 Holstein Friesian bulls were assigned to one of two groups: 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 (ADLIB; n=30). 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 0.5 kg/day. At the end of this period, 15 animals from each treatment were slaughtered. All remaining animals were slaughtered at the end of Period 2. At each slaughter time-point liver tissue was harvested and total RNA was extracted. The expression of the following component genes of the somatotropic axis was measured, transcription factors: *SOCS3; JAK2; STAT5B;* IGF-1, associated binding proteins: *IGF1; IGFBP1-6,* growth hormone receptor and associated acid labile subunit: *GHRtot; GHR 1A; ALS* by q-RT-PCR with *RPS9*, *CAP1*, *SRRM2, PSMD2* and *ACTB* as reference genes. Gene expression values were normalised to the reference gene and converted to values relative to the greatest cycle threshold (Ct) within each data set. 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 0.5 kg/d for RES and 1.8 kg/d for ADLIB treatment. During re-alimentation an ADG of 2.1 and 1.4 kg/d was observed for the RES and the ADLIB groups, respectively. Least square means of back-transformed expression data are presented in Table 1. Treatment x period interactions were evident for *IGFBP2*, and *GHR1A*, with higher expression of *IGFBP2* and lower expression of *GHR1A* observed in RES compared with ADLIB during Period 1, but no difference (P>0.10) between treatments for either gene in Period 2. Period affected the expression of *SOCS3, GHRtot, IGFBP6* and *ALS*: manifested as lower expression of *SOCS3* and *GHRtot* and higher expression of *IGFBP6* and *ALS* in Period 1 compared to Period 2. RES animals showed lower expression of *IGF1* and higher expression of *IGFBP2* in Period 1, while *IGFBP1* expression was reduced in RES animals compared to ADLIB animals across both periods.

Table 1 Effect of treatment (T) and period (P) on the expression of genes in the liver of the somatotropic axis.

	RES		ADLIB			Significance		
	Period 1	Period 2	Period 1	Period 2	SEM	T	P	T*P
SOCS3	1.93	3.03	2.38	3.08	0.46	NS	*	NS
JAK2	3.97	4.64	2.99	4.01	0.72	NS	NS	NS
STAT5B	1.58	1.80	1.82	1.93	0.48	NS	NS	NS
IGF1	2.18	2.56	3.44	3.02	0.38	*	NS	NS
IGFBP1	5.17	3.41	3.28	2.24	0.63	*	*	NS
IGFBP2	8.17	4.96	2.66	4.59	0.86	**	NS	**
IGFBP3	1.55	1.60	1.88	2.06	0.34	NS	NS	NS
IGFBP4	1.50	1.92	1.14	2.33	0.41	NS	NS	NS
IGFBP5	4.22	4.10	4.23	3.06	0.64	NS	NS	NS
IGFBP6	3.69	2.21	3.40	2.75	0.41	NS	*	NS
ALS	8.98	6.95	8.57	7.73	0.53	NS	*	NS
GHRtot	2.23	3.95	1.93	3.46	0.55	NS	**	NS
GHR1A	3.13	4.37	4.36	3.59	0.42	NS	NS	*

*P<0.05; **P<0.01; ***P<0.001; NS = P>0.05.

Conclusion Higher expression of *IGFBP2* during Period 1 in RES animals is indicative of its role as a key regulator of the availability of systemic *IGF1*. These data combined with lower mRNA expression of *GHR1A* suggests un-coupling of the somatotropic axis following a period of restrictive feeding. The decrease in *IGFBP2* and increase in *GHR1A* expression during Period 2 in RES animals suggests a re-coupling of the somatotropic axis upon re-alimentation. This study provides an insight into the molecular mechanisms regulating reduced and compensatory growth in hepatic tissue of cattle.

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Dry matter intake, growth rate and residual feed intake of two divergent breeds of finishing steers offered either a concentrate-straw based diet or a silage based diet *ad libitum*

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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. Furthermore, owing to the direct link between food intake and methane output, improving feed efficiency is a potential strategy for the mitigation of greenhouse gas emissions. This study investigated growth, voluntary feed intake and feed efficiency of two divergent breed types of finishing steers fed one of two commercially relevant diets in the UK.

Materials 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 steers (n=72) were group-housed in even numbers of each breed type across 4 pens, and each diet type was allocated to 2 pens. Treatments were balanced for age at start of test (AgeST). Individual feed intake was recorded using electronic feeders, and feed offered *ad libitum*. Following a 4 week adaptation period, feed intake was recorded for 56 days. Liveweights were measured weekly, and ultrasonic fat depth at the $12^{th}/13^{th}$ rib (FD) was measured at the end of test. Growth was modelled by linear regression of weight against test date, to describe average liveweight gain (LWG), mid-test liveweight (LW) and mid-test metabolic liveweight ($MLW=LW^{0.75}$). Feed conversion ratio (FCR) was calculated as average dry matter intake per day (DMI kg/day)/LWG. Residual feed intake (RFI) was calculated as deviation of actual DMI (kg/day) from DMI predicted based on linear regression of actual DMI on LWG, MLW and FD. To test for breed, diet and breed x diet effects, ANOVA was used (Genstat 14^{th} Ed.), and blocked by pen.

Results Mean values for parameters determined in this study are presented in Table 1. CHx steers were significantly heavier (mean LW 550 *vs.* 479 kg), than LUI steers. Although CHx steers had higher LWG compared to LUI steers, the differences were not significant. LUI steers had significantly larger fat depth than the CHx steers. There were no significant differences between breeds for DMI (kg/day); however, concentrate-fed animals consumed significantly more feed (11.54 *vs.* 10.57 kg/day, P<0.05) than forage-fed animals. Whether expressed as g DM/kg LW or g DM/kg MLW, LUI steers consumed significantly (P<0.001) more DM than CHx steers on both diets. No significant differences were identified for FCR, however, CHx steers fed the forage diet had significantly lower RFI values (interaction, P<0.05) than the other treatment groups.

Table 1 Effect of breed, diet and its interaction (breed*diet) on growth, feed intake and feed efficiency of Charolais sired (CHx) and Luing (LUI) steers fed either a forage based or concentrate based diet.

	Forage		Concentrate		s.e.d.			Significance		
	CHx	LUI	CHx	LUI	Breed	Diet	Breed*Diet	Breed	Diet	Breed*Diet
AgeST (days)	396	392	394	392	6.27	6.95	9.36	NS	NS	NS
LW (kg)	537^{b}	476^{a}	563^{b}	482^{a}	14.10	5.88	15.27	***	NS	NS
MLW (kg)	111.4^{b}	101.7^{a}	115.5^{b}	102.6^{a}	2.24	0.96	2.44	***	NS	NS
LWG (kg/day)	1.60	1.49	1.70	1.62	0.05	0.06	0.08	NS	NS	NS
FD (mm)	6.7^{a}	7.7^{b}	6.2^{a}	7.1^{ab}	0.34	0.73	0.81	**	NS	NS
DMI (kg/day)	10.48^{a}	10.67^{ab}	11.83^{c}	11.24^{bc}	0.27	0.12	0.29	NS	*	NS
DMI/LW(g/kg)	19.6^{a}	22.6^{bc}	21.0^{ab}	23.5^{c}	0.32	0.49	0.58	***	NS	NS
DMI/MLW(g/kg)	94.1^{a}	105.1^{bc}	102.4^{b}	109.6^{c}	1.28	1.96	2.35	***	NS	NS
FCR (kg, kg)	6.65	7.26	6.98	7.06	0.21	0.25	0.33	NS	NS	NS
RFI (kg)	-0.76^{a}	0.09^{b}	0.21^{b}	0.45^{b}	0.13	0.16	0.21	***	NS	*

*<0.05, **<0.01, *** <0.001, NS = not significant (P>0.05). ^{a,b,c}Numbers with the same superscript are not significantly different.

Conclusions Comparison of RFI results in particular suggest that forage-fed animals utilized feed more efficiently than concentrate-fed animals, especially the CHx steers. No significant differences were identified for FCR across either diet or breed treatments. However, CHx steers had significantly lower RFI values (more efficient) compared with the LUI steers, highlighting the importance of RFI as an efficiency measure that can take factors such as growth rate, body weight and its composition into account, beyond more traditional measures such as FCR. CHx steers were most efficient on the forage diet, consuming significantly less per day than the concentrate-fed CHx animals, for the same levels of LWG.

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Influence of age and processing on the *in vitro* fermentation characteristic of *Panicum maximum* and *Pennisetum purpureum*

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Introduction Forages being the basic diet of ruminant animals must be as much as possible well digestible and the nutrient well utilized to affect the productivity of livestock in terms of weight gain, reproduction, but seasonal shortages in feed availability for ruminant is a basic constraint in livestock production. The feed made available to animals during dry season must therefore be tested for its digestibility and nutritive value to retain the productivity of the animals using the *in vitro* gas production.

Material and methods Guinea grass **(***Panicum maximum*) and Elephant grass (*Pennisetum purpureum*) of four and eight weeks regrowth prior flowering collected in triplicates were staged to provide forage regrowth either 4 or 8 weeks at the time needed either for pelleting or for fresh green chop. The forages for pelleting was harvested with a flail cut harvested and were chopped into 50-60 mm using a reel cutter. They were immediately dehydrated at 120^0 C (panicum) and 150^0C (pennisetum). The temperature difference was to achieve approximately similar moisture since pennisetum has larger stems than panicum. After dehydration the dried forage was milled with a hammer mill fitted with 3 mm sieve and pelleted using a 6mm mesh size to produce pelleted hay of average length of 40 mm. Water was used as the binding agent. *In vitro* gas production, metabolizable energy and organic matter digestibility was completed according to the procedure described by Menke and Steingass (1988). Short chain fatty acids **(**SCFA**)** (Getachew *et al*., 1999).

Results Pelleted Pennisetum at 8 weeks old had the highest (P<0.05) ME of 6.18 MJ/kg DM while the least was recorded when Pennisetum was harvested fresh at 8 weeks old. The OMD and SCFA content of the grasses were similar (P>0.01) when either pelleted at 4 and 8 weeks or green chopped at 4 and 8 weeks old. While age at harvest did not affect SCFA of the grasses, OMD was higher (P<0.01) 4 week old grasses compare to 8 weeks old either pelleted of green chopped. At 24 hours, the volume of gas produced by pelleted grasses was higher (P < 0.01) than the fresh grasses. Forages at older ages decline in digestibility (Kabuga and Darko, 1993) which has been attributed to an increase in structural cell wall components and decline in leaf to stem ratio.

Table 1 *In vitro* gas production characteristic and post incubation parameters of the experimental forage grasses

Forage diets	b (ml/200mgDM)	c (ml/hr)	Absg (ml)	ME (MJ/kg DM)	OMD (%)	SCFA (µmol/g)	24 hrs gas Volume (ml)
P.maximum							
4weeks Pelleted	32.43	0.03	1.41	5.22^{b}	46.41^{a}	0.36^{ab}	17.50^{ab}
8weeks Pelleted	40.02	0.27	1.23	5.60^{a}	45.10^{a}	0.38^{a}	18.50^{ab}
4weeks green chopped	30.77	0.91	0.82	3.85^{bc}	34.19^{bc}	0.08^{bc}	6.00^{bc}
8weeks green chopped	18.57	1.22	0.56	3.72^{bc}	31.16^{c}	0.07^{c}	5.50^{c}
P.purpureum							
4 weeks Pelleted	44.98	0.03	1.12	6.18^{a}	47.47^{a}	0.50^{a}	23.50^{a}
8 weeks Pelleted	43.23	0.44	0.85	4.61^{abc}	36.41^{abc}	0.24^{abc}	20.00^{a}
4weeks green chopped	37.12	0.02	0.45	5.79^{a}	46.78^{a}	0.42^{a}	12.50^{b}
8 weeks green chopped	28.32	1.29	0.33	3.51^{c}	30.80^{c}	0.04^{c}	4.00^{c}
SEM	19.51	0.24	0.89	0.25	1.77	0.04	1.82

Conclusion For improvement of animal performance, grasses could be pelleted especially in preparation for dry season shortage and harvest at four weeks for optimum digestibility.

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Effects of four animal manure and age at harvest on the preference of three tropical grasses fed to yearling calves

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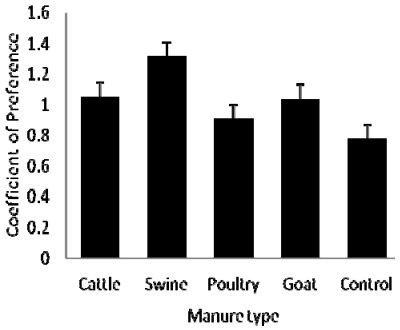
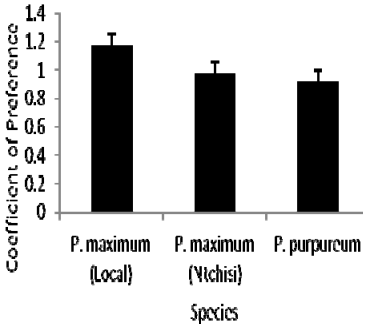
Introduction Ruminant livestock activities are gaining attention among stock owners in the last two decades in South west Nigeria, as this led to increase in pasture production with the use of inorganic fertilizers that have been investigated to have residual health effect on the final consumer as well as it cost and unavailability. This study is aimed at investigating the acceptability of the forages fertilized with animal waste and age at harvest.

Materials and methods The experiment was conducted at the Organic Research farm, Federal University of Agriculture, Abeokuta, Nigeria to evaluate the effect of animal manures and age at harvest on the preference of three tropical grasses fed to yearling calves. The grass species used are *Panicum maximum* (Local), *P. maximum* (Ntchisi) and *Pennisetum purpureum* that were fertilized with different animal manures namely cattle, goat, swine, poultry and as well as control. At each age at harvest (4, 8, 12 and 16 weeks after planting), forage materials were harvested a day before introducing them to the animals to allow for wilting under shade. 2 kg of each forage material were served from each treatment. Fifteen calves with five calves of N'dama breed per replicate between the age 9 and 10 month old with weight ranging between 75.9 to 79.8 kg were used. The calves were sourced from the cattle unit of the Teaching and Research Farms, Federal University of Agriculture, Abeokuta; they were tagged for easy identification. The treatments were assigned within 5 consecutive days of data collection (Reid *et al.*, 1992). The calves were confined in a feeding pen made of concrete and they were fed together. The forages were introduced into 15 different feeding troughs of about 70 cm x 40 cm x 23 cm in dimension which were placed in the feeding pen and with two troughs being empty at each end to nullify any border bias. The forages were randomly placed in the troughs on daily basis so as to disallow the calves from being familiar with forages in each trough. The animals were allowed to have access to the forages for 30 minutes after which the forages were withdrawn from them to determine the preference of forages offered. A known weight of grasses (2 kg) were randomly collected from the experimental plot comprising 15 treatments at each age at harvest and were distributed into 15 different feeding troughs. The feed were offered to the calves at 08.00hr and withdrawn by 08.30hr.The preference for the forages were calculated as the percentage of the forage consumed relative to forage offered for five days. The forage preferred were assessed from the coefficient of preference (COP) value, calculated from the ratio between the intakes for the individual forage, divided by the average intake of the forages (Babayemi *et al*., 2006). The forage was therefore inferred to be relatively acceptable if the COP is greater than unity. Data collected were subjected to analysis of variance and the treatment means separated using Duncan's Multiple Range Test using SAS 9.0 version and thereafter presented graphically.

Results The coefficient of preference (COP) of the grasses as recorded (1.31) in this study shows that the swine manure fertilized grasses was significantly higher ($P<0.05$) than others as the unfertilized grasses recorded the least (0.77) value (Figure 1a).The COP of the grasses as affected by the species recorded the highest value for *P. maximum* (Local) (Figure 1b). The age at harvest effect on the COP of the grasses showed that the grasses harvested at 8 WAP were significantly higher than those harvested at other ages (Figure 1c).





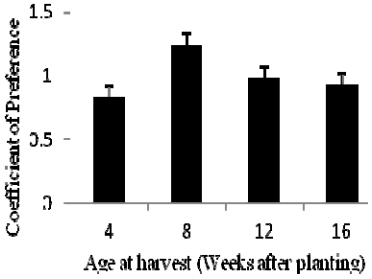


Figure 1 Preference as affected by manure type **Figure 2** Preference as affected by species **Figure 3** Preference as affected age at harvest

Conclusions It could be concluded that grasses fertilized with swine manure were the most prefered, *P. maximum* (Local) was the most prefered of the three grasses and grasses harvested at 8 weeks after planting were the most prefered as they were above unity.

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Feed intake, animal performance and Net Feed Efficiency (NFE) in young Stabiliser breeding bulls

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Introduction Improving the efficiency with which cattle produce animal protein (beef) for human consumption is recognised as one of the major routes to improving the economic sustainability of suckler beef farms. It is also widely recognised that improving the efficiency of beef production is the most viable way to reduce the greenhouse gas (GHG) emissions from these animals. Net Feed Efficiency (NFE) is a measure of feed use efficiency that allows identification of individual future breeding stock that are more efficient for any given level of animal productivity over the longer term. The objective of this study was to determine voluntary feed intakes, animal performance and NFE in young Stabiliser bulls prior to their selection as future breeding stock.

Material and methods A total of 82 Stabiliser bulls between 10-13 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 (391), barley straw (37), barley (209), sugar beet pulp (168), wheat distillers grains (148), molasses (39) and minerals (8) - (DM: 621 g/kg; ME: 11.6 MJ/kg DM; CP: 140 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 bull 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 bulls as the difference between actual DMI against estimated DMI using a multiple regression model including metabolic LW ($LW^{0.75}$), LWG and fat depth 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 feed intake, animal performance, FCR and NFE measurements are shown in table 1 whilst individual NFE values for all 82 bulls are shown in Figure 1. No significant differences between the three groups were seen in mean $LW^{0.75}$, LWG or fat depth parameters as expected in NFE derivation studies. However, low NFE bulls ate less and were significantly more efficient (P<0.05) in terms of both FCR and NFE compared to the high NFE group of bulls.

Table 1 Feed intake, animal performance and NFE in young Stabiliser bulls grouped on the basis of NFE standard deviation

	Low NFE (n=26)	MID NFE (n=31)	High NFE (n=25)	s.e.d.	Significance
DMI (kg/d)	12.04[a]	12.73[b]	13.91[c]	0.235	**
DMI (g/kg LW)	21.2[a]	22.5[b]	23.8[c]	0.355	***
DMI (g/kg $LW^{0.75}$)	104[a]	110[b]	117[c]	1.28	***
Mean LW (kg)	568	568	586	15.08	
Mean $LW^{0.75}$ (kg)	116	116	119	2.32	
LWG (kg/day)	1.89	1.79	1.93	0.084	
Mean fat depth (mm)	6.0	6.8	6.2	0.518	
FCR (kg DMI/kg LWG)	6.50[a]	7.26[b]	7.53[b]	0.374	*
NFE (kg DMI/day)	-0.77[a]	0.00[b]	+0.80[c]	0.080	***

[a,b,c] Values within rows not sharing common superscripts differ significantly

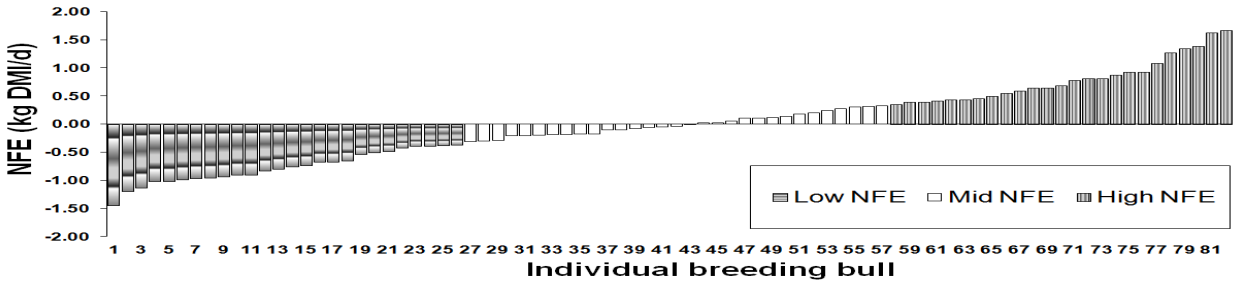

Figure 1 Individual bull NFE values (kg DMI/day)

Conclusions Low NFE bulls consumed 13% less feed, had 14% better FCR and cost £24 less to feed over the 12 week period (at £155/t DM) on the NFE unit compared with high NFE bulls without significant differences in LW, LWG or carcass fat depth. Measurement of NFE offers significant opportunities to select future breeding stock with improved feed efficiency characteristics.

Acknowledgements This work was funded by a Technology Strategy Board project grant under the SAF-IP programme.

Effect of preimplantation factor (PIF) on the lipoteichoic acid (LTA) and lipopolysaccharide (LPS) prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) responses of bovine mammary epithelial cells (BMEC) *in vitro*

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Introduction *Escherichia coli* and *Staphylococcus aureus* are common mastitis causing pathogens in the UK. *E. coli* infections often cause clinical symptoms together with a strong immune response, while *S. aureus* infections frequently induce sub-clinical mastitis. It has long been a concern that the use of antibiotics in agriculture provides a platform for transferable antibiotic resistance genes to the human population. Therefore, there is considerable interest in the development of non-antibiotic treatments for production diseases. PIF is a novel embryo-secreted peptide which is found only to be secreted by viable embryos and absent in nonviable embryos. Synthetic PIF (sPIF), when applied to a human endometrial stromal cell line significantly changed >500 gene expressions including immune pathway genes (Paidas *et al.* 2010). The objective of this experiment was to determine if sPIF had an immunomodulatory effect on a bovine mammary epithelial cell line (BMEC) treated with LTA and LPS (respective cell wall components of *S. aureus* and *E. coli*) *in vitro*, and thus potential as a therapeutic agent for mastitis. $PGF_{2\alpha}$ is a mediator of inflammation and it is therefore a useful biomarker for measuring the inflammatory response to pathogen associated molecular patterns (PAMPs) from cell culture supernatants.

Materials and methods Cell cultures of a cloned BMEC (Rose *et al.* 2002) were cultured in DMEM supplemented with 20% FBS until 80% confluence was reached. LTA (InvivoGen: tlrl-pslta) or LPS (InvivoGen: tlrl-peklps) (20µg/mL) were added to culture medium at 0h along with two concentrations of sPIF (50nM and 100nM). Cultures were stopped at 10h, 16h and 24h; media were collected and frozen at -20°C. Culture supernatant $PGF_{2\alpha}$ concentrations were measured by radio-immuno assay. Data was logarithmically transformed and analysed using a three-way (PIF, PAMP and time) analysis of variance.

Results There was a significant increase ($P<0.01$) in the secretion of $PGF_2\alpha$ from BMEC that were treated with 100nM sPIF (control: 3.64µg/L, 100nM sPIF: 4.68µg/L, Figure 1) and there was also a significant increase ($P<0.001$) in the secretion of $PGF_{2\alpha}$ from BMEC that were treated with LTA (control: 3.64µg/L, LTA: 4.86µg/L, Figure 2). Secretion of $PGF_{2\alpha}$ significantly increased between time points 10h and 16h ($P<0.001$) (10h: 3.35µg/L, 16h: 4.73µg/L, Figure 3). There was no significant interaction between sPIF, PAMP or time point. The secretion of $PGF_2\alpha$ from cells treated with LPS was not significantly different from control cells.

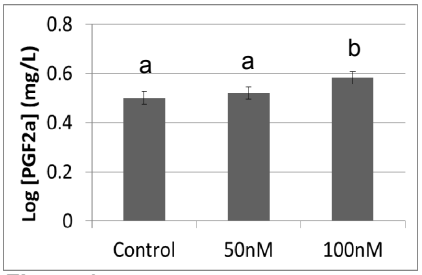

Figure 1

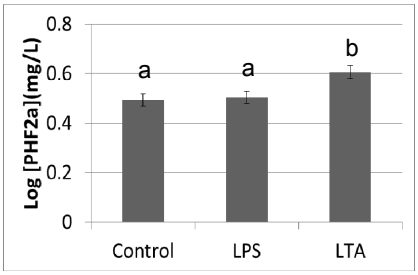

Figure 2

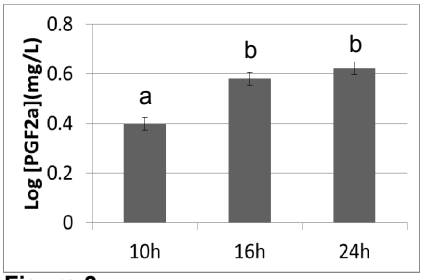

Figure 3

Figures The effect of two concentrations of sPIF (50nM and 100nM) **(Figure1),** PAMP **(Figure 2),** and time point (**Figure 3)** on the concentration of Log [$PGF_{2\alpha}$] (µg/L).

Conclusion At 100nM concentration, sPIF significantly increased the secretion of $PGF_2\alpha$ from BMEC indicating that sPIF, at 100nM concentration, was pro-inflammatory. The treatment of BMEC with LTA also caused a significant increase in $PGF_{2\alpha}$ secretion, however LPS did not. Secreted $PGF_{2\alpha}$ accumulates with time explaining the significant effect of time on the concentration of $PGF_{2\alpha}$. $PGF_{2\alpha}$ concentrations are increased in naturally occurring and experimentally induced mastitis and are also increased in primary BMEC cultures treated with phenol extracted LPS (Piotrowska-Tomala *et al.* 2012). The lack of $PGF_{2\alpha}$ response from BMEC in our results could be explained by the ultra-pure form of *E. coli K12* LPS used in the cultures. Further investigation of the effect of sPIF on expression of other inflammatory mediators would better inform the understanding of how, or if, sPIF affects the inflammatory response of BMEC treated with LTA and LPS.

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Investigating host responses and disease pathogenesis in the duodenum of broilers exposed to crude *Clostridium perfringens* toxin *in situ*

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Introduction Necrotic enteritis (NE) is an enteric disease of broilers, which costs the poultry industry $2 billion annually. The main causative agent is *Clostridium perfringens* type A which produces several toxins involved in the pathogenesis. Host responses to the disease are poorly characterised because of a) the complex pathogenesis and b) difficulties in reproducing the disease experimentally. An *in situ* broiler model was recently developed which allows closer study of host-pathogen interactions in this multi-factorial disease (Athanasiadou *et al*, 2011). In the current study the model is used to investigate biological pathways involved in the pathogenesis and host responses following exposure to crude *C. perfringens* toxin.

Materials and methods Fourteen 3-week old broilers were subjected to full surgical anaesthesia (non-recovery) and underwent intestinal surgery to create 4 chambers around the duodenal loop using ligatures. Either crude *C. perfringens* type A toxin (n=9) or a toxin-free preparation (n=5) was infused into the 4 chambers. The birds were maintained for 4 hours after infusion; intestinal segments were removed from one of the chambers at 0.5, 1, 2 and 4 hours after infusion, for histological and gene expression analysis. Formalin fixed sections were stained with hematoxylin and eosin for heterophil quantification. Gene expression related to cell death (FAS & GIMAP8), antigen recognition (IRAK-4) and cell signalling (BCL6 & NBL1) were quantified using sybr green qPCR to determine temporal host responses. Each gene was normalised with β-actin and transformed using a Johnson's transformation to normalise the variance. The transformed data were then analysed using repeated measures ANOVA for treatment and time effects. A one-way ANOVA was carried out at each time point to further explore interactions.

Results Heterophil numbers were not affected by toxin exposure throughout the experiment ($p=0.730$); their numbers increased over time in both treatments ($p<0.001$). FAS expression tended to be higher in toxin infused birds throughout the experiment ($p=0.095$); the difference was mainly attributed to up-regulation at 0.5 h in toxin infused birds ($p=0.071$). GIMAP expression also tended to be higher in toxin infused birds ($p=0.099$); the difference was attributed to increased GIMAP expression at 4h post toxin infusion ($p=0.004$). IRAK-4 expression was unaffected by toxin exposure ($p=0.625$). Expression of BCL6, an innate immune inhibitor, was significantly higher in toxin infused birds ($p=0.051$); overall levels were reduced over time ($p=0.005$). A similar pattern was detected in NBL1 expression where it was higher in birds infused with toxin ($p=0.083$) and the overall expression was reduced over time ($p=0.003$).

Table 1 Transformed mean gene expression values as calculated by repeated measures ANOVA. Different letters within a time point denote statistical significance ($p<0.05$) and different symbols within a time point denote a statistical tendency ($0.05<p<0.1$).

Gene	0.5h		1h		2h		4h	
	Control	Toxin	Control	Toxin	Control	Toxin	Control	Toxin
FAS	$0.77^{\#}$	1.21^{*}	-0.42	-0.52	-1.44	-0.39	0.21	0.19
GIMAP8	0.10	0.12	-0.43	-0.27	-0.70	-0.46	-0.54^{a}	0.58^{b}
IRAK-4	0.70	-0.19	-0.40	-0.60	0.46	1.07	-0.17	-0.33
BCL6	$0.38^{\#}$	1.16^{*}	-0.65	-0.35	-1.15	-0.33	$-0.37^{\#}$	0.17^{*}
NBL1	0.94	0.90	-0.22	0.06	-1.49	-0.24	-0.58^{a}	0.08^{b}

Conclusions FAS and GIMAP8 both have roles in cell death (Lavarik *et al.,* 2012. Krücken *et al.,* 2005). Cell death can be apoptotic, when it is programmed and causes little damage to surrounding cells or necrotic, with increased inflammation and damage of surrounding cells. FAS is a pro-apoptotic gene; up-regulation of FAS in toxin-infused birds 0.5h post infusion implies that at the very early stages of infusion the toxin may modulate cell death by apoptosis. This was not sustained at later stages of this work and in previous studies (Athanasiadou *et al*, 2012), which is indicative of a switch towards necrotic cell death early in the disease pathogenesis. Although little is known about GIMAP8 function, it appears to have an anti-apoptotic role (Krücken *et al.,* 2005). GIMAP8 expression was significantly increased at 4 hours in toxin infused birds and may indicate an attempt of host cells to prevent further death and damage. NBL1 and BCL6 both participate in preventing early inflammation (Yu *et al.,* 2005. Chen *et al.,* 2004). BCL6 inhibits cytokine responses from macrophages, which are involved early in immune responses. Cytokines from macrophages attract inflammatory cells to damaged tissue. NBL1 inhibits monocyte movement from blood into tissues where they mature into macrophages. Increased expression of both genes in toxin-infused birds may reflect another anti-inflammatory response in toxin infused birds. This work indicates ways in which the host responds to minimize pathogenic processes initiated by *C. perfringens* toxins.

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The role of natural killer cells following vaccination of neonatal calves with *Mycobacterium bovis* BCG

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Introduction Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, is an infectious disease of increasing incidence in the UK and currently there are no available vaccines. Experimental vaccination of neonatal calves with *Mycobacterium bovis* BCG provides significant protection against bovine TB. The improved efficacy of BCG in neonates may be attributed to the increased numbers of circulating innate effector cells observed in neonatal calves, particularly natural killer (NK) cells. Bovine NK cells are characterised by their constitutive expression of NKp46 (CD335) and can be subdivided into two subsets based on their differential expression of CD2. NK cells, through interactions with *Mycobacterium bovis*/*M. bovis* BCG infected dendritic cells (DC) are hypothesised to prime optimal Th1 immune responses, which are essential for protection against mycobacteria. The aim of the present study was to determine the frequency, phenotype and function of NK cells following BCG vaccination. Furthermore, to understand the nature of the interaction between NK cells and DC, co-cultures were established between the two innate immune cell populations.

Material and methods Six neonatal calves were vaccinated subcutaneously with BCG vaccine SSI (Danish 1331 strain) and peripheral blood samples were taken at various time points following vaccination. Six age-matched naïve animals were used as controls allowing NK cell responses in both vaccinated and non-vaccinated neonates to be compared. The percentage of NK cells present within peripheral blood following BCG vaccination was determined by labelling peripheral blood mononuclear cells (PBMC) with mouse anti-bovine CD335 monoclonal antibody. To identify if redistribution of NK cell subsets occurred as a result of vaccination, CD335+ cells were further labelled with mouse anti-bovine CD2 monoclonal antibody. Likewise, NK cell phenotype was investigated by labelling CD335+ cells with antibodies against CD44, MHC class II, CD62L and CCR7. The functional capacity of bovine NK cells following BCG vaccination was determined by stimulation with phorbol 12-myristate 13-acetate (PMA)/Ionomycin/Brefeldin A for 4 hours followed by fixation and permeabilisation. NK cells were then assessed for their expression of intracellular IFNγ. Overnight in vitro co-cultures between NK cells derived from weekly intervals following BCG vaccination and autologous monocyte-derived DC were set up. The expression of cell surface and intracellular molecules by both DC and NK cells was then determined by flow cytometry. All samples were analysed using CellQuest Software on the FACSCalibur, acquiring 50000 events.

Results Following vaccination of neonatal calves, changes in the percentage of NK cells were present between the BCG vaccinated and non-vaccinated groups with a significant increase in NK cells observed at 4 weeks post BCG vaccination ($p=0.042$). Furthermore, NKp46+ CD2- NK cells were more frequent in the calves that had received BCG in comparison to the naïve animals; however BCG had no significant effect on the distribution of the NK cell subsets. Changes in NK cell phenotype and function, particularly IFNγ expression were also evident. A significant increase in the percentage of NK cells expressing IFN-γ was seen at day 3 ($p=0.0142$) and 4 weeks ($p=0.0286$) post BCG vaccination. Preliminary data from the in vitro NK cell – DC co-cultures provides evidence for a reciprocal interaction occurring between the two cell populations. Work in the laboratory is on-going to validate the above findings.
Conclusions Neonatal vaccination of calves with BCG results in an increase in the percentage of NK cells within the peripheral blood at 4 weeks post vaccination. Furthermore NK cells from BCG vaccinated calves have an altered phenotype and show increased expression of IFNγ, particularly at day 3 and week 4 post vaccination, suggesting that NK cells have a role during the innate immune response following BCG vaccination of neonatal calves.

Discussion The role of NK cells during post BCG vaccination immune responses has not been elucidated and due to their increased prevalence in neonates, these may be an essential part of the protective immune response when BCG is delivered to neonatal calves. Ultimately, further understanding of the immune mechanism whereby BCG exerts protective immunity could allow targets for improved vaccination to be identified, leading to enhanced vaccine efficacy and protection against bovine TB.

Acknowledgements The authors gratefully acknowledge funding from BBSRC and Pfizer Animal Health

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Experimental vaccination with recombinant mutant *Fasciola hepatica* cathepsin L1 in cattle – preliminary results

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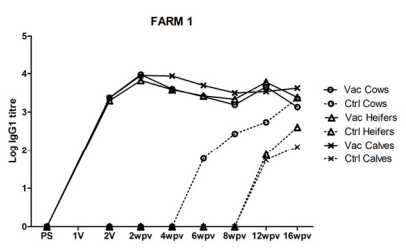
Introduction Fasciolosis is an important parasitic disease throughout the world. The cathepsin L1 (CL1) is a proteinase secreted by the trematode *Fasciola hepatica* that is involved in host invasion, nutrition, immune modulation and was indicated as good option for vaccination (Dalton *et al*., 2003). A recombinant mutant CL1 (rmFhCL1), with preserved antigenic properties but enzymatically inactive, was successfully expressed in yeast, making possible the production of that protein in a quantity big enough for a commercial vaccine (Collins *et al*., 2004). An experimental vaccination protocol with rmFhCL1 provided good protective immune response against liver fluke infection, decreasing the fluke burden and impairing helminth development in vaccinated animals (Golden *et al*., 2010). That experimental protocol is now being tested in a larger scale, under field conditions, and the preliminary results are presented here.

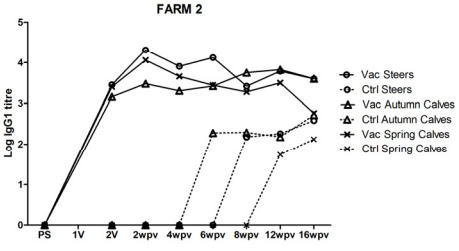
Material and methods For this field trial, animals from three different farms within Republic of Ireland, all with previous reports of *F. hepatica* infection, were tested by indirect ELISA for liver fluke and 210 animals were selected. Animals were grouped according to age and location. In farm 1, cows (n=32), heifers (n=26) and Spring born female calves (n=32); in farm 2, steers (n=30), Autumn born male calves (n=30) and Spring born male calves (n=30); in farm 3, Spring born female calves (n=30) were selected. The vaccine consists of 200μg of rmFhCL1 in 2ml of adjuvant (Montanide 70VG®, Seppic). Vaccinated animals received two doses of the vaccine, by SC route, in the left cervical region, with an interval of three weeks and were kept in commercial field conditions, naturally exposed to the infection. All animals will be monitored for 48 weeks after the second vaccination. Blood samples are collected periodically from each animal in three different tubes: a plain tube, for serum collection and ELISA testing; an EDTA tube, for haematological (lymphocytes and eosinophils) analysis; a heparin tube, for liver enzymes (GLDH and γGT) evaluation. After the booster dose, four samplings were carried out with an interval of two weeks and subsequent samplings with four week intervals. Individual faecal samples were collected every three months for faecal egg counts. Quantitative indirect ELISA was performed with pools of serum samples grouped according to age, location and status (vaccinated or control). Body weights of the animals were recorded for productivity analysis, as well as the milk yield of the cows. All routine treatments were provided to the animals as required, with the exception of flukicide treatment.

Results All vaccinated animals became seropositive three weeks after the first vaccination. There was possibly an increase in humoral immune response after the second vaccination (graphs 1, 2 and 3). Control groups became positive at 6wpv, indicating exposure of the animals to natural infection. In addition, the natural infection boosted the serological response of vaccinated animals (graphs 1 and 2). The exception is farm three (graph 3), where the controls are still negative, indicating the absence of infection in all animals. The infection of seropositive animals was confirmed by positivity in faecal analysis, when *F. hepatica* eggs were visualised.

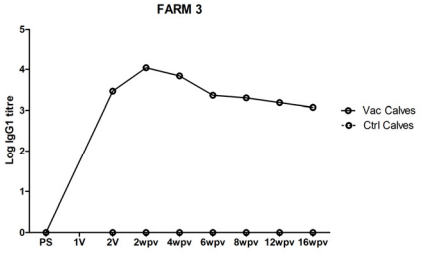
Graph 1 - Serological immune response in animals from farm 1, vaccinated with rmFhCL1 and controls, exposed to natural infection



Graph 2 - Serological immune response in animals from farm 2, vaccinated with rmFhCL1 and controls, exposed to natural infection



Graph 3 - Serological immune response in animals from farm 3, vaccinated with rmFhCL1 and controls, exposed to natural infection



Conclusions Cattle from different ages vaccinated with rmFhCL1 showed high levels of specific humoral immune response anti-*F. hepatica*. Natural exposure of the animals was confirmed by seroconversion of controls and detection of liver fluke eggs in faeces of those controls. A better picture on the levels of protection will be available by the time of this presentation, when the liver analysis data from the first slaughtered group may be shown.

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Genotypic and phenotypic characterisation of Irish mastitis-associated *Staphylococcus aureus*

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Introduction Mastitis, which is inflammation of the milk producing tissue of the mammary gland, is an important animal health and economic problem of the dairy industry. The majority of mastitis cases are of bacterial origin and a wide variety of pathogens may be associated. *Staphylococcus aureus* is traditionally considered to be the predominant mastitis causing pathogen in Ireland. Molecular epidemiological studies have shown that specific genotypes of *S. aureus* are particularly suited to infecting cattle suggesting that these isolates have adapted or acquired distinct virulence factors for interacting with the bovine host (Herron-Olson *et al.,* 2007). In this study, we aim to profile the genetic diversity of Irish mastitis-associated *S. aureus* using Multi Locus Sequence Typing (MLST), identify the prevalent clonal complexes (CC), profile their antimicrobial susceptibility status and to characterize these isolates for their bovine mammary epithelial cell adhesion and internalization properties using Flow Cytometry.

Material and methods Milk samples were collected over a one-year period from cows displaying symptoms of clinical mastitis on 32 farms in Ireland. From these milk samples a total of 137 *S. aureus* isolates were recovered. Genotypic characterization of the isolates was carried out using MLST with all PCR conditions and oligonucleotide sequences provided on the *S. aureus* MLST database (http://saureus.mlst.net/). For each isolate, a consensus sequence was generated for each of the seven loci examined by MLST, using the sequence analysis software Bioedit (http://www.mbio.ncsu.edu/bioedit/bioedit.html). These consensus sequences were subsequently compared to those in the MLST database to assign allele numbers for each locus. For each isolate, the alleles at each of the seven loci defined its sequence type (ST). Groups of related genotypes were identified using the eBURST algorithm (http://eburst.mlst.net/). Irish mastitis-associated *S. aureus* STs were also compared with all STs of bovine origin in the MLST database. Antimicrobial susceptibility profiling was performed using the Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk susceptibility testing for bacteria isolated from animals for the following antibiotics: Amoxicillin + Clavulanic Acid (AMC), Oxacillin (OX), Ampicillin (AM), Penicillin (P), Cefalexin (CN), Ceftiofur (XNL), Kanomycin (K), Neomycin (N), Clindomycin (CM), Erythromycin (E), Tetracycline (TE) and Enrofloxacin (ENR). A whole cell IgG affinity blot determined the presence of Protein A on the cell surface of the *S. aureus* isolates. Flow Cytometry was utilised to determine the ability of the *S. aureus* isolates to adhere to and internalize into bovine mammary epithelial cells. For the adherence and internalisation studies BME-UV bovine mammary epithelial cells were infected with CFDA-SE stained *S. aureus* at an MOI of 10:1 for 3h. The infected cells were washed, fixed and stained with DAPI and any externally adherent *S. aureus* were counterstained with Alexa Fluor 633 IgG. Samples were analysed using an Attune Cytometer.

Results MLST analysis was performed for 137 *S. aureus* isolates from which 55 different STs were identified. Of these, 46 were novel STs that had never been sampled previously. The MLST results show a high level of genotypic diversity in the *S. aureus* isolates, both nationally and on individual farms. Despite the large number of novel STs only five clonal complexes (CC) were identified; CC97, CC71, CC151, CC5 and CC1 indicating a phylogenetic relationship among many of the isolates. CC97, CC71 and CC151 are all known bovine-specialised clonal types and the majority of isolates clustered into these groups with only four isolates clustering with CC5 and CC1. A small number of isolates did not cluster and remained singletons. The antimicrobial susceptibility status for the 137 isolates to twelve commonly used antibiotics was determined. Overall, low levels of antimicrobial resistance were observed although approximately 50% of the isolates were resistant to Ampicillin and Penicillin. Antimicrobial susceptibility results were compared with ST results from MLST and resistance to Ampicillin and Penicillin was associated with the CC71 genotype. The presence of protein A was determined in the 137 *S. aureus* isolates and following this, the ability of isolates belonging to CC97 (n=34), CC71 (n=30), CC151 (n=44), CC5 (n=1), CC1 (n=3) ST136 (n=11) and singletons (n=14) to adhere to, and invade BME-UV cells was quantified. Phenotypic differences between isolates were observed.

Conclusions MLST analysis showed extensive genotypic diversity in Irish mastitis-associated *S. aureus* isolates. However, the majority of isolates were highly related and belonged to 5 clonal complexes. Three of these clonal complexes are known to be bovine adapted. Antimicrobial susceptibility testing of the *S. aureus* isolates showed that many displayed Penicillin and Ampicillin resistance and this resistance was associated with CC71. The *S. aureus* isolates were phenotypically characterised for the presence of protein A and their ability to adhere and internalise into bovine mammary epithelial cells by flow cytometry.

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Potential role for Interleukin-17 in bovine *Streptococcus uberis* mastitis

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Introduction *S. uberis* is one of the most important causes of mastitis in dairy cows. Despite its importance pathogenesis is poorly understood (1). IL-17A is the prototypic member of a family of cytokines involved in control of extracellular bacterial infections.
IL-17A stimulates the recruitment of and enhances the killing ability of neutrophils in human and mice (2) and has been shown to up-regulate genes that encodes for antimicrobial peptides in epithelial cells of the bovine mammary gland (3). Multiple cell types are capable of expressing these cytokines, although $CD4^+$ T helper type 17 (Th17) lymphocytes are thought to be the predominant cellular source (4). The aim of the study was to characterize the IL-17A response to intramammary challenge with *S. uberis* and to determine whether IL-17A is capable of enhancing clearance of *S. uberis* by bovine polymorphonuclear leukocytes (PMN).

Material and methods Six mid-lactating Holstein cows were intramammarily challenged with 200- 700 cfu of *S. uberis* strain FSL Z1-048. Clinical data and samples were collected for 14 days. Viable bacteria and somatic cell count in milk were determined. Influx of T cells subsets in the mammary gland was studied by flow cytometry. The effect of IL-17A on PMN isolated from blood of 4 lactating cows was studied *in vitro*. PMN were stimulated with three different concentrations of recombinant bovine IL-17A for 2 h then co-incubated with an equal number of pre-opsonised *S. uberis* bacteria for 1.5 h. Viable bacteria were counted and percentage survival of bacteria was calculated.

Results All the cows challenged (n = 6) developed clinical mastitis. The response to the intramammary challenge was characterized by clinical signs, reduced milk production and increased body temperature. Somatic cell count increased by 36 h post challenge (PC), reached the peak 42 h PC and remained elevated throughout the study. *S. uberis* viable bacteria were isolated in milk by 12 h PC and reached the maximal concentration at 36 h PC thereafter concentration decreased and 5 quarters cleared the infection by the end of the study. IL-17A was detected in 4 animals between 72 h and 168 h PC. The increase of IL-17A concentration coincided with the major influx of $CD4^+$ lymphocytes observed at 96 h PC. All the animals in which IL-17A was detected cleared the infection spontaneously by the end of the study. Il-17A was undetectable in the animal that did not clear the infection. In individual quarters the increase in concentration of IL-17A coincided with a decrease in concentration of *S. uberis* bacteria in milk. Stimulation of PMN isolated from blood with recombinant bovine IL-17A increased the ability of the cells to kill *S. uberis*. After incubation with PMN alone 33.5±3.8% of bacteria survived whereas after incubation with PMN stimulated with 10 ng/mL of recombinant bovine IL-17A survival was 20.4±1.3% ($P = 0.029$).

Conclusions The results show that IL-17A is produced in the mammary gland in response to the IMI with *S. uberis* and the concomitant increase in milk concentrations of $CD4^+$ T cells and IL-17A suggests that $CD4^+$ T cells may be the major cellular source of IL-17A during infection. Results also suggest that IL-17A may play a role in the clearance of the infection by enhancing S. *uberis* killing by PMN.

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Testing the potential anti-inflammatory properties of a naturally occurring iminosugar compound using a novel equine endometrial tissue explant *in vitro* screening tool

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Introduction Chronic and persistent inflammation is a contributing factor to the pathology of many diseases and has significant impact upon fertility in livestock. An *in-vitro* tissue culture model using equine endometrial tissue explants has been developed that models persistent mating induced endometritis in the mare. This uses bacterial lipopolysaccharide (LPS) to induce altered release patterns of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which is used as a biomarker for inflammation. Iminosugars are small (<500Da) simple amines resembling monosaccharide sugars in which the ring oxygen is replaced with nitrogen; iminosugars are known to interact with a variety of proteins and sugar receptors. Iminosugars have application in the treatment of a variety of diseases and show potential for immunomodulation; to date no research has explored potential anti-inflammatory properties. The aim of this study was to test a naturally occurring iminosugar compound for anti-inflammatory properties using the *in-vitro* equine endometrial explant model.

Materials and methods Endometrial tissue explants were extracted from mares post slaughter encompassing different stages of the oestrous cycle (n=12). Explants were harvested from mares undergoing: anoestrous (n=4), luteal (n=4) and follicular (n=4) stages of oestrous. Explants were cultured in triplicates; control explants were cultured in absence of LPS or iminosugar compound; treatment explants were challenged with: 3µg/mL LPS; 25µg/mL iminosugar compound; 25µg/mL iminosugar compound and 3µg/mL LPS. Culture media was sampled 24 and 72 hrs after challenge and $PGF_{2\alpha}$ concentrations were measured using radioimmunoassay. Mean $PGF_{2\alpha}$ values data were square root transformed, split by time and analysed by ANOVA using SPSS 19th edition software, with a Bonferroni post hoc test for significant difference between treatment means.

Results Explants released significantly greater $PGF_{2\alpha}$ 72 hrs compared to 24 hrs after challenge ($P<0.05$). Explants challenged with LPS secreted significantly greater $PGF_{2\alpha}$ than control explants and explants challenged with iminosugar compound only at 24 hrs after challenge ($P<0.05$); but had not secreted significantly greater $PGF_{2\alpha}$ than explants challenged with iminosugar compound and LPS. Explant challenged with LPS secreted significantly greater $PGF_{2\alpha}$ than control and all other treatments 72 hrs after challenge ($P<0.05$) (Figure1).

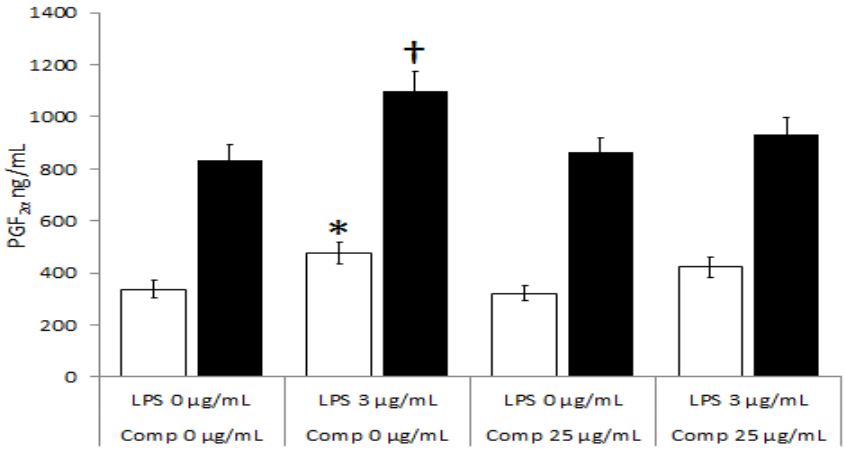

Figure 1 mean release of $PGF_{2\alpha}$ by equine endometrial explants 24□ and 72■ hours after treatment with combinations of LPS and iminosugar compound (+SEM).
Values differ significantly to control at * 24hrs and † 72 hrs ($P<0.05$).

Discussion
The iminosugar compound appeared to modify the secretion of $PGF_{2\alpha}$ by endometrial explants challenged with LPS, over the time course of the culture. There were significantly lower $PGF_{2\alpha}$ concentration values from explants challenged with iminosugar compound and LPS at 72 hrs compared to the LPS only challenged explants, but at 24 hours there was no significant difference in $PGF_{2\alpha}$ concentration values. This suggests that the compound requires longer than 24 hrs for a significant effect to be seen and the iminosugar compound may have potential for treatment of inflammation in the acute to chronic phases and for mitigation of persistent inflammation.

Conclusion Further research is warranted to explore the anti-inflammatory properties of iminosugar compounds. The effect upon release patterns and interaction with other inflammatory mediators e.g. Interleukins 1β and IL-8; TNF-α; and other prostanoids particularly PGE_2, should be examined. The molecular mechanisms by which effects are achieved also require investigation.

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The effect of *Bacillus subtilis* on *Campylobacter* in the caeca of broiler chickens

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Introduction *Campylobacter coli* and *C. jejuni* cause campylobacteriosis and are the leading cause of bacterial gastro-enteritis in the industrialised world. These bacteria thrive in the chicken (*Gallus gallus domesticus*) gut and most poultry flocks are thought to be colonized with *Campylobacter spp.* with 81.3% of poultry meat contaminated after slaughter (Pezzotti *et al* 2003). As the use of prophylactic antibiotics is no longer allowed, there is a need to investigate alternate methods of modulating the chicken gut microbiome and reducing the load of pathogenic bacteria. Probiotics have been shown to reduce the load of pathogenic bacteria. *Bacillus subtilis* strain KD1 isolated from broilers, when used as a feed supplement, was linked to the maintenance of a normal intestinal microflora with an increase in lactobacilli, known for their anti-inflammatory and anti-cancer properties, and a decrease in *Escherichia coli* (Wu *et al*. 2011). This study investigated the effect of the GRAS registered (Westers *et al*. 2004) *B. subtilis* (*Bacillus natto* Sawamura) as a probiotic feed supplement on levels of thermotolerant *Campylobacter spp.* in broiler chickens. A second complimentary paper reporting growth weights and performance is also reported (Horton *et al*. 2013).

Material and methods Ross 308 broiler chicks (n = 200) were weighted, with 10 euthanised as the day 0 initial slaughter group with organs dissected and weighted. The remaining chicks (*n*= 190) were divided into two groups and fed either commercial feed or feed supplemented with *B. subtilis* spores to provide 10^7 CFU/d as a starter, grower and finisher ration. The broiler facility consists of 4 self-contained berths, each of which were split into two pens. Treatment was allocated at random to each of these pens, with 22 – 25 chicks per pen. Temperature, lighting, humidity and ventilation were all controlled and set-up to simulate commercial broiler production. *Campylobacter* positive top-litter (100g) from 4 broiler farms was introduced to all pens. Appropriate feed and water was provided *ad libitum* via bell feeders and nipple drinkers with feed intake per pen monitored daily. On days: 14, 21, 28 and 36 five birds from each pen were weighed, euthanised and caecal luminal contents weighed into 30mL sterilin tubes. A subsample of this caecal content was then weighed into a test tube containing 9mL maximum recovery diluent and serially diluted logarithmically four times. From each of these five serial dilutions, 100µL was spread in triplicate onto plates containing modified cefoperazone charcoal deoxycholate agar (mCCDA). Plates were then incubated microaerobically (5% O_2, 10% CO_2, 85% N_2) at 42°C for 48h and colonies counted. *Campylobacter* spp. were identified based on colony morphology, microscopy and results from catalase, oxidase and hippurate hydrolysis tests. Colony forming units per gram (CFU/g) were calculated, converted to log+1 values, and statistically analysed using multivariate ANOVA with bird as the experimental unit, treatment as diet (Control vs. Intervention) * day of slaughter * pen and blocked according to berth (Genstat ®).

Results Birds fed commercial feed (control) carried greater loads of *Campylobacter* spp. than birds fed feed supplemented with *B. subtilis* spores (intervention), except for day 36 where loads were similar, as shown in figure 1. The overall mean results for CFU/g were significantly different (P<0.01), with control birds having on average 3.47 log CFU/g and intervention birds having 3.14 log CFU/g.

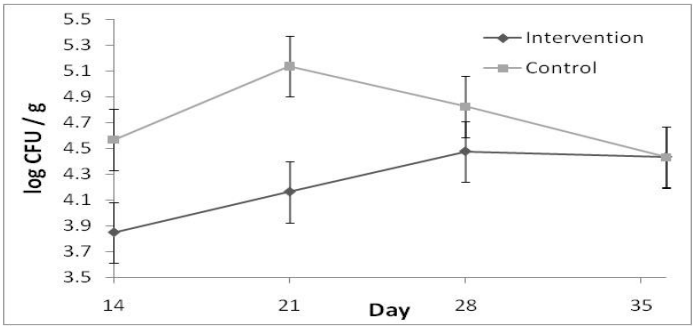

Figure 1 Effect of feed on *Campylobacter* loads in the caeca of broiler chickens (Error bars are s.e.d.).

Conclusions The results show that diet had an affect on loads of *Campylobacter* present in the luminal contents of broiler chicken caeca, with the greatest difference in *Campylobacter* loads seen on days 14 and 21. This indicates that *B. subtilis*, when used as a food supplement, has the potential to reduce levels of *Campylobacter* present in chicken caeca.

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Mastitis pathogens isolated from conventionally or organically managed dairy cattle according to lactation number

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Introduction Mastitis is a common disease of dairy cattle, causing economic loss (Seegers, Fourichon, & Beaudeau, 2003), by reducing milk yield and increasing production costs. Some researchers have found less mastitis in organic dairy herds compared to conventional herds (Hamilton *et al*., 2006), while others reported poor udder health and higher levels of somatic cell count (SCC) in organic herds compared to conventional herds (Hovi & Roderick, 2000). The objective of this research was to assess the effect of lactation number on the frequency with which differing types of mastitis related pathogens are isolated from milk collected from conventionally (Con) and organically (Org) managed cows and use this to assess potential management strategies to reduce specific mastitis pathogens and improve the opportunity to increase the animal longevity in organically managed dairy cattle.

Materials and methods The experiment was completed at the dairy cattle research unit in accordance with the ethical procedures of Massey University, Palmerston North, New Zealand, between August 2010 and May 2011. Individual milk samples were collected from 46 Org and 51 Con lactating Holstein Friesian and Friesian x Jersey cross bred dairy cattle. All milk samples were collected aseptically by hand stripping of 20 ml of milk, prior to machine milking, from all the individual functioning quarters of each individual cow into an aseptic plastic tube, directly following the top was attached. Milk samples were collected from all quarters of all cows at 1, 7, 14, 130 and 260 days in milk and transferred directly into a cool box and then to the pathology laboratory at Massey University, which is (ISO) accredited for mastitis pathogen identification and this was completed according to standard procedures for each pathogen type. Milk SCC count was assessed using 20 ml samples of whole milk collected from individual cow on a monthly basis, which was assessed using NIR analysis (Livestock Improvement Company, NZ). The data was found to be normally distributed, with the exception of geometric mean somatic cell counts, and analyzed using a pro GLM MIX procedure in SAS 9.2 with management (organic or conventional) and number of lactations were included in the model as fixed effects, while individual animal was included as a random effect to account for repeated measures. Differences between means were assessed using pairwise least significant difference test, using individual errors and a confidence interval of 95%.

Results Con. managed cows in ≥5 lactation were more frequently infected by Bacillus, spp, while Org cows in 3+4 lactation were more frequently infected by Strep uberis. There was no significant effect of management system on the frequency of Staph aureus.

Table 1 Pathogens isolated from milk samples collected from individual quarters of cattle in lactation 1, 2, 3+4 & ≥5 under organic (Org) and conventional (Con.) herd management

		Dairy system		Sig
		Organic	Conventional	
Bacillus spp	1	4.0 (1.80)	7.2 (2□60)	NS
	2	6.2 (1.98)	9.9 (2.79)	NS
	3+4	10.0 (3.96)	7.1 (2.08)	NS
	≥5	2.9 (1.06)[b]	10.9 (2.67)[a]	***
Strep uberis	1	3.6 (1.71)	2.0 (1.16)	NS
	2	1.9 (0.97)	2.7 (1.21)	NS
	3+4	7.8 (3.54)[a]	2.2 (0.99)[b]	**
	≥5	4.4 (1.41)	2.9 (1.15)	NS
Staph aureus	1	0.6 (0.64)	0.6 (0.64)	NS
	2	0.8 (0.64)	0.9 (0.70)	NS
	3+4	0.9 (0.98)	1.1 (0.72)	NS
	≥5	2.6 (1.1)	1.9 (0.97□	NS

** and *** - Means in the same row with differing superscripts differ significantly at P<0.01 and P<0.001 respectively

Conclusions Org cows were more frequently infected by Strep uberis and subjected to premature culling. Con cows in ≥5 lactation were more frequently infected by Bacillus spp, most possibly due to older age, poorer udder conformation and contamination.

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Chemical composition and fibre fractions of cowpea husk fermented with single and mixed culture of fungi for use in rabbits' rations

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Introduction Agro - industrial by -products (AIBPs) such as cowpea husk can be valuable fibre source in rabbit's diets. These AIBPs are lignocellulosic in nature. However, their nutritive value can be improved through biotechnology which involves solid state fermentation followed by biodegradation of the products using appropriate fungi species. This procedure holds bright prospects for on – farm adaptation by small to medium scale rabbit enterprises who have minimal resourses and may not be able to afford use of expensive purified enzymes nor be able to support high technological procedures on their farm in order to ensure maximal output. The aim of this preliminary investigation is to evaluate the chemical composition and fibre fractions of cowpea husk fermented with single and mixed cultures of some selected fungi in vitro.

Materials and methods Cowpea husks (CH) were collected from designated centres, sundried (≤ 90% DM) and then, 200g of milled (1.0 mm sieve) CH was measured into six different beaker each and replicated four times. The measured cowpea husks were moistened (23mls/10g) with distilled water (Adedire *et al*, 2012). and the spore solutions of respective fungi species were added at the rate of 20mls/100g 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. oligosporu*s (TRH) (Adedire *et a*l, 2012) and then mixed together thoroughly. The crop residues were allowed to ferment anaerobically for 72 hours. Triplicate samples of fermented and unfermented products were analysed to determine crude protein, ether extract, crude fibre, ash on dry mater basis according to A.O.A.C (2000) methods; neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) according to Goering and Van Soest (1970); hemicelluloses as the difference between NDF and ADF; cellulose as the difference between ADF and ADL and non fibrous carbohydrates (NFC) using the equation:
NFC = 100 – (CP + EE + ASH + NDF) according to Calsamiglia *et a*l 1995. Data obtained were statistically analyzed with the one-way ANOVA using SPSS (15) and means were separated sing Tukey comparism test.

Results Fermentation increased the crude protein and the ash content of the fermented samples while the values of ADF and ADL were significantly reduced. Cellulose and NDF contents remained largely similar. It was also observed that fermentation degraded the fibre content and enhanced the soluble fibre fractions (hemicelluloses and NDF) of the fermented product.

Table 1 Chemical composition and fibre fractions of fermented and unfermented cowpea husk (%)

	UCH	ASP	RHZ	TRI	ARH	ATR	TRH
Crude protein	12.06^{a}	14.23^{b}	14.50^{c}	15.04^{de}	14.90^{d}	15.02^{de}	15.10^{e}
Ether extract	0.69^{a}	1.20^{b}	1.20^{b}	1.21^{b}	1.20^{b}	1.20^{b}	1.21^{b}
Total ash	7.02^{a}	10.03^{b}	10.20^{b}	10.01^{b}	10.02^{b}	10.02^{b}	10.01^{b}
Crude fibre	35.04^{g}	33.72^{f}	33.01^{e}	31.86^{b}	32.59^{c}	32.63^{d}	31.72^{a}
NDF	35.63^{a}	36.74^{b}	36.82^{c}	38.00^{f}	37.01^{d}	37.78^{e}	38.22^{g}
ADF	34.85^{g}	32.00^{f}	31.92^{e}	30.29^{b}	31.62^{d}	30.02^{a}	30.64^{c}
ADL	6.86^{d}	4.21^{c}	4.19^{c}	4.02^{b}	4.02^{b}	4.00^{b}	3.76^{a}
Hemicellulose	0.78^{a}	4.74^{c}	4.42^{b}	7.71^{f}	5.39^{d}	7.76^{g}	7.58^{e}
Cellulose	27.99^{g}	27.79^{f}	27.73^{e}	26.27^{b}	27.60^{d}	26.02^{a}	26.88^{c}
NFC	50.65^{g}	37.80^{f}	37.28^{e}	35.74^{b}	36.87^{d}	35.98^{c}	35.46^{a}

UCH – unfermented cowpea husk. Means within each role with different superscript are significantly different ($P<0.05$)

Conclusion These results have shown the potential usefulness of fermented cowpea husk as protein and fibre sources in rabbits' feed. The fibre conten in the products can be tolerated by rabbits if included in rations at percentage that will meet the stated nutrient requirements of the rabbits.

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Copra cake as a partial replacement for maize and fish meal in non ruminant diets using the Albino rat as a model

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Introduction The use of by-products in developing countries is increasingly becoming popular although agricultural by-products, which are in abundance, are underutilized (Orunmuyi *et al*., 2006). This is due to the nutritional values not known by the end users who are the rural farmers. Even where the nutritional values are known, the levels of inclusion are not known. This sometimes led to detrimental effects on the animals and make farmers reluctant to utilize them subsequently. Copra cake (CC) a by-product after extracting oil from the dried meat of a matured coconut is a potential dietary supplement. Its inclusion has the tendency of improving feed usage due to the high Arginine:Lysine ratio effect in alleviating heat stress. The objective of this study was to assess the nutritive value and effect of CC as a partial replacement for maize and fishmeal on performance and carcass characteristics of the Albino rat.

Material and methods A study was carried out at the Livestock Section of the Department of Animal Science, KNUST, Kumasi, Ghana. Thirty entire male rats with average initial weight of 85.5 g were used in a completely randomized design (CRD) feeding trial over a 5-week period. The rats were randomly allocated to five dietary treatments (formulated to be iso-caloric and iso-nitrogenous) with 6 replicates per treatment and fed diets containing 0, 60, 120, 180 and 240 g copra cake/1000 g with maize, wheat bran, fish meal and soya bean meal as major ingredients. The treatments were designated T_1 – T_5 respectively. The rats were housed in individual well ventilated shelves. Each was kept in a plastic container covered with wire mesh, which allowed free circulation of air. Fresh water was supplied every morning to the rats and their containers also cleaned. The rats were given *ad libitum* access to water and feed. 75 ml of water was given daily and the leftover measured the next morning before refilling. Feed was supplied from a known bulk amount (150 g) at the beginning of the week and the left over at the end of the week was subtracted to obtain the weekly feed intake and subsequently the daily intake. Weekly weights of the rats were taken after the initial weight at the start of the study. Average daily gain (ADG) and feed conversion efficiency (FCE) were determined from the intake data. On termination of the study the rats were slaughtered and the weight of the viscera, spleen, liver, kidney, lungs and the GIT (full and empty) recorded. The data obtained from the intake measurements were subjected to analysis of variance (ANOVA) using Genstat (2008) version 8 for windows 7 and differences between means were detected using the Least Significance Differences (LSD) test.

Results The data on the performance of the rats is presented in Table 1. It can be seen that there were no significant ($P>0.05$) differences between the 5 treatment groups in the daily feed intake and the final body weights. There was, however, a numerical increase in the final weight as the level of copra cake increased, with treatment 5 recording the highest (209.9 g) while the control, treatment 1 recorded the lowest (181.6 g). There were significant ($P<0.05$) differences between treatment 5 and 1 with regards to ADG and FCE. T_5 recorded the least feed intake of 9.64 g, the highest gain and best FCE compared to T_1. The organ weights were similar for T_5 and T_1.

Table 1 Effect of dietary Copra Cake on the growth performance of Albino rats.

	Dietary Treatments					
Parameters	T_1	T_2	T_3	T_4	T_5	SEM
Mean final weight (g)	181.6	186.95	196.1	207	209.9	12.57
Mean daily feed intake (g)	10.28	9.74	10.25	10.92	9.64	0.65
Average daily gain ADG (g)	2.76^{b}	2.89^{b}	3.16^{ab}	3.46^{a}	3.56^{a}	0.21
FCE (feed/gain)	3.72^{a}	3.37^{ab}	3.24^{ab}	3.15^{ab}	2.71^{b}	0.22
Mean heart weight (g)	0.82^{a}	0.58^{b}	0.72^{ab}	0.80^{a}	0.83^{a}	0.05
Mean liver weight (g)	7.90^{ab}	6.81^{b}	8.31^{ab}	7.40^{b}	9.26^{a}	0.53
Mean kidney weight (g)	1.48^{a}	1.12^{b}	1.54^{a}	1.51^{a}	1.66^{a}	0.10
Mean spleen weight (g)	1.06^{a}	0.83^{ab}	0.91^{b}	0.97^{b}	1.23^{a}	0.07

SEM- Standard Error of Means: $^{a, b, c, d}$means within rows with different superscripts differ significantly ($p<0.05$).

Conclusions From the study, the inclusion of copra cake in the diet up to 24 % could replace maize and fishmeal in monogastric diets and improve performance. Feeding copra cake in the diet has no adverse effects on the organ weights.

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Comparative utilization of different fibre feedstuffs by weaning/growing pigs in the tropics

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Introduction There have been efforts geared towards increasing animal protein supply at a reduced cost for human consumption in Nigeria. According to Adesehinwa *et al* (1998), pig production represents the fastest means of correcting animal protein shortage in Africa. Because pigs are omnivores, they can convert household wastes and all sorts of feed to high quality protein. For this reason, there is a need to raise these animals efficiently and at the lowest cost possible. Due to the serious competition between the feed industry and other sources of foods which has been resulting in high cost and scarcity of conventional feed ingredients (Onyimonyi and Ugwu, 2007) like maize, soybean meal (SBM), groundnut cake (GNC), fish meal etc, most pig farmers use agro-industrial by-products such as palm kernel cake (PKC), brewers' dried grain (BDG), wheat offal (WO), rice bran (RB), corn bran (CB) as basal feed ingredients. The objective of this study was to compare the effects of including these agro-industrial by-products in the diets of weaning/growing pigs on their performance and economy of production.

Material and methods Five feedstuffs viz: PKC, WO, CB, RB and BDG were used as sources of fibre in the diets of the experimental animals. Five experimental diets were formulated to contain between 19.5-21.5% crude protein and metabolisable energy of 2,720-2,968 Kcal/kg. Each of these feedstuffs was used at 25% of the diets. Diet 1 contained PKC, diet 2 contained WO, diet 3 contained CB, diet 4 contained RB and diet 5 contained BDG. The fixed ingredients which constituted 75% in all the diets were made up of 45% maize, 15% groundnut cake, 10% soybean meal, 2% fishmeal, 2.25% bone meal, 0.5% salt and 0.25% vitamins/minerals premix. Growth trial which lasted for 28 days was conducted using 20 weaner pigs with the initial mean weight of 13.05±1.59. They were randomly allocated to the five experimental diets formulated according to completely randomised design. The proximate composition of the experimental diets was determined using AOAC methods (1995). . On daily basis, the animals were fed the experimental diets at 5% of their body weight and on weekly basis each animal was weighed. At the end of the study, average daily feed intake, average daily gain, feed/gain ratio and feed cost/kg weight gain were determined to evaluate the performance and economy of production of the animals fed different fibrous feedstuffs. Routine management practices were observed. All data on performance and economy of production were analysed statistically using SAS (2000).

Results Table 1 showed the performance and economy of production of the experimental animals. The average daily gain which ranged from 0.20kg to 0.39 kg was highest ($p<0.05$) with diet 2 which had wheat offal and lowest with diet 5 which had brewers' dried grain. The feed cost per kg weight gain was significantly ($p<0.05$) lower for diet 2 (£0.70) when compared with the highest cost of £1.01 for diet 5.

Table 1 Performance and economy of production of experimental pigs fed experimental diets

	Diets				
	1	2	3	4	5
Initial body weight (kg)	13.5±2.21	12.88±1.25	13.25±1.38	12.88±1.25	12.75±1.86
Final body weight (kg)	26.0±3.97^{b}	27.1±1.33^{a}	23.6±1.63^{c}	24.1±3.26^{b}	20.25±3.1^{d}
Average weight gain (kg/day)	0.34±0.05^{b}	0.39±0.02^{a}	0.28±0.01^{c}	0.30±0.05^{b}	0.20±0.03^{d}
Average daily feed intake (kg)	0.92±0.14^{a}	0.90±0.06^{a}	0.85±0.08^{b}	0.84±0.12^{b}	0.73±0.11^{c}
Feed/Gain Ratio	2.72±0.12^{a}	2.38±0.23^{a}	3.01±0.27^{c}	2.90±0.18^{b}	3.65±0.17^{d}
Feed cost/kg weight gain (£)	0.76±0.03^{b}	0.70±0.01^{a}	0.9±0.01^{c}	0.78±0.03^{b}	1.01±0.01^{d}
Calculated Metabolisable Energy (Kcal/Kg)	2,796.6	2,720.7	2,878.2	2,968.2	2,748.2
Crude Protein (%)	21.25	21.50	19.50	19.75	21.25
Crude Fibre (%)	5.32	4.32	5.32	4.45	7.32

$^{a, b, c, d}$ means along the same row having different superscript differ significantly at $p<0.05$.

Conclusion Findings from this study showed that wheat offal was better utilized in all the growth parameters monitored. Also the wheat offal based diet 2 gave the least feed cost per kg weight gain. Conclusively, for best growth rate and feed cost per kg weight gain in pigs, wheat offal should be used as a fibre feedstuff.

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Agro-industrial by-products in the diet of growing rabbits in Northern Ghana

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Introduction The use of agro-industrial by products as a source of protein could minimize the challenge of obtaining locally available concentrate for rabbit production especially in the dry season. Rabbits are a group of livestock with the potential of supplying the protein needs of families particularly in the developing nations. When compared with other domestic livestock, rabbit meat is reported to contain lesser amounts of cholesterol, fat, energy and protein per kilogram. The purpose of this study was to determine the effect of agro-industrial by products on the growth of rabbits.

Material and methods The study was carried out at the Nyankpala campus of the university for development studies, tamale, Ghana. Nyankpala is about 18 km west of Tamale in the Tolon district. The area is in the guinea savannah zone characterized by a unimodal rainfall pattern. Rains begin in April, rising to a peak in August–September and ending in October or November. Rainfall averages 1060 mm per annum. Twenty weaned rabbits of mixed breed with an average weight of 785.5±1.03g were sourced at 8 weeks from a rabbit farm in Ejisu near Kumasi in Ghana. The completely randomized design was used in grouping the animals into 5 groups of 4 and placed on 5 different diets. The feeding trial lasted for 49 days. The dietary treatments were 70% maize, 70% Milled mango seed kernel (MMSK), 70% Brewers spent grain (BSG), 70% Corn mill residue (CMR) and 70% Maize bran (MB). The other 30% was made up of soya bean meal (29%) and di-calcium phosphate, vitamin premix and salt (1%). The rabbits were housed in wire mesh cages each raised 1m above the ground. Each animal was given 100g of feed a day for the 49days.Water was given *ad libitum.* Data collected included feed intake, weight gain, blood profile and carcass characteristics. The experimental diets were analysed for the crude protein, ether extract, ash and dry matter (DM) content according to the procedure of AOAC (2000). The meat products were analysed for ether extract. The NDF and ADF were analysed using the detergent method described by Van Soset *et al*. (1991). The gross energy content (GE) of the feed was determined using a bomb calorimeter. The carbohydrate (CHO) fraction was calculated using the formulae proposed by Sniffen *et al*. (1992) (100-CP-Fat-Ash), organic matter (DM-Ash) Data collected was analysed using analysis of variance from Genstat 13th edition. Means was separated using Duncan's multiple range tests at 5%.

Results The OM was in the range of 609g/kg to 857g.kg and that of carbohydrate was within the range of 563g/kg to 768.5g/kg. The crude protein from the experimental diet was within the range of 168g/kg and 330g/kg. The highest ADF fraction was obtained in BSG and the least from maize. There was no significant (P>0.05) difference in final live weight gain and average daily weight gain among the treatments diets. Significant difference was observed in packed cell volume (PCV), red blood cell (RBC) and haemoglobin (Hb) with the highest recorded in MB. The total WBC was also significantly different with the highest obtained in CMR and the least in MMSK. There was no significant difference (P>0.05) in dressing percentage, hot carcass, cold carcass weight and fat composition.

Table 1 Effects of the treatment diet on intake, growth, blood profile and carcass characteristics of rabbits

Parameters	Treatment						
	Maize	MMSK	BSG	CMR	MB	s.e.d	P-Value
Live-weight (g)	1189	1039	1173	1275	1404	271.6	0.740
Final weight gain (g)	524	234	490	345	540	158.6	0.064
Average daily weight gain (g)	10.70	4.77	9.99	7.04	11.02	3.236	0.640
Daily dry matter intake (g)	52.6	61.0	82.1	60.8	70.1	14.58	0.100
Gain/Feed	0.197^{b}	0.083^{a}	0.120^{ab}	0.124^{ab}	0.160^{ab}	0.047	0.040
PCV(g/l)	26.33^{a}	31.00^{ab}	39.50^{cd}	35.00^{ab}	41.50^{d}	2.392	0.001
HB (%)	8.81^{a}	10.30^{ab}	13.15^{cd}	11.65^{bc}	13.80^{d}	0.800	0.001
RBC(µL)	3.423^{a}	4.030^{ab}	5.135^{cd}	4.550b^{c}	5.394^{d}	0.311	0.001
WBC (%)	6.97^{ab}	5.77 ^{a}	10.15^{b}	10.30^{b}	8.55^{ab}	1.528	0.038
Neutrophils%	48.00	44.00	49.50	48.50	51.00	2.513	0.124
Lymphocytes%	48.00	53.33	49.50	50.75	47.00	3.109	0.326
Eosinophils%	3.00^{ab}	2.00^{ab}	1.00^{a}	0.75^{a}	1.50^{a}	0.555	0.008
Monocytes%	1.00^{b}	0.67^{ab}	0.00^{a}	0.00^{a}	0.50^{ab}	0.325	0.036
Dressing (%)	52.0	49.1	48.9	49.6	55.3	6.22	0.817
Hot Carcass Weight (g)	788	632	729	617	1028	324.2	0.724
Cold carcass Weight (g)	739	606	685	583	959	303.6	0.746
Water holding capacity	97.21^{a}	99.72^{b}	99.68^{b}	99.89^{b}	99.03^{b}	0.463	0.010
Fat content of meat	8.3	0.7	1.3	3.8	3.1	4.39	0.518

Conclusion Maize bran could be incorporated in the diet of rabbits at 70% without any detrimental effect on grower rabbits.

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Estimating lameness incidence in UK herds with use of hoof trimming records

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Introduction Lameness in dairy cattle is one of the most important causes of economic losses in the dairy industry and is a significant welfare problem. Diseases of the feet account for ~90% of lameness cases and is often caused by claw disorders and subclinical laminitis (Sogstad *et al.*, 2005). A wealth of data on various lameness measures exist (e.g. farmer recorded, mobility scoring, foot trimming, classifier recorded, veterinary); however at present in the UK it is not exploited to achieve its potential value to the industry, such as its use in genetic evaluations because most of it is distributed across a number of databases. A descriptive analysis was carried out on hoof trimming data together with a preliminary genetic analysis.

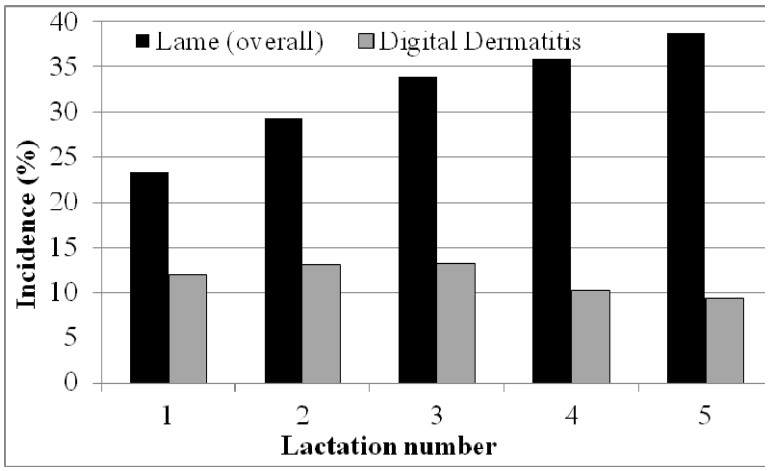

Figure 1 Incidence of lameness (overall) and Digital Dermatitis in lactations 1 to 5

Methods Hoof trimming data collected from March 2010 to July 2012 was supplied by SKS Footrimming Service Ltd, with use of Hooftec software. Data was recorded on each limb and included treatment type (e.g. wrap, block) or the cause of lameness (e.g. sole ulcer). Hoof trimming data was matched to milk recorded data, obtained from Milk Recording Organisations. Data were pooled together across trimming events over the lactation in which a cow was trimmed and overall lameness (combining all problems) and separate lameness problems were scored as a binary trait (1 if affected on any limb and 0 if not affected). Cows with milk records and present in the same herd at the time of a cow with a hoof trimming record were added to the dataset as contemporaries and these were assumed to have no lameness present. The observation period for lactation length was from 0 to 305 days. Incidence of lameness was defined as the number of lactation records with a lameness event divided by the total number of lactation records. After editing, the dataset contained 7,267 animals and 9,707 records in 5 lactations. Genetic parameters were estimated in ASReml (Gilmour *et al.,* 2006) using a linear animal model.

Results Problems were more common on back feet (> 80%) than front feet (<20%). The overall incidence of lameness (when all problems were grouped together) was 30.1% and 33.6% of animals were affected with lameness at some point during the period studied. The five main problems recorded were digital dermatitis, sole ulcer, sole bruise, bad claw, and wall ulcer with 12%, 10%, 9%, 8%, and 6% of cows affected at least once during a lactation. Incidence of lameness problems generally increased with lactation number, ranging from 23% in lactation 1 to 39% in lactation 5 (Figure 1). However, the incidence of digital dermatitis increased up to lactation 3 but declined in lactations 4 and 5. The incidence of lameness ranged between 0.1% and 21.1% within the herds studied.

Conclusions The overall incidence of lameness in this study was 30.1% which is similar to 26% found by Barkema *et al*. (1994). Generally the incidence of lameness (overall) increased with lactation number which was also found in similar studies (Barkema *et al.*, 1994; Sogstad *et al*., 2005). An estimate of lameness incidence using farmer recorded data was 16% (Pritchard *et al.,* 2013), but in general farmer recorded data is expected to be under recorded as it tends to be limited to those cows most severely affected or with obvious cases, particularly those requiring antibiotic treatment. This indicates that, in addition to farmer recorded data, hoof trimming data is a very useful source of information and if collected on a wider scale could be of value to genetic evaluations to reduce lameness. The heritability estimates for lameness (all problems combined), digital dermatitis, bad claw, sole bruise, and sole ulcer ranged between 0.02-0.03. Despite the low heritability genetic progress can still be made (e.g. improvement in fertility using traits with heritability of 0.01-0.04 in the fertility index), together with improvement in management practices to reduce lameness.

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Does vaccinating pigs against porcine circovirus type 2 at three *versus* four weeks of age affect overall performance? – a preliminary study

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Introduction It is a primary concern for pig farmers to maintain high levels of growth performance and reduce mortality levels in their herds; this can be achieved predominantly by reducing disease. Porcine Circovirus Type 2 (PCV2) is prevalent in the majority of pig farms in the UK and has a number of associated diseases, due to its role as an immunosuppressant, which are triggered by factors such as co-infections or external stresses (Krakowka *et al.* 2002; Jacobsen *et al.* 2009). A number of PCV2 vaccines are now commercially available and have been shown to be effective in decreasing the occurrence of associated diseases. However the appropriate timing of vaccination is required to provide maximal protection. This appropriate timing may not always be convenient for farmers leading to variations in piglet age at vaccination. Although the PCV2 vaccine is licensed for use in pigs from three weeks of age, producers often prefer to administer vaccinations at weaning (four weeks of age). Little is known about the effects of vaccinating pigs at either three or four weeks of age on overall growth performance, from weaning through to slaughter. The aim of this study was to investigate the effects of vaccinating piglets at three weeks of age or at four weeks of age on overall growth performance.

Materials and methods Two hundred and eighty nine Large White cross pigs were used in this study. At three weeks of age half of the pigs in each litter were vaccinated against PCV2 with a single 1 ml dose intramuscularly. All piglets in a litter were weighed and placed in rank order, for the first litter, a coin was flipped to determine whether the heaviest pig was vaccinated or not, thereafter alternate pigs were vaccinated. All pigs were ear tagged for individual identification. Pigs that were not vaccinated at three weeks of age were vaccinated at weaning one week later (four weeks of age) again with a single 1 ml dose intramuscularly. All pigs were weighed at weaning and prior to slaughter. Pigs were weaned into weaner accommodation where they stayed until approximately 10 weeks of age. They were then moved into finishing accommodation as is normal farm practice. ADG from weaning to slaughter and age at slaughter were calculated. A Kolmogorov-Smirnov normality test was used to test for normal distribution (SPSS Statistics 20). A General Linear Model (SPSS Statistics 20) was used to analyse the results as a two treatment model to detect any differences in growth performance between the pigs. The individual pig was the experimental unit.

Results Age of vaccination did not affect ADG from weaning to slaughter (97.2±0.54 kg) ($P<0.05$). There was no difference in weight ($P>0.05$) or age ($P>0.05$) at slaughter when comparing pigs vaccinated at three or four weeks of age.

Table 1 Performance of pigs vaccinated at three or four weeks of age

Age of vaccination	3 weeks	4 weeks	SE	P-Value
Week 3 weight, kg	6.2	6.1	0.11	ns
Week 4 weight, kg	7.8	7.8	0.13	ns
Slaughter weight, kg	96.4	98.1	0.78	ns
Slaughter age, d	157.6	158.6	0.851	ns
ADG wean to slaughter, kg	0.678	0.687	0.005	ns

Conclusion
There was no difference in performance throughout the study, thus it can be concluded that the PCV2 vaccine is equally effective in pigs vaccinated at either three or four weeks of age with regards to growth performance.

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Semen characteristics of Landrace boars fed diets supplemented with selenium and soyabean oil

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Introduction The reproductive ability of the male comprises of production of semen containing normal sperm cells in adequate numbers together with high libido (*Oyeyemi* et al., *2000*). Boars that could produce high quality semen allowing greater dilution to attain larger numbers of artificial insemination doses are of importance (Alltech, 2002). Selenium is a component of glutathione peroxidase and an anti-oxidant that protects cellular components against free radicals. Fatty acids not only serve as structural components of cells but also take part in cellular metabolism. Proper nutrition in boars could help to alleviate some of the negative effects of ambient environment thus providing opportunity for them to attain their full genetic potentials. The objective of this study was to determine the effects of feeding selenium and fatty acid (soyabean oil) to Landrace boars on their semen quality with a view to providing base-line information to small scale resource poor pig farmers in Abia State, Nigeria.

Material and methods Landrace boars (n=24; mean weight=41.52 kg; and age 7 months) were used in a2x2 factorial experiment with 4 treatments: T1 (control), T2, T3 and T4 and 6 boars per treatment. T2, T3 and T4 were fed concentrate supplemented with 4 mg selenium (selenite), 3 ml soyabean oil and 4 mg selenium +3 ml soyabean oil /100 kg feed, respectively for 56 days before data collection. The concentrate diet in all the groups contained 18.5% CP and 2626.6 ME (Kcal/Kg). Semen was collected twice a week from each boar for 5 consecutive weeks using gloved hand technique. Semen volume was recorded, motility was determined using an aliquot of semen dropped on a glass slide and viewed under microscope. Sperm concentration was assessed by adding 10 % formal buffer diluent to another aliquot to immobilize sperm cells, which were counted using a Neubauer chamber haemocytometer. Normal and abnormal sperm cells were assessed on slides containing sperm cells stained with eosin negrosin viewed under lignt miroscope. Testis circumference was determined using measuring tape. The data was analysed using repeated measures analysis.

Results and discussion The results of the semem characteristics of the boars are presented in Table 1. Semen parameters of the control group (T1) were ($P<0.05$) lower than most of the semen parameters of the boars fed supplemented diets. The interaction (T4) between selenium and soyabean oil on the semen characteristics of boars were ($P<0.05$).

Table 1 Semen characteristics of the boars

Parameters	T1 (Control)	T2	T3	T4	SEM
Ejaculate volume (ml)	175.00^{a}	186.86^{b}	175.63^{a}	187.50^{b}	2.63
Motility (%)	68.25^{a}	70.63^{b}	70.13^{b}	72.50^{c}	0.53
Concentration ($x10^{9}$/ml)	0.26^{a}	0.28^{b}	0.29^{b}	0.31^{c}	0.01
Total sperm ($x10^{9}$)	45.06^{a}	51.50^{b}	50.65^{b}	57.09^{c}	0.51
Normal sperm cells (%)	76.25^{a}	79.25^{ab}	78.75^{ab}	81.75^{b}	1.37
Abnormal sperm cells (%)	23.75^{a}	20.75^{b}	21.25^{b}	18.25^{b}	0.52
Testis circumference (cm)	8.00^{a}	8.28^{a}	8.13^{a}	8.40^{b}	0.84

Conclusion. The results showed that combined effects of selenium and soyabean oil improved semen quality of boars compared to the others, suggesting higher reproductive characteristic of boars in this group while boars fed T1 (control) had the lowest semen quality.

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Effects of environmental temperature on semen characteristics of New Zealand White rabbits in the tropics

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Introduction High ambient temperature has been shown to impair fertility in male rabbits as a consequence of heat stress, a number of reports indicate alteration in androgen production and subsequent spermatogenesis. Such detrimental effects of seasons are usually high when temperature is combined with relative humidity(RH) which results in thermal stress and consequently lead to changes in biological functions of the animal. A temperature of 21^0C is known as the comfort zone for rabbits, and "their productive and reproductive performance could be impaired when temperature-humidity index(THI) is over 28.0 ^{o}C(Marai *et al*.,2009). This is because rabbits lack functional sweat glands which makes heat loss very difficult above the thermo-neutral zone (Okab, 2007). THI serve as a function of environmental thermal comfort level. However, this particular influence in reproductive functions has not been fully elucidated in the tropics. The aim of this research study was to evaluate the effects of temperature-humidity index on rabbit semen quality in the tropical climate.

Materials and methods Six Isocaloric dietary treatments that differed in either Protein (14%CP, 18%CP and 22%CP) or Selenium content (0.7 and 0.4 mg of Se/kgDM) were offered to mature New-Zealand White rabbit bucks of 22-24 weeks of age, weighing between 2.0 – 3.6kg. Eight animals were allotted to each treatment and fed adlibitum for the period of 24 weeks (6 months).The design of the experiment is 2 x 3 factorial. Total of 288 ejaculates was collected monthly, using artificial vagina between April and Sept (average temperatures range 25.5^{o}C - 32.0 ^{o}C).Ejaculate samples were evaluated according to standard routine. Meteorological data of the study area was recorded throughout the experimental period using digital thermometer (Mextech TM-1). THI was computed using the standard formular by Marai *et al* (2009). Data were analysed by analysis of variance (Minitab) using GLM procedures to determine the effect of temperature- humidity index.

Results A significant reduction (P=0.001) in reaction time (3.8sec) which indicated higher libido, was noted in July (THI 25.33^{o}C) shown in Table 1. This could be because of the lower THI value. Since environmental temperatures (THI) above 28^{o}C cause heat induced physiological stress and consequently leads to degeneration of germinal epithelium (Marai *et al*., 2009) and reduced libido. The relatively lower(P<0.05) mean value of sperm concentration (82.9 $\times 10^6$) in July (THI 25.33), most likely because of the hypoactivity of accessory gland secretions, thereby affecting the ejaculate output, due to mild hyperthermia. There was a significant reduction (P<0.05) in sperm motility (65.7%) in May (THI-26.30^{O}C), and also a significant positive correlated effect of THI on abnormal sperm cells(P=0.001), these were probably due to disruption of membrane fluidity as a consequent effect of homeothermy following aftermath influence of heat in April(THI 30.00^{O}C), which could alter the hormonal secretions and spermatogenesis via hypothalamic-pituitary gonadal axis.

Table 1 Effects of Period/Temperature on Semen Characteristics of New Zealand White Rabbits

Month	THI(^{O}C)	Reaction time(sec)	Semen volume(ml)	Sperm motility(%)	Sperm conc($\times 10^6$)	Live sperm(%)	Abnormal sperm(%)
April	30.00	9.3^a	1.1	74.5^{ab}	85.6^{ab}	76.3	14.7^a
May	26.30	4.8^c	0.9	65.7^b	114.3^{ab}	79.1	11.1^b
June	25.11	5.4^{bc}	1.0	73.6^{ab}	125.8^a	80.2	12.2^{ab}
July	25.33	3.8^c	1.3	76.5^{ab}	82.9^b	80.5	11.1^b
August	25.00	4.0^c	0.9	77.2^a	103.5_{ab}	76.9	7.7^c
September	27.01	7.0^b	0.8	71^{ab}	102.8^{ab}	81.8	10.9^b
s.e.d		0.463	0.129	2.812	10.029	1.717	0.634
P		0.001	NS	0.045	0.023	NS	0.001

Conclusion There was indication of moderate thermal effect on ejaculate parameters, considering the correlated changes in THI values, even though the adverse effects of THI were not immediate. Hence caution should be taken in exposure to an environment with THI above 26.0^{O}C in sub-humid tropics. Therefore it is expedient to develop strategies to offset detrimental effects of heat stress on semen quality in male rabbits.

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Effects of triple super phosphate supplementation on *in vitro* degradability of rice straw and ammonia nitrogen concentration

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Introduction Phosphorus (Phos) is very important for normal rumen metabolism, reproduction, skeletal growth and production. Phos plays active roles for the formation of phospholipids in the cells, nucleic acid and co enzyme. It also plays a vital role in energy metabolism. Rumen microbes have specific Phos requirements to degrade the cell walls of feedstuffs. Also, rumen microbes need Phos to maintain metabolism and growth. Bacterial proliferation is strongly dependent on a sufficient supply of Phos. There was a significant linear response in the voluntary feed intake in calves supplemented with Phos. Low Phos intake frequently occurs in young stock and dry cows are lack of supplementation or concentrates feeding. Most of the normal forages consumed by ruminants are less in Phos content than the requirement (Khan and Chaudhry, 2012). In herds reared at pasture, Phos can be considered, worldwide, the major mineral deficiency causing economic impact. Therefore, increasing use of poor quality roughages and by-products, generally deficient in Phos, animals need to be supplemented with this mineral to meet their nutritional requirements. Phos is generally the most costly mineral to supplement in animal diets. So farmers from developing countries are looking for alternative source of Phos that is cheap and easily available. Triple super phosphate (TSP) can be used as an alternative source. It is known as fertilizer. The chemical composition of TSP is Ca $(H_2PO_4)_2.CaSO_4.2H_20$. TSP contains 48% phosphorus penta oxide (P_2O_5) and 21.12 % Phos. It was not only the sources of Phos but also provides calcium and sulpher. All these three minerals are associated with each other and helped phosphorus to function properly. Therefore the present study was carried out to examine the effect of TSP supplements to enhance the utilization of low quality forages by ruminants.

Material and methods In vitro trial was conducted for 2 days in which rice straw was used as basal forages. The rumen fluid (RF) was collected from immediately after slaughtered a mature cow. RF was stained through two layers of the muslin cloth into pre warmed flask. The RF was mixed with pre warmed buffer at 1:3 (RF: buffer) ratio. The incubations of rice straw were conducted in 50-ml centrifuge tubes each containing about 0.4 g of ground (1mm) sample. TSP was added as a source of P with the forage at six different levels (0, 2, 4, 6, 8 and 12 g/kg). Then 40 ml of buffered RF were added to each tube. The tubes were sealed with rubber stoppers fitted with pressure release narrow glass rod. Incubation was conducted at 38°C in a water bath. After 0, 6, 12, 24 and 48 h the tubes were collected from water bath and submerged in an ice box to stop further fermentation. The liquid and residue was separated by filtering with filter cloth. The supernatant of the buffered rumen fluid was collected to determine ammonia concentration in rumen fluid and 20 ml of supernatant were acidified with 10 ml of 1 (N) HCl and kept in a tube. Residues were washed with distilled water and used to determine DM degradability. Acidified sample were distillated with 40 ml 40% NaOH solution into kjeldahl flask. Twenty ml 2% boric acid solution were placed into distillation set. After the distillation the sample were titrated with 0.1N HCl.

Table 1 DM degradability and NH_3-N concentration in rumen fluid with rice straw at different incubation time

Level of P (g/kg)	DM degradability (g/kg)						NH_3-N concentration (mg/L) in rumen fluid					
	Time (h)					SEM	Time (h)					SEM
	0	6	12	24	48		0	6	12	24	48	
0	103.5	150.0	167.5	175.0	287.5	20.9	89.3	173.2	199.5	236.3	241.5	18.5
2	108.8	152.5	292.5	301.3	326.3	29.5	94.5	257.3	252.0	262.5	273.0	22.4
4	136.3	201.3	316.2	325.0	351.2	27.8	99.8	262.5	273.0	273.0	278.3	23.0
6	132.5	265.0	355.0	366.3	428.8	34.6	99.8	278.3	278.3	299.2	309.7	25.9
8	166.3	306.2	343.7	347.5	458.7	33.2	99.8	278.3	288.8	309.7	325.5	27.4
12	182.5	361.3	405.0	453.8	562.5	37.3	110.3	330.8	346.5	346.5	351.7	31.3

Level of phosphorus; $p<0.001$, Time; $p<0.001$

Result The DM degradability and NH_3-N concentration increased significantly ($P<0.001$) with higher level of TSP and longer incubation time. The DM degradability increased due to the presence of Phos that helps in increased microbial activity during the process of digestion. The higher degradability of DM causes higher rumen metabolism. The DM degradability of rice straw without any treatment was very low due to various ligno-cellulose bonds. TSP as a source of Phos played an important role in synthesis of protein and was crucial for the production of sugar phosphate, adenosine di phosphates and tri phosphate that helped to increase the number of amylolytic, pectinolytic and cellulolytic bacteria species. Due to increased microbial activity and higher protein synthesis DM degradability were increased with higher level of TSP. TSP also played a vital role in energy metabolism. The activity of bacterial fibrolytic enzymes is strongly dependent on the supply of available Phos.

Conclusions Low quality forages in developing countries are shortage in CP and other minerals. Phos is one of them. To fufill the CP requirement urea became popular in many regions. For Phos deficiency TSP could be an alternative source. Further research on this tropic can help in future.

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Effects of dietary inclusion of discarded beetroot *beta vulgaris* on the growth performance of Bonsmara steers

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Introduction Approximately 80% of the red meat produced in South Africa derives from feedlot operations where high concentrate diets are fed (Strydom *et al*., 2008). However, this situation is unattainable under poor communities due to high feed costs. It is well established that by-products from the processing of fruit and vegetables, traditionally considered as an environmental problem, can be successfully used in animal nutrition to reduce feed costs (Negesse *et al*., 2009). Discarded beetroot (DB) (*Beta vulgaris var. esculenta*) is amongst the food by-products that are available (± 300 kg/d) in the fresh produce markets in South Africa, which are under-utilized. Research has shown that beetroot is rich in sucrose, contains metabolizable energy (ME) of 11.2 MJ ME/kg DM, and can be fed to ruminants as a supplementary feed (Clark *et al*., 1987, Rodriguez-Sevilla *et al*., 1999). In contrast, Shyamala and Jamuna (2010) reported 61 g tannins/kg DM in beetroot, which can reduce feed intake by ruminants (Silanikove *et al*., 2001). The objective of the present study was to determine the optimal inclusion levels of DB on the growth performance of Bonsmara steers reared under feedlot operation.

Material and methods Batches of discarded beetroot (DB) were collected from the fresh produce markets (Tshwane and Johannesburg) and brought to the Animal Production Institute for chopping, chemical analysis and animal feeding experiment. Diets containing 0, 100, 150, and 200 g/kg DM DB were formulated and fed *ad libitum* to 24 Bonsmara steers (150±10 kg live weight) that were randomly allocated to four treatments in a completely randomised design (n=6). Twenty one (21) days were allowed for adaptation, followed by 81 days growth and data collection. Animals were weighed at the start of trial and thereafter at weekly intervals until the end of the trial. Feed samples were collected on a weekly basis for the analysis of nutritive value. Dry matter intake, average daily gain (ADG) and feed conversion rate (FCR) were determined. Data of the means for the chemical composition and growth performance of steers were analysed in a completely randomized design for ANOVA using Genstat (2005). The differences among treatment means were compared with least significant difference (LSD) and significance was declared at 5 % probability level.

Results Chemical analyses showed that DB contained (g/kg DM) 144 dry matter, 150 crude protein and 12.7 ME MJ/kg DM. Although steers on the control diet had higher ($P < 0.04$) feed intake compared to those fed diets containing DB, the latter treatments improved ($P < 0.05$) daily gains and reduced FCR compared to the control.

Table 1 Growth performance of Bonsmara steers fed experimental diets (n = 6)

Parameter	DB inclusion (g/kg)					
	0	100	150	200	P	SEM
Initial Body Weight (kg)	150	150	151	149	0.81	10.92
Final Body Weight (kg)	361[b]	388[a]	382[a]	383[a]	0.02	12.64
Average Feed Intake (kg/d)	8.8[a]	8.4[b]	8.3[b]	8.4[b]	0.04	0.76
Average Daily Gain (kg/d)	1.9	2.0	1.9	1.9	0.71	13.54
Feed Conversion Rate (kg/kg)	4.8[a]	4.1[b]	4.0[b]	4.3[b]	0.01	0.046

[a b]Means followed by different superscripts within rows differ significantly ($P<0.05$)

Conclusions It was concluded that DB can be included up to 200 g/kg in the diet of growing steers without adversely impacts on animal growth. Further work to evaluate this finding on nutrient digestion, blood metabolites and carcass quality is needed.

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Seasonal weight loss in dairy goats from the Canary Islands: assessment of production losses

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Introduction Seasonal weight loss (SWL) is the major constraint to animal production in tropical regions. In this work, we used two different goat breeds from the Canary Islands (Spain) with common ancestors and contrasting levels of adaptation to SWL: *Majorera* (adapted to nutritional stress) and *Palmera* breed (susceptible to SWL) (Cardoso and Almeida, 2013). The ultimate objective of this work is to quantify the losses in these two breeds when subjected to experimentally induced weight loss.

Material and methods The study was conducted during May/June 2012 at the experimental farm of the Faculty of Veterinary Medicine of the Universidad de Las Palmas de Gran Canaria - ULPGC (Arucas, Gran Canaria, Spain) with 10 *Majorera* and 10 *Palmera* adult dairy goats (3 lactations with kidding in late February) obtained from the experimental flock of the *Pico* Research Station (*Valle Guerra*, Tenerife, Spain). The animals were always housed in group, including during meals. Goats were divided in four sets, two for each breed: an underfed group in which animals were fed on wheat straw *ad libitum* (restricted diet, so a 15-20% reduction of their initial body weight would be attained by the end of the experimental period), and a control group in which animals were fed *ad libitum* on maize, soy 44 (crude protein 44%), dehydrated lucerne, dehydrated beetroot, lucerne hay and a vitamin-mineral supplement, in accordance with the guidelines issued by INRA (Martínez-de la Puente *et al.*, 2011). Goats were weighted on days 0, 9, 13, 14, 15, 16, 17, 20, 21, 22 and 23 of the trial. Animals were milked once-daily in a milking parlour equipped with recording jars (4 litters ± 25 per ml) and the milk yield was recorded on the same days of weight record and also on day 10.

Results The relative live weights and milk yields is presented in figures 1 and 2, respectively. The animals from both control groups had an increase of their weights of approximately 7% and 4% for *Majorera* and *Palmera*, respectively. In relation to the experimental groups, both had a reduction of their live weights of approximately 14% and 13% for *Majorera* and *Palmera*, respectively. Both control groups increased their milk yields during the studied period in 31% and 20% (*Majorera* and *Palmera*, respectively). Concerning the experimental groups both had their values reduced for approximately 88% of their initial milk yield.

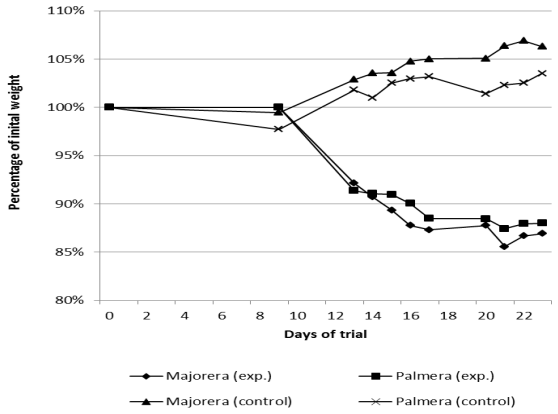

Figure 1 Relative evolution of live weighs for the *Majorera* and *Palmera* goats in the trial. Control groups have significant differences regarding to underfed groups from day 9 to 23 ($p<0.05$).

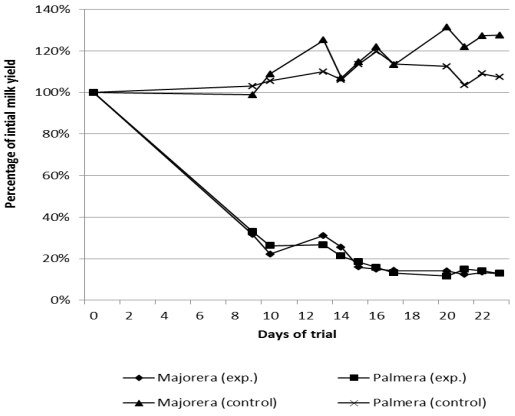

Figure 2 Relative evolution of milk production for the *Majorera* and *Palmera* goats in the trial. Control groups have significant differences regarding to underfed groups from day 9 to 23 ($p<0.05$).

Conclusions Both breeds had similar losses when subjected to weight loss. In the near future, mammary gland biopsies from the animals of the trial herein described will be studied to compare their transcriptomes and proteomes using omics tools. In conclusion, this project results will allow the identification of candidate genes and proteins associated with SWL tolerance, which will be used to the improvement of small ruminant production in tropical and Mediterranean climates.

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Changes in newborn lamb proteomic profile upon colostrum ingestion: an iTRAQ proteomics study

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Introduction Colostrum contains a complex mixture of proteins that participate in the protection of the neonate, mainly immunoglobulins. Due to characteristics of ruminant placenta, the transfer of immunoglobulins from the dam to the foetus is not enough to ensure the survival of the newborn. As a consequence, colostrum intake and protein absorption plays an essential role in Passive Immune Transfer, and ultimately in the newborn survival rate. The aim of this study was to determine which proteins present in the colostrum are absorbed by newborn lambs during the first 14 hours after birth using the iTRAQ (isobaric tag for relative and absolute quantitation) method.

Material and methods Two groups of lambs (of 4 each) were fed colostrum at two different time points after birth. The first group (C group) was fed with colostrum, at 2, 14 and 26 hours after birth, while the other group (DC group) received colostrum at 14 and 26 hours after birth. At the end of the colostrum feeding period (26 hours after birth), each animal (from both groups) received the same amount of fresh colostrum from a pool with 64.74 mg of IgG/mL. Blood samples were collected before feeding at 2 and 14 hours after birth, and the obtained plasma was frozen at -80ºC until further analyses. Samples were homogenized using TES buffer (10mM Tris-HCl (pH 7.6), 1mM EDTA, 0.25M sucrose). After protein quantitation, 100 µg of protein from each sample were obtained after precipitation with 6 volumes of ice-cold acetone at 15.000 x g for 10 minutes at 4ºC. Samples were treated according to Danielsen *et al*. (2011) and then they were labelled according to the manufacturer (Applied Biosystems Inc., Foster City, CA). Finally, samples were combined to create 4-plexed samples. An Agilent 1100 Series capillary HPLC equipped with a Zorbax Bio-SCX Series II was used to fractionate protein mixtures generated from the digestion of 50 µg of protein and an increasing NaCl solution was used for elution. Fractions were collected every minute for 65 minutes and then combined according to their peptide loads into 9-10 pooled samples. Pooled samples were then separated by a reverse phase liquid chromatography using an Agilent 1100 Series nano-flow HPLC system. LC-MS/MS analyses were performed on a QSTAR Elite mass spectrometer (AB Sciex). The collected files were used to interrogate an in-house assembled sheep and goat database consisting of sequences from TrEMBL, Swiss-Prot and NCBInr (Updated November 2012) using Mascot 2.3.02 (Matrix Science). Results were analysed using MS Data Miner v.1.1 (MDM; Dyrlund *et al*., 2012) and a final report was generated, comparing all identified and quantified proteins from the 6 sets of iTRAQ data. Statistical analysis was performed using SAS, Version 9.00 (SAS Institute Inc., Cary, NC). The SAS PROC MIXED procedure for repeated measurements was used to evaluate the effect of colostrum intake (C group vs. DC group) at 2 and 14 hours after birth. A Bonferroni's test was used to evaluate differences between groups.

Results From this result, a total of 31 proteins were selected, as they followed a normal distribution in at least 3 of the 4 biological replicates in each of the studied groups. A statistical analysis was performed in the selected proteins, as described. A total of 8 proteins were found increased in the Colostrum group at 14 hours after birth, as shown in table 1.

Table 1. Identification and function of colostrum proteins overexpressed in the colostrum group (C).

Protein	Function
Apolipoprotein A-IV, B-100 and E	Fat absorption (Intestinal synthesis). Reduces gastric acid secretion. Immunomodulatory effect
Ceruloplasmin precursor	Iron metabolism and Copper transport
Fibrinogen Alpha Chain	Coagulation. Increase during acute-phase reaction. Promotion of adhesion, migration, chemotaxis and phagocytosis of monocytes and macrophages
Immunoglobulin M	Immune function
Tetranectin	Regulation of plasmin activation. Neutrophil migration to the infection
Trypsin inhibitor	Reduction of biologically active trypsin. Protein structure protection

Conclusions Early colostrum intake increased eight plasma proteins in the C group, demonstrating the importance of the non-immunoglobulin proteins that play a fundamental role in the activation and attraction of immune cells, the low gastric secretion, among others. These results can be used to decrease lamb mortality rates and increase the economic benefit of the sheep producers.

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Food Security

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"Food security is the sustainable production of sufficient amounts of high quality, affordable, safe food required to underpin health and wellbeing of human populations world-wide".

The "perfect storm" described by the UK's Chief Scientist, Professor Sir John Beddington, predicted that the world will need to produce 50% more food (FAO, 2008) and energy (IEA, 2008), together with 30% more available fresh water (IFPRI, www.ifpri.org) by 2030, in order to feed the estimated world population of 7 billion. This will have to be achieved whilst mitigating against and adapting to climate change, especially warming of the planet.

Securing world food supplies will require the co-operation and participation of global leaders and politicians in ensuring optimal world trade, stable economies and conflict-free regions. Food producers, large and small, will have to produce significantly more food using fewer input resources, especially land, and resulting in reduced levels of waste, both pre- and post-harvest. The term used for this concept is sustainable intensification. The role of the scientific community extends to economists, engineers, ecologists, social scientists and those involved in crop and livestock production. There is increasing interest in providing "sustainable ecosystems services" from land across the world. Food safety, food sourcing and, topically, food authentication are also important in maintaining food supplies.

The livestock sector sometimes appears somewhat less well aligned than it should with the scientific communities involved in food security. This may result from the perception of some that livestock have mainly a negative effect on the environment and global warming. It is however well recognised that the demand for food from livestock is rapidly increasing both in the emerging economies and in developing countries, as their incomes grow and expectations change.

Professor Sir John Beddington and Professor Sir Robert Watson stated during The Foundation for Science and Technology discussion on the theme of "Achieving food security in the face of climate change" in London in 2011 that the two most important answers to food security were Technology and Rural Development. There is an exciting opportunity for those involved in veterinary research and teaching to contribute to both aspects of this agenda. Technologies in livestock production include the breeding of livestock most adapted to the environment in question, and resistant to relevant diseases, while producing meat, milk or fibre in an efficient manner. Interventions including vaccines and therapies must be integrated into optimal health and production management systems and adopted widely. Exotic and endemic diseases need to be detected rapidly, anticipated where possible, and their biological and environmental impact reduced.

There is no single answer to technology development and adoption. Vaccines will include native protein and recombinant approaches, diagnostics will involve both traditional approaches and rapid molecular techniques, especially those that may be used pen-side. The role of currently-available drugs for therapy and prevention remains controversial in some cases, and unsustainable in others. The need for new drug development also will be key to producing future food supplies.

The delivery of knowledge and technology requires a focus on rural development to ensure that scientific findings reach those who need to adopt them most rapidly. This requires new thinking in terms of approach, with GALVmed a public private partnership funded by Bill and Melinda Gates and DFID, leading the way. The best pathway to delivering existing and new technologies varies with the product in question. In the case of products for livestock keepers, a private good, the pathway should be commercial. Those products which benefit human health benefits through control of disease in livestock, a public good, also need to be supported. The question remains as to who should pay.

Livestock scientists in the UK therefore have an important role to play – we should focus on producing our own food supplies in the most biologically and environmentally efficient ways possible, while developing our research so that we may both use our own technologies and export them worldwide to feed the future population.

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The role of veterinary vaccines in global food security

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Many studies have shown that meat consumption will increase in the foreseeable future as world population increases and as emerging economies become more affluent. This trend is already evident for example in the 'BRIC' countries: Brazil, Russia, India and China. However livestock are a significant source of 'greenhouse' emissions of methane and carbon dioxide and therefore are potentially contributing to global climate change. Nevertheless livestock are essential because they are capable of utilising marginal land, they produce high quality protein and they provide livelihoods for the world's poorest communities. Thus improvement of productivity is necessary to minimise their environmental footprint as well as to produce food as economically as possible. Specifically it was argued a decade ago that in 50 years time the human race would need 100% more food and that 70% of this must come from performance improving technologies.

It has been claimed that livestock production efficiency is reduced 20% by disease. The impact of disease is exerted in various ways including lost productivity (morbidity, mortality), cost of medicines, increased labour, lost local and international trade, human disease in the case of zoonoses, the need for biosecurity measures and environmental impact. Increased international focus on food security has led to increased attention to livestock disease control and prevention. This includes both enzootic production disease and trans-boundary diseases. Thus there is much current interest in the wider application of vaccines to control and prevent such diseases. Vaccines induce protection by a combination of humoral (antibody) and / or cell mediated immune responses. An ideal vaccine could be described as multivalent, providing specific immunity against both clinical disease and infection of a number of diseases and protective to neonates in the face of maternal derived immunity. It would also have a long duration of immunity, without the need for boosters, there would be no adverse reactions, it would be administered by needle free delivery, cheap to manufacture and of reproducible quality. In fact very few available veterinary vaccines meet more than one of these criteria. Conventional vaccine technology is very old, using whole organisms which are either inactivated (dead) or live (attenuated) and many such vaccines are still effective although some diseases have been more problematic, for multiple reasons. In short there are still many unmet needs. Molecular biological techniques have also been extensively applied in veterinary immunology and there are a number of recombinant and other 'high tech' products on the market.

The veterinary vaccine market is worth about US$5.5 billion globally and accounts for about 25% of the total market in veterinary medicines. Livestock vaccines account for about 60% of the vaccine market with companion animal products representing 40%. The vaccine market is growing at about 3.5% in real terms annually. It is predicted that the demand for vaccines will continue to grow for the foreseeable future because of increased international animal production, due to demand from emerging economies as discussed above, increased international animal (also food and people) movements and trade and, increased demand for preventive measures (prophylaxis). Other reasons include increased regulatory hurdles for pharmaceuticals in food animals e.g. antibiotics, anti-parasitics, the threat of bioterrorism, emerging disease threats e.g. Avian influenza, Bluetongue, Schmallenberg, Rift Valley fever including the role of climate change and, advances in technology including host pathogen interactions, genomics and proteomics. These trends are clearly reflected in national and international government policies and investment in research and development in infectious diseases.

Whilst we have witnessed the emergence of economies in Asia and Latin America, the development of the African market has been much slower. The total Sub Saharan African (SSA) animal health market is estimated to be around $500 million and growing at about 10% with South Africa accounting for about half of this ($250 million). About 30% of this market is thought to be in vaccines. Thus the SSA veterinary vaccine market outside South Africa is estimated at around $75 million. It is suggested that East Africa has the largest market share and is one of the most accessible in terms of its growth potential. Although there are several commercial veterinary vaccine manufacturers in South Africa, there are very few non-government manufacturers elsewhere in SSA. However there is an increased focus on public-private initiatives to improve animal health in Africa (see below). Much of the evidence suggests that the African livestock market is poised for growth and a major prerequisite will be for safe and effective vaccines for both trans-boundary and production diseases.

The Global Alliance for Livestock Veterinary Medicines (GALVmed) was set up in 2006 as a not-for-profit organisation to develop veterinary products for the benefit of poor livestock keepers in developing countries. Initial funding was provided by the UK Department for International Development and then the Bill and Melinda Gates Foundation. Four disease projects were initially prioritised i.e. East Coast fever, Rift Valley fever, Newcastle disease and Porcine cysticercosis, although the circumstances and product gaps were very different in each case. In 2012 GALVmed was awarded a further 5 years' funding by these two donors to pursue a larger development portfolio, but also including work on product distribution, adoption and other links in the value chain, as it had quickly become apparent that product development alone was not the solution to availability to poor farmers. GALVmed is a public private partnership and one key to its niche role is the application of animal health industry methodology and rigour to the product development process and intellectual property management.

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Food security: responding to emerging issues in food fraud and authenticity

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Introduction In order to protect consumer interests to combat the growing problems of food fraud and adulteration, scientific expertise and technologies are constantly being developed. Such methods have been applied during recent high profile cases of food fraud and adulteration in the media such as the use of illegal Sudan dyes, addition of bovine material to chicken, and species determination of meats. Analytical approaches employed at the Food and Environment Research Agency (Fera) to screen for meat fraud are discussed.

Stable isotope and elemental analysis Analysis of stable isotopes in foods can reveal economically motivated adulteration such as verification that chicken has been 'corn-fed' and differentiation between organic and conventional egg production methods. In more sophisticated applications of multi-element stable isotope analysis, the geographic rearing location of animals used in meat production can be determined(1).

Liquid chromatography-mass spectrometry (LC-MS) Targeted LC-MS analysis involves screening for pre-determined components in a sample. Such applications have, for example, been used to interrogate processed foods for the presence of illegal Sudan dyes or to determine the inclusion and species origin of meat binding products or 'glues' prepared from bovine or porcine thrombin(2).

Proteomics In high resolution LC-MS applications, proteomic methods can be applied to identify unique proteins or peptides to determine food components. Proteins can be useful markers of authenticity in highly processed samples in which the DNA is denatured and lost. A proteomics method was developed at Fera in collaboration with BioArCh, University of York to determine the species origin (pork, beef, horse, fish or poultry) of gelatine and other highly processed hydrolysed collagen proteins. Such proteins are incorporated into many foods as thickeners and to enhance 'mouth feel'. The method employs a proprietary database containing species and phylogeny data which is used to match unique peptides which are specific to a species or to a tissue (skin or bone). The method was recently employed to investigate the suspected addition of hydrolysed collagen to samples of chicken fillets as a plumping agent(3). The fillets, labelled as 'chicken only' or as 'Halal-slaughtered', were shown to be adulterated with hydrolysed proteins derived from bovine collagen material. In a variation of the same method, the species contained in meat and bone meal can be determined; a method which may be useful in supporting the relaxation of the European Commission Extended Feed Ban.

Metabolomics Meats allowed to mature for longer periods of time carry a premium price at market. Beef, for example, can be labelled as being matured for 21 days to promote sales. Metabolomic methods were developed at Fera to verify labelling claims linked to beef ageing and storage temperature. Samples of meat stored under ideal (4°C), sub ideal (-20°C) and above ideal (20°C) temperatures for wet ageing were analysed daily over 28 days by ^{1}H NMR spectroscopy to generate a non-targeted profile. The ^{1}H NMR spectra were analysed statistically using principle components analysis (PCA) to determine underlying trends. Several analytes that varied with both temperature and age were identified and could be used to verify the age of a sample(4).

Fingerprinting techniques Finally, DNA fingerprinting techniques can be applied to authenticate foods. One application of DNA techniques is the identification of meat and fish species in a sample(5).

Conclusion A wide variety of scientific techniques using sophisticated tools are employed to screen for authenticity and/or adulteration in the food and feed chain. The services at Fera are underpinned by scientific expertise and internationally recognised quality standards to support brand and consumer protection.

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Why worry about our farm animal genetic resources?

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The status of UK FanGR There are currently around 700 breeds of farm livestock in the UK - about 9% of all global livestock breeds (FAO, 2007; Defra, 2013). Of these, 235 are UK native breeds – so the UK has one of the richest native Farm Animal Genetic Resources (FAnGR) populations globally. Over 75% (179 breeds) of UK native breeds are at risk of extinction, as a result of small breeding population size, geographical concentration, or both. Threats to breeds at risk include economic pressures on livestock keepers who may cease to keep breeds at risk, the spread of fewer, specialised breeds, nationally and globally, and the threat from existing and emerging diseases.

Why worry about our FAnGR? Ensuring that our FAnGR – whether 'at risk', or not - are conserved and used sustainably is important for several reasons: 1) they are an important component of wider biodiversity which intrinsically deserves protection; 2) having diverse FAnGR will help us to respond to new challenges e.g. feeding a dramatically growing global human population, while limiting the environmental impact of livestock production; potentially developing our FAnGR to adapt to climate change; 3) they are of great economic importance, being the foundation for livestock products in the UK's £72 billion food and drink industry; 4) many of our FAnGR, especially grazing animals, have a key role in managing the farmed environment and landscapes; 5) they are of great social, cultural, and heritage importance; and 6) we have formal commitments to manage these resources – at domestic, European, and wider international levels.

How should we respond? There is a strong tradition of caring for animal genetic resources in the UK – thanks largely to the activities of individual breeders, breed societies, charities and other non-governmental organisations. Over the last 15 years, this has been augmented by a growing involvement of government, recognising the increasing threats to FAnGR and the obligations to protect them. This involvement has included support for a series of technical advisory committees, including the current Farm Animal Genetic Resources Committee (see www.defra.uk/fangr). These have assisted in the production of reports supporting conservation and sustainable use of FAnGR (e.g. UK National Action Plan on FAnGR (2006), UK Country Reports on FAnGR for 2002 and 2012); established robust definitions for, and categorised, FAnGR, especially identifying breeds at risk; influenced animal health policy so that it affords additional protection to FAnGR; influenced environmental and biodiversity policies so that they recognise the role of FAnGR; advised on research priorities and numerous other livestock policy issues. Key roles remain in many of these areas, for instance in promoting better characterisation of our FAnGR, in developing more automated mechanisms for monitoring their status, and establishing a comprehensive approach to the *ex-situ* cryoconservation of the UK's native breeds.

The role of science and technology Science and technology have a key role in protecting our FAnGR. Many of the tools available to assist in conservation and sustainable use of FAnGR are science-based. Recent research commissioned to further underpin conservation and sustainable use of FAnGR includes projects on: molecular characterisation of breeds; the role of animal genetics in mitigating greenhouse gas emissions; quantifying the contribution that FAnGR and selective breeding can make to improving nutrient use efficiency; developing biodiversity indicators for native cattle and sheep breeds; developing a co-ordinated strategy for *in situ* and *ex situ* conservation of FAnGR; and the development of a DNA-based tool to authenticate traditional-breed meat products (see www.randd.defra.gov.uk for more details). New scientific knowledge and new technologies will offer both opportunities and threats to the conservation and sustainable use of our FAnGR. New technologies are likely to include: 1) reproductive technologies, including improved technologies for sex ratio control, improvements in the efficiency of livestock cloning, the potential for manipulation of cells *in vivo* and selection *in vitro*; 2) genomic selection and related techniques - potentially offering faster genetic gain in mainstream breeds, and offering potential advantages in selection for 'hard to measure' traits, many of which contribute to robustness; 3) statistical genetics techniques that will help in both selection and in genetic resource/diversity management; 4) biobanking and related cryopreservation technologies – new techniques for conserving biological material, and recreating viable populations from this material, offer major opportunities for cost effective conservation of FAnGR; and 5) direct genetic modification of livestock, including via the new approach of genome editing (Defra, 2013).

Conclusion For the many reasons outlined, is clear that we do need to be concerned about the status of our FAnGR. Protecting, conserving, characterising, sustainably using and developing our FAnGR, and the systems in which they are used, is a challenge, especially given the range and diversity of FAnGR present in the UK. While there is growing awareness of the issues in industry, government and academia, and progress has been made in many areas – some illustrated elsewhere in this session and conference - much remains to be done.

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Sustainable genetic management of small populations

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Management of small populations must involve good husbandry routinely, but the small size of the population will place the continued existence of the population into the future at risk. This is important when the population may be a nucleus or elite group of a commercial breed, perhaps managed by a breeding company, or may be a breed outside the mainstream where conservation is an important objective. In the latter case, such populations will more likely be managed by a breed society, which acts as a voluntary co-operative. These risks may be catastrophic, such as those posed by pathogenic disease, which may bring about the disappearance of large swathes of the population, or genetic where an overly narrow gene pool may bring about the reduction in fitness of the population and, consequently the value of the population for its purpose.

Coping with the genetic risks is associated with theories of inbreeding. It is perhaps surprising that the measure of risk faced by the population is not best measured by the widely calculated inbreeding coefficient *per se*. It is often asked what the safe level of inbreeding is, and sometimes levels are proposed, and breeders become concerned on how to keep populations below this level. However there is no 'safe' level of inbreeding coefficient, and in populations maintained without an influx of new parents, the values of inbreeding coefficient will inevitably cross any threshold, even though temporary decreases may be observed due to circumstance. This is not necessarily a problem as inbreeding is a natural process. Instead the risks are more strongly associated with how fast inbreeding is changing in the population, the rate of inbreeding, ΔF, which is also often described by effective population size $N_e = (2\Delta F)^{-1}$. High ΔF, low N_e, promotes more rapid changes in frequencies of alleles such as lethal recessives, reductions in genetic variance, greater random drift in breed attributes, and promotes more severe inbreeding depression. Therefore ΔF is important in determining the scale of depression even though classical theory simply relates depression to the inbreeding coefficient.

ΔF is directly related to the sum of squared contributions a cohort of contemporaries make to the gene pool in the long term, not just the next generation. Therefore if the cohort is small in numbers each member will make a large contribution, since the future gene pool will be shared between them, however even if the cohort is large in number but a few are extremely popular and the remainder make only relatively small or no contribution, then ΔF will still be large, and it is in this way that a breed that is large in census size will be small genetically and face the same genetic risks of a breed that is small in census size. A major determinant of ΔF is the *minimum* of the number of male parents and number of female parents used in a generation, *not* the total number of parents used in a generation, since each sex will contribute half the future gene pool. In livestock the sex with fewer parents is typically male, and very large numbers of females cannot overcome problems arising from a small number of males. It is important to note that contributions of *every* generation are important. Founders are sociologically important, but are an arbitrary group genetically. Over time, contributions of all ancestral generations, including founders, become more and more difficult to change, and the greatest potential impact on the health of the future gene pool comes from the good management of the contributions of the most recent generation.

One of the challenges for a breed society, and geneticists alike, in managing genetic risks is that ΔF is a property of the group of ancestors, and so decisions by individual breeders are only effective in influencing ΔF in so far as they are part of a co-ordinated strategy by the society. For example, genetic bottlenecks (overly narrow parental contributions) cannot be avoided by simply looking at the inbreeding coefficients of parents or potential offspring (another limitation in using the coefficient). Genetic theory and application have advanced so that breeders can simultaneously select and mate parents to maximise gain whilst maintaining ΔF to a particular value, but these tools are only really effective for breeding companies where they have full control over selection and mating. The co-ordination across a whole breed society is much more difficult, but there are steps that can be taken. Whilst these may be challenging to implement, a breed society is a group that is concerned with the future of the breed. For example, societies can amend registration, with advance notice, that allow only a quota of full registrations for offspring from an individual parent. A second step is to promote openness of problems, the ready identification of recessives, analysis of any restricting breed 'standards' so that breeders can use properly evaluated information to move away from problems, and to do so in a way that does not make a small breed smaller. It is important to be aware that modern genomics has developed to a point that it is cost effective for a breed to banish the curse of the recessive at a very early stage before it is a serious problem, providing breeders and societies are alert and pro-active. A further step is to organise breeder groups that promote the flow of all segments of the breed through the breed, and to publicise how the society has done in relation to inbreeding compared to how well it might have done had optimised procedures been followed – there will always be a gap determined by practical and sociological factors, but auditing this gap may promote awareness.

Reduction of risks includes protection from catastrophe, as mentioned above. In principle this can often be achieved by ensuring the populations are dispersed. Dispersal of breeds can raise issues such as the importance of *in situ* conservation relative to *ex situ* conservation of live animals, and in some cases this distinction is critical where it is clear that the differences in the environment of conservation and that of development may rapidly alter the gene pool. One such example is the North Ronaldsay sheep where outside its native environment the breed is at risk of selective mortality against the ability to absorb Cu from its diet, which is one of the adaptations that allow it to survive on the seaweed in its native environment. One degree of freedom in combating catastrophes associated with pathogenic disease has been achieved by changing government policy to give special consideration to breeds at risk through small census size or geographical concentration; in the course of epidemics (such as FMD) dispensation from protective culling will be allowed *providing* there is evidence of appropriate quarantine procedures being applied. A further important measure to reduce risks to small populations is the provision of biobanks, of semen, embryos or DNA, but this topic is large in its own right.

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Finding the genetic lesion which causes Foal Immunodeficiency Syndrome (FIS) in the Fell and Dales pony and the results of diagnostic testing 2010 - 2012

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Introduction Foal Immunodeficiency Syndrome (FIS), formerly known as Fell Pony Syndrome, was first reported in 1996 as a unique condition leading to the wasting and death of foals at a young age. It is specifically seen in Fell and Dales ponies, and characterised by diarrhoea, cough and failure to suckle. Despite initially being responsive to treatment, these opportunistic infections persist and were shown to be due to a primary B-cell deficiency associated with reduced antibody levels. Concurrently, affected foals develop a progressive anaemia, as a result of which foals die or are humanely destroyed at 1-3 months of age. FIS has a pattern of inheritance typical of a recessive inherited disease, and research was undertaken to identify the primary genetic lesion by Laura Fox-Clipsham, a PhD student at the Animal Health Trust and Liverpool University. Mapping investigations using microsatellite and SNP markers were used to locate the chromosome region where the mutation lay. Targeted sequencing was then used to identify the precise mutation which caused the disease (Fox-Clipsham *et al*., 2011). This work led to the development of a diagnostic test which can be performed on pulled mane or tail hair, and used to identify non-symptomatic carriers of FIS, and also to definitively diagnose foals suspected of FIS. Crucially the identification of carriers allows the selection of breeding combinations to avoid the risk of FIS-affected foals; affected foals only result from the breeding of two FIS carriers. The researchers involved attended regular meetings with both the Fell and Dales Pony Societies, where the use of the test to avoid affected foals was discussed. Concerns about depletion of the gene pool were discussed with breeders and it was emphasised that due to the very high carrier levels carrier ponies should still be used for breeding with genetically clear ponies.

Materials and methods Commercial testing was performed using DNA extraction from hair roots followed by PCR amplification and sequencing.

Results A commercial diagnostic test was developed towards the end of 2009, and was launched in the spring of 2010. The testing figures for 2010-2012 have recently been collated and submitted for publication in Veterinary Record. The adults tested comprise a large proportion of the breeding population for both the Fell and Dales ponies.

Table 1 FIS test results in the Fell Pony since the launch of a commercial diagnostic test

	Year	Number tested	Clear (%)	Carrier (%)	Affected (%)
Adults	2010	565	290 (51%)	275 (49%)	N/A
	2011	179	103 (58%)	76 (42%)	N/A
	2012	106	60 (57%)	46 (43%)	N/A
Foals	2010	142	72 (51%)	58 (41%)	12 (8%)
	2011	108	60 (56%)	39 (36%)	9 (8%)
	2012	71	32 (45%)	38 (54%)	1 (1%)

Table 2 FIS test results in the Dales Pony since the launch of a commercial diagnostic test

	Year	Number tested	Clear (%)	Carrier (%)	Affected (%)
Adults	2010	180	158 (88%)	22 (12%)	N/A
	2011	53	44 (83%)	9 (17%)	N/A
	2012	36	32 (89%)	4 (11%)	N/A
Foals	2010	10	9 (90%)	0 (0%)	1 (10%)
	2011	4	3 (75%)	1 (25%)	0 (0%)
	2012	1	1 (100%)	0 (0%)	0 (0%)

Conclusions The implementation of a diagnostic test has drastically reduced the incidence of FIS affected foals. The carrier levels in the general population may differ from those illustrated above, however, as a guide they seem to indicate that the carrier levels have decreased only slightly since testing began. This would suggest that, as recommended, the use of carriers for breeding has continued. The elimination of the mutation should be a long-term objective which should be undertaken gradually over many generations.

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Development of coordinated *in situ* and *ex situ* UK farm animal genetic resources conservation strategy and implementation guidance

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Introduction Defra project GC0146, with the above title, seeks to provide an up to date record, analysis and evaluation of effectiveness of current breeding programmes and conservation strategies (including both in situ and ex situ methods) as they operate in UK native farm animal genetic resources (FAnGR), both mainstream and those at risk. Evidence-based guidance for implementation of effective and compliant activities for FAnGR conservation and sustainable development are being developed. The current status of cryoconservation is reviewed, together with the relevant aspects of animal identification and the policy background to FAnGR. Throughout, the project makes reference to emergent issues and to overseas practice, when appropriate. The project is of 6 months' duration (completion March 2013).

Material and methods All aspects of the project have employed literature review, web searching and personal contacts. In addition, breeding plans and conservation activities are being analysed by reference to pedigree registers and unpublished data. While excellent science-based guidance for population management is available, and there is extensive evidence of good practice, knowledge appears scattered and piecemeal, and the project seeks to gather this information in one place and review it.

Results: review documents The review of policy background considers international agreements and UK national policies and support measures; there are important differences between the constituent countries of the UK. The review also draws attention to the opportunities that the reform of the Common Agricultural Policy provides for supporting conservation of FAnGR, and emphasises how FAnGR do not receive the same degree of support as plant genetic resources. The place of FAnGR in mainstream biological conservation is affirmed. The reviews of cryoconservation and of animal identification, are also designed to support policy makers and to draw attention to areas where research is needed. These reports include costings. The implementation guidance is designed to be accessible, for breeders, breed societies and umbrella organisations, and to facilitate compliance with Zootechnical Regulations and policy in general.

Results: numerical data In addition to the project reports and guidance outputs, material has been assembled in a form suitable for the purposes of the current project but also to serve as a platform for the development and testing of scientific hypotheses of value for sustainable conservation. New data have been collated on the following numbers of native breeds: cattle 20, sheep 33, goats 5, pigs 5, equines 6, poultry 32. Introgression into mainstream breeds has received particular attention. Investigations have been made into methods of assessing effective population sizes and of gathering and utilising demographic information.

Conclusions The project, which was designed to achieve specific outcomes relevant to Defra's responsibilities for UK FAnGR, is also producing results of broader relevance, to be disseminated in the scientific media.

Acknowledgements The help and support provided by the Defra Project Officer and the Project Steering Group, as individuals and collectively, are gratefully acknowledged. The co-operation of breed societies has been fundamental to the progress of the research.

The future for native breeds in creating a sustainable livestock industry

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This paper will argue that future sustainable livestock production systems will call for evermore intelligent approaches to the simultaneous achievement of quality systems that minimise environmental burdens, sustain high standards of animal health and welfare and deliver nutritionally valuable products into well organised food supply chains. That should mean a role for native farm livestock breeds which have over hundreds of years been bred to suit their unique landscape.

The UK is particularly rich in livestock breeds. However between 1900 and 1973 we lost 26 native breeds plus many more varieties of poultry. There has been no loss of breed since RBST formed in 1973.The fact remains though that there are currently 56 breeds at risk on the RBST Watchlist.

The UK's native breeds are the essential building blocks to underpin genetic diversity. There are a number of "success story" breeds where due to a number of different factors they are no longer at risk and their future is assured. Their success shows how such breeds can make a contribution to British agriculture. For example the British White cattle breed are now thought to be more numerous than at anytime in their history whilst the Beef Shorthorn has received a massive fillip due to the interest being shown by Morrisons,a major retailer. For sheep, breeds such as the Wiltshire Horn can fit very well into Easy Care systems whilst Hebrideans can have an important role in conservation grazing.

The success of the above breeds illustrates perhaps the increasing awareness that too much emphasis on high output/high input breeds is dangerous as they may be less well adapted to a changing environment. The payment structure for agri-environmental schemes has also led to renewed interest in traditional livestock breeds.

The paper will continue with short sections on why mainstream farmers should consider native breeds and look at some of the options for restoring a breed's popularity. Market differentiation and segmentation could prove a much added stimulus. It will conclude by suggesting that livestock production systems will have polarised by 2030 into three broad sectors-high output systems, low input/medium output and niche markets. This approach will lead to a sustainable livestock industry which at the same time recognises the importance and relevance of native breeds.

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Why Morrisons is investing in Britain's native breeds

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Morrisons Supermarkets has launched a major new beef supply initiative through their wholly-owned abattoir subsidiary, Woodhead Brothers. Since September 2011 the Morrisons Traditional Beef Scheme has been sourcing 350 British native breed cattle every week (18,000 per year) in return for special native breed premium. All British native beef breeds and their crosses, including rare breeds, receive a 10ppkg premium over Woodhead Brothers base price, while Beef Shorthorns and their crosses receive 20ppkg in total, or up to £76 / head.

The Scheme supplies a new range of "Traditional British Beef" available in over 370 of Morrison's 500 supermarkets across the UK. The beef is matured for up to three weeks for extra flavour, while the prime cuts are matured on the bone in specially designed chillers, cementing Morrison's already strong reputation for British beef.

These British native breed cattle have boosted output from the supermarket's three Woodhead Brothers abattoirs (in Colne, Lancashire; Turriff, Aberdeenshire and Spalding, Lincolnshire) by approximately 10% to almost 200 thousand head per year.

The special premium for shorthorns reflects the supermarket's long-standing commitment to the Shorthorn breed on its own farm at Dumfries House in Scotland, where it has developed a special feeding protocol specifically to enhance the inherent eating quality of native breeds and produce a premium product.

Frank Milnes, Secretary of the Shorthorn Society, commented: "There were 27,000 Beef Shorthorn and Beef Shorthorn-cross calves registered by BCMS last year. This record number shows how the commercial value of the breed is more widely recognised than ever before"

As part of the alliance formed between Morrisons and the Beef Shorthorn Society, Morrisons has agreed to promote the use of Shorthorn bulls among its suppliers while the Society has made DNA recording of all registered sires compulsory; a move which will guarantee the integrity of the product while also making groundbreaking new performance measurements possible once carcass data can be linked to individual sires.

More recently Morrisons has also begun to work with the RBST to help conserve the critically rare Whitebred Shorthorn. At the instigation of HRH the Prince of Wales, a small breeding herd has been established at their Dumfries House farm, which is run as a joint venture with HRH. With the recent resurgent of the Beef Shorthorn, will the Whitebred Shorthorn be next?

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Proteomics in search of new biomarkers of osteoarthritis: from culture systems to animal models and patients

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Introduction Osteoarthritis (OA) is disease of the whole joint, affecting articular cartilage, sub-chondral bone, synovium and peri-articular tissues. OA is the most common form of degenerative joint disease in humans and companion animals. Although OA is primarily associated with ageing, there are other key contributing factors, including obesity (which increases mechanical stress and systemic levels of adipose-derived pro-inflammatory mediators), a history of joint trauma or repetitive use, genetics, heritable metabolic disorders, muscle weakness, underlying anatomical and orthopaedic disorders (i.e. congenital deformity, hip dislocation), joint infection, crystal deposition, previous episodes of gout or rheumatoid arthritis and various disorders of bone turnover and blood clotting. The metabolic alterations that occur in obesity along with the pro-inflammatory factors produced by white adipose tissue in the chronically overweight are thought to be major factors in the progression of the disease. Although OA does not affect production animals, its incidence is rising in companion animals due to the presence of a similar set of risk factors (particularly ageing and obesity). The diagnosis of OA relies on the manifestation of the symptoms (i.e. joint pain, stiffness and swelling) and the use of conventional radiography, which is the reference technique for determining the severity of joint attrition and cartilage loss. However, clinical symptoms and radiographic signs usually develop in late stages of joint disease, when significant tissue damage and cartilage loss has already occurred. Therefore, there is an acute need for tests that enable early diagnosis. Biomarkers are anatomic, physiologic, biochemical, or molecular parameters associated with the presence and severity of specific diseases. They are detectable by using a variety of methods, including physical examination, laboratory assays, and imaging and can be used as indicators of pharmacologic responses to therapeutic interventions. The OA Biomarkers Working Group, which was recently established by the Osteoarthritis Research Society International (OARSI) and the US Food and Drug Administration (FDA) has recently classified OA biomarkers into two groups. “Dry” biomarkers include imaging parameters (i.e. from radiographs, magnetic resonance imaging (MRI), and ultrasound), questionnaires and data from visual analogue scales (VAS). “Wet” biomarkers are soluble biochemicals and include RNA, DNA, carbohydrates, proteins, protein fragments, peptides and metabolites. Despite the interest in OA biomarkers, there are currently no reliable, quantifiable and easily measured markers that provide an earlier diagnosis of OA during the asymptomatic and pre-radiographic stages of the disease. For example, many of the existing biomarker assays identify fragments of the extracellular matrix of cartilage (i.e. fragments and neoepitopes of type II collagen, aggrecan and smaller proteoglycans) (Williams *et al*., 2011). Identification of these molecules in urine or serum does not consistently correlate with radiographic changes and symptoms. Furthermore, biomarkers of type II collagen and aggrecan also originate from the spine and other joints and may not even be specific for OA. Consequently, there are no reliable assays that can inform prognostic evaluations and monitor responses to existing therapies. The lack of such biomarkers has also hampered the development of disease modifying osteoarthritic drugs (DMOADs) (Mobasheri, 2012). Current pharmacological management of OA is dominated by analgesics, non-steroidal anti-inflammatory drugs (NSAIDs) and intra-articular injection of hyaluronic acid (viscosupplementation) or corticosteroids. Analgesics and NSAIDs are commonly administrated to all patients, irrespective of the stage of the disease. However, clinical trials have shown that only 20 to 60 percent of patients respond to treatment. Many patients do not benefit from the drugs that are administered and long-term use of NSAIDs is associated with adverse reactions and gastro-intestinal side effects. Therefore, identification of more sensitive biomarkers of pre-radiographic joint tissue turnover will enable a more rational, targeted and individualised approach to disease management. The availability of new tools and biomarker reagents is currently a major bottleneck in the biomarker pipeline, with consequences for the development of DMOADs. The recent proliferation of post-genomic technologies has resulted in rapid growth and progress in biomarker research. Current research on OA biomarkers focuses on identification of biomarkers in urine, serum and synovial fluid using proteomics, metabolomics, lipidomics, advanced imaging techniques, genomics, epigenomics and transcriptmoics. This presentation will focus on how proteomics is currently being applied to identify early biomarkers of joint inflammation and tissue damage using 2-D and 3-D culture systems, animal models, veterinary species and longitudinal studies of cohort of human patients (Gharbi *et al*., 2011; Mobasheri and Henrotin, 2010; Mobasheri, 2011). Improvements in proteomics technology and throughput will facilitate diagnostics, drug discovery and clinical trials (Ruiz-Romero and Blanco, 2010).

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Biomarkers and stem cells: Translating discovery to clinical practice

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Recent studies suggest that a subset of cancer cells with the ability for self-renewal and differentiation into different cell lineages is responsible for tumour progression, metastasis and resistance therapy. These cells, designated as tumour-initiating cells, tumour-propagating cells or cancer stem cells, are of great interest for cancer prognostication and therapeutics. A number of cell surface and intracellular markers indicating cancer stem cell characteristics have been identified in in certain cancer types, presenting an opportunity to use them as biomarkers.
However, these markers present their own challenges and there is a significant need for the improvement in clinical and experimental methodologies to detect stem cells in clinical samples, and validate their utility in clinical practice. Of particular relevance is the potential of these cells as predictors of the disease outcome, metastasis potential response to treatment and as indicators of early relapse.
A problem facing researchers and oncologists in this field has been to define markers that can identify these cell types either in the tumour, or as circulating cells initiating metastatic disease. A range of cell-based markers has been described to isolate populations of tumour initiating cells from a range of solid and liquid tumours[1]. However, it is clear that there is no one universal marker that can identify "pure" tumour-initiating cell populations. All marker studies to-date have only managed to produce enriched populations for in vitro analysis. This raises an important point, as it would be ideal to be able to identify such populations for:

- Drug screening and to identify therapeutic targets
- Identify circulating cells that have the potential to create metastatic deposits and
- Ascertain whether circulating tumour-initiating cells are of diagnostic or prognostic importance.

It is unlikely that a universal marker will be identified, and it is possible that populations will have to be isolated and characterized based upon a combination of surface markers, gene expression signatures and functional characteristics. Unfortunately, this is complicated by the reliance of these cells on their microenvironment in vivo, which has a profound effect on cellular behaviour. In recent years it has emerged that carcinoma cells rely on a mechanism "borrowed" from normal stem cell biology for invasion and metastatic spread. In this, cells at the leading edge of a tumour, undergo an epithelial to mesenchymal transition (EMT) where the cells adopt the phenotypic characteristics of mesenchymal cells. This allows for invasion and migration to distant sites. Emerging evidence suggests that the EMT process requires a fall in E-Cadherin expression, and an increase in expression of polycomb markers such as BMI-1. This is an emerging field, but the implications for biomarker discovery are clear:

- This may offer a new opportunity to identify metastasising cells in the circulation by a new set of markers;
- There may be an opportunity to identify new therapeutic targets
- There may be an opportunity to characterize the niche environment for both EMT and MET to occur, affording the opportunity for new biomarker discovery.

Conclusions The emerging area of cancer stem cells is opening up a whole new area of cancer and biomarker discovery. Although the past 10 years has seen an explosion in our understanding of the molecular events in cancer, it is clear that we have to return to basic cell biology to further understand the processes of cancer biology and metastasis. It is emerging that stem cell programmes such as EMT and MET are central to disease progression and prognosis. From studies on these areas, it is anticipated that new biomarkers will emerge that will allow more accurate disease predictions and measures of treatment success.

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Gel based proteomics and protein ID using mass spectrometry: a brief introduction and importance to animal and veterinary sciences

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Introduction Proteomics may be defined as the study of the proteome: proteins being expressed in a particular organelle, cell, tissue, organ (or fluid), organism or population. For the last fifteen years, proteomics has been of key importance and wide use in numerous fields such as plant sciences, pharmaceutical studies, cell biology, etc., just to name a few. Albeit the fact that proteins are a key component of animal production and of food products of animal origin, in Animal and Veterinary Sciences, the use of proteomics, is still very limited, due essentially due to an unawareness or lack of knowledge on the principles of proteomics and associated technologies as well as to the high costs and difficult access to these state-of-the-art technologies by most researchers working in animal and veterinary sciences (Eckersall *et al*., 2012). To address this important issue, we have created COST action FA1002 – Proteomics in Farm Animals (www.cost-faproteomics.org) aiming precisely at increasing the awareness of the potential of proteomics to researchers in the field and creating conditions for applications and collaborations between scientists in Europe and associated countries. Here, we present a brief introduction to the most currently used technologies in Proteomics: Gel based proteomics and protein identification using mass spectrometry, providing two examples on how proteomics may be used: muscle characterization in farm animals and the study of *Ehrlichia ruminantium*, a bacteria causing the tick-borne disease heartwater.

Gel-based Proteomics Protein electrophoresis, particularly 2DE – Two Dimensional Electrophoresis is the workhorse of proteomics studies. It involves denaturing whole or fractioned protein extracts and run them in 2-dimensional gels where proteins are separated by their isoelectric point first and then by their molecular mass, creating a spot pattern in the gel that is characteristic of the tissue/fluid being studied. Gels are then analysed using specific software that provides an indication of the spots (proteins) being under or over-expressed. Proteins are then identified using mass spectrometry and the biological implications of the differential expression ascertained. Mass Spectrometry (MS) Protein identification is a key component of proteomics. One of its major roles is the identification of differentially expressed proteins arising from gel-based proteomics studies. The most frequently used technology is MALDI TOF/TOF – Matrix Assisted Laser Desorbtion Ionization – Time Of Flight. Briefly, proteins excised from 2DE are in-gel digested with an enzyme (trypsin) creating a peptide pattern characteristic of that protein that is compared to peptide patterns of tryptic protein digests available in databases. For further information, refer to Soares *et al.* (2012) where this issue is addressed.

Case study 1: Muscle expression profiles in rabbit and sheep breeds with different levels of adaptation to nutritional stress
Seasonal Weight Loss is the major limitation to animal production in the tropics and the Mediterranean. In this case study we will conduct an overview of the effect of weight loss on animal productivity and their relation to studies at the level of of protein expression in rabbits (Almeida *et al*., 2010) and sheep (Van Harten *et al*., 2013; Almeida *et al*., 2012, 2013, Scanlon *et al*., 2013), leading to differences in important metabolic and structural proteins.
Case study 2: *Ehrlichia ruminantium*: from EB proteome description to virulence biomarkers? Heartwater is an important tick-borne disease in sub-Saharan Africa and a few islands in the Caribbean and the Indian Ocean that severely limits ruminant production. Here we address the first conducted proteome characterization of the infectious form of this bacteria, the EB (Elementary Body), recently published (Marcelino *et al*., 2012b), and advances in virulence studies using proteomics.

Conclusions This presentation provides an introduction to the most relevant techniques in proteomics, demonstrating the potential in the field.

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An overview of proteomic approaches for farm animal research

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Introduction Proteomics has been used now for several decades and it is considered as a fast-evolving technology. Novelties are directly linked with new features in mass spectrometry and automation. Proteomics aims at identifying and quantifying large numbers of proteins expressed in a particular system: detection of disease markers, study of posttranslational modifications, cataloguing the highest number of proteins present in a cell type, analysing the interactions between cell compartments, and many other biological questions can be addressed and the panel of techniques used to reach the goal are numerous (Lottspeich *et al*, 2010). While the first 2D gels performed in the seventies allow resolving few hundreds of proteins from E. coli, nowadays the number of detected proteins has been drastically increased and considerable progress has been made for their identification and/or characterization. However, researchers are still facing many challenges: complexity of the samples, reproducibility, quantitative analysis, dynamic aspects and so on. For example, highly abundant proteins (such as albumin) can hide those that are present in lower amount but being essential such as transcription factors; salts used during the extraction steps or in culture medium can also bring problems in gels but also in the mass spectra…Another aspect that should be considered is the availability of protein sequences in public databases, that can create difficulties.

Examples In this presentation, different methods will be briefly described in order to give an idea on how proteomics can complement data acquired by other means, or via other 'omics' approaches. In short, the use of gels and its potentiality in farm animal proteomics together with the gel-free approaches will be commented, with pros and cons.
As a proteomics platform, our team is dealing with many different samples, ranging from algae to human. Regarding animal studies, we had the opportunity to work with samples obtained from sheep, cows, guinea pigs, mice, .. (Sheridan *et al*, 2013; Condell *et al*, 2012; Siller-Matulla *et al*, 2012; Quesada Calvo *et al*, 2011). Animal pathogens and toxicological studies have been the focus of several projects. Until now, most of the studies were performed with 2D DIGE technique, based on fluorescent labelling and allowing a correct quantification of the protein abundances, followed by identification with MALDI-TOF-TOF. Progressively, the use of gel-free approach will be integrated to the platform to complement previous studies, for example to improve the coverage of membrane protein analysis or to allow in-depth analysis of PTMs. Illustrations of proteomics applications will be discussed:

- protein expression patterns during intra-mammary infections in mononuclear cells of cow blood.
- Metabolic disorder in lambs treated in utero with exogenous steroids
- Salmonella tolerance to the biocide active agent triclosan
- Proteomic analysis in mouse models of asthma

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Metaproteomics- investigating the impact of the gut micro-biota on pig gut health

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Accumulating evidence indicates that micro-flora inhabiting the gastrointestinal tract of mammals contributes importantly to controlling host biology. The bacteria take part in regulating mechanism like nutrient uptake, growth and development of intestinal tissues, and in regulating energy metabolism and immune response of the host.
Investigating the impact of gut-biota on host biology is a challenging task, complicated by the enormous complexity of the mammalian gut micro-biome, currently estimated to consist of 500-1000 different microbial species, of which many are not yet well characterized.

However, recent advances in next generation genome sequencing, has provided a major leap towards investigating the composition of the complex universe of gut micro-flora. These meta-genomics mappings have not only increased our knowledge of the diversity and variation of mammalian gut flora, but also offers an opportunity to correlate meta-genome patterns to changes in physiology, cell biology and molecular changes observed in tissues and body fluids.
In our laboratory we have developed a variety of porcine models to study the interplay of gut flora and proteome changes in the porcine gut epithelium. Pig is an excellent model for studying the role of human micro-flora because the gastrointestinal tract as well as the gut flora of pig and man show great resemblance, and also because pig is an omnivore, hence relevant experimental models can be developed where nutrition and gut flora can be manipulated to mimic well that of humans.
Examples of studies where integration of proteomics, microbiology and meta-genomic research aims to characterize the impact on gut bacteria on porcine health will be presented.

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Activated metabolic pathways in the fatty liver of early lactating dairy cows

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Introduction The early lactation period of high-yielding dairy cows is characterized by mobilization of body reserves including fat, protein and glycogen, and enter a negative energy balance (NEB). With increasing body fat mobilization, non-esterified fatty acid (NEFA) concentrations increase in plasma and fat accumulates in the liver during early lactation (Hammon *et al*. 2009). However, dairy cows respond to NEB with only a small increase in hepatic fatty acid oxidation (Grum *et al*. 1996) accompanied by the very limited hepatic VLDL export of triacylglycerides, both contributing to the development of compromised liver health such as hepatic steatosis. The underlying metabolic mechanisms contributing to liver disease are not very well understood but the involvement of oxidative stress may contribute to it. Therefore, the aim was to investigate the expression of hepatic enzymes involved in fatty acid oxidation in parallel with those belonging to the antioxidative stress response system in the liver of cows with a different extent of body fat mobilization during the periparturient period.

Material and methods Nineteen multiparous German Holstein cows (2^{nd} to 4^{th} lactation, >10,000kg/305d in a previous lactation) housed in a tie stall from week 7 ante partum (ap) until 5 weeks post partum (pp). Cows were total mixed rations according to their physiological state: week -7 to -4:, 5.87 MJ NE_L, week -3 to 0: 6.49 MJ NE_L), and week 1 to 5: 7.06 MJ NE_L). While individual feed intake was measured daily, body mass (BM), body condition score (BCS), back fat thickness (BFT), and milk yield were determined weekly. Blood samples were taken once weekly and around parturition twice weekly to measure plasma concentrations of glutamate dehydrogenase (GLDH) and aspartate transaminase (AST). The liver of the animals was biopsied at five consecutive times at days -34, -17, 3, 18, 30 relative to calving. A further liver sample was obtained from slaughter at day 40 pp and immediately frozen in liquid nitrogen. The total liver fat content (LFC) was analysed, and based on the average amount of LFC pp, cows were retrospectively allocated to high (H; LFC >24.4% DM; n=10)) and low (L; LFC <24.0% DM; n=9) fat mobilizing groups. Ground frozen liver tissue (50 mg) was extracted as described earlier (Kuhla *et al*., 2009) and prepared for proteomic analysis. Briefly, extracts were applied to 2-dimensional (2D) gel electrophoresis followed by colloidal Coomassie staining. 2D gels were processed using Decodon Delta 2D software. Normalized spot volumes were analysed by the Mixed Model of SAS (version 9.2) with fixed effects for group and time relative to parturition. All spots that were differentially expressed between groups ($p<0.05$) were picked, tryptic digested and analysed on a 5800 MALDI TOF/TOF analyser.

Results Dry matter intake increased after calving but was lower in H than in L cows throughout the whole experimental period. Liver fat content was highest at day 18 pp in both groups but was significantly higher in H than in L cows. Plasma GLDH and AST were not different between groups but were approximately 1.4 fold higher in H than in L cows at day 18 pp. In the proteome approach, we were able to identify 170 protein spots among them were several proteins involved in fatty acid transport, mitochondrial and peroxisomal fatty acid degradation as well as in defence against oxidative stress The expression of medium chain acyl-CoA dehydrogenases (the first enzyme of the beta-oxidative pathway) and peroxisomal enoyl-CoA hydratase were higher after parturition as compared to the prepartal period but was also higher in H than in L cows. Enoyl-CoA hydratase and 3-hydroxy acyl-CoA dehydrogenase, the next two downstream enzymes of the beta-oxidative pathway, were also higher expressed pp, but lower expressed in H than in L cows. Moreover, 3-ketoacyl-CoA thiolase, the 4^{th} enzyme of the beta-oxidative pathway, was higher expressed pp only in L but not in H cows. The amount of catalase, which detoxifies H_2O_2 generated during peroxisomal fatty acid degradation, continuously decreased from d -17 to d +30 relative to parturition in both groups but remained higher in H than in L cows. A similar course of expression was observed for peroxiredoxin-6 which also degrades H_2O_2 and for Cu/Zn superoxide dismutase, which degrades peroxide radicals to H_2O_2. Moreover, DJ-1 protein, which protects against oxidative stress by scavenging hydroperoxyl radicals continuously increased after calving and remained higher in H than in L cows. On the other hand, expression of glutathione-S-transferase also decreased from d -17 to d +30 relative to parturition but was lower in H than in L cows.

Conclusions Our results clearly show that cows highly mobilizing body fat in early lactation are not able to completely oxidize fatty acids. This is associated with an increased oxidative stress and a higher extent of fatty liver.

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Proteomics in biomarker discovery in cattle, chicken and Atlantic salmon

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Background Proteomics has not been widely used in farm animal health and production research despite the objective of livestock and dairy farming and recent developments in aquaculture being the production of nutritious protein for human consumption. However there have been increases in the application of proteomics in the farm animal research over the last few years, coinciding with significant publications across a spectrum of application in many farmed species (Eckersall & Whitfield, 2011; Eckersall *et al* 2012). One area which has great potential for applications in veterinary medicine and animal science is in the discovery, identification and utilisation of protein biomarkers of disease. While the electrophoresis and mass spectrometry based technologies may not be appropriate for rapid and economic diagnostic procedure, the biomarkers identified can be developed using conventional technology into assay systems of sensitivity and specificity to the benefit of animal health and welfare. Three areas where proteomics have enabled biomarker discoveries have been in the investigation of the serum or plasma proteomes of chicken and Atlantic salmon and in analysis of bovine nasal secretion.

Proteomics and bovine nasal secretion Nasal secretion has been collected using a novel absorbent based technique which can provide up to 5-10 ml of secretion with a protein concentration of up to 30 g/L. The proteome of this fluid was investigated using 2 dimension electrophoresis, followed by spot excision, trypsin digest, mass spectrometry and MASCOT database searching to identify the most abundant protein in the secretion (Rodrigues *et al.*, 2012, pp63-66). A number of serum proteins were identified including albumin, serotransferrin, fibrinogen fragments and apolipoprotein A1. Lactoferrin and odorant-binding protein were also identified which have anti-bacterial and odour detection activity respectively relevant to their presence in nasal secretion. Glutathione S-transferase was also present in the nasal secretion, an enzyme which acts in the detoxification of xenobiotics via glutathione reactions. A high level of γ-glutamyl transferase activity is present in bovine nasal secretion which also affects glutathione metabolism suggesting that this pathway has a significant role in the protection of the nasal epithelium.

The chicken plasma proteome The chicken plasma proteome has been compared between groups with gait scores 1 (slight defect) and gait score 3 (unsteady gait) and increased acute phase protein concentrations. Following 2 dimension electrophoresis and mass spectrometry of the protein excised from the polyacrylamide gel it was found that PIT 54 (the haptoglobin analogue in chicken), ovotransferrin, α1 acid glycoprotein and haemopexin were raised in the plasma samples from the chicken with gait score 3 and an acute phase response (Rodrigues *et al.*, 2012, pp177-180). However high abundance proteins of chicken plasma obscure change in the lower abundance proteins and more proteome differences could be detectable with analysis following removal of the protein such as albumin, fibrinogen, immunoglobulin and apolipoprotein A1.

Biomarkers of pancreas disease of Atlantic salmon Pancreas disease of Atlantic salmon is caused by infection with salmon α-virus which causes pathologic lesions in white and red muscle and the heart as well as the pancreas of infected fish. Analysis by 2 dimension electrophoresis of serum from a Trojan experimental infection of salmon with salmon α-virus revealed that many serum proteins changed their abundance in serum during the 12 weeks following infection. Identification of protein by trypsin digest and mass spectroscopy demonstrated that increases in a number of proteins such as creatine kinase, pyruvate kinase and glyceraldehyde 3-phosphate dehydrogenase were related to damage in particular tissue. Biomarkers associated with red muscle damage were the most responsive, coinciding with the peak in tissue damage (Rodrigues *et al.*, 2012, pp87-90). In contrast, proteins related to innate immune responses to infection such as complement C3 and haemopexin were increased at later stages of the infection.

Conclusion Proteomics is a powerful technology with multiple applications in the identification and characterisation of protein biomarkers of blood and other biological fluids. There are manifold applications for this approach in farm animal and aquaculture production.

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Applying proteomics to investigate bacterial pathogens of veterinary importance

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Proteomics offers alternative methods to assess pathogenic mechanisms of infection which can be specifically applied to animal models of disease. These concepts are illustrated using two different, yet significant, bacterial pathogens of veterinary importance.

Leptospirosis

Pathogenic species of *Leptospira* cause leptospirosis, a disease of many domestic animal species including dogs, cattle, sheep and pigs. Leptospires colonize the renal tubules of reservoir hosts of infection, from which they are excreted via urine into the environment. *Rattus norvegicus* is a reservoir host for *Leptospira interrogans* and experimentally infected rats remain clinically normal, yet persistently excrete large numbers of leptospires from colonized renal tubules via urine, despite a specific host immune response. Whilst persistent renal colonization and shedding is facilitated in part by differential antigen expression by leptospires to evade host immune responses, there is limited understanding of kidney and urinary proteins expressed by the host that facilitates such biological equilibrium. Urine pellets were collected from experimentally infected rats shedding leptospires and compared to urine from non-infected controls spiked with *in vitro* cultivated leptospires for analysis by 2-dimensional difference gel electrophoresis (DIGE). Differentially expressed host proteins include membrane metallo endopeptidase, napsin A aspartic peptidase, vacuolar H+ATPase, kidney aminopeptidase and immunoglobulin G and A. Loa22, a virulence factor of *Leptospira*, as well as the GroEL, were increased in leptospires excreted in urine compared to *in vitro* cultivated leptospires. Urinary IgG from infected rats was specific for leptospires. Results confirm differential protein expression by both host and pathogen during chronic disease and include markers of kidney function and immunoglobulin which are potential biomarkers of infection.

Enzootic Abortion of Ewes

Chlamydophila abortus, the aetiological agent of enzootic abortion of ewes (EAE), replicates in trophoblast cells leading to their destruction and dissemination of the bacterium to foetal organs. To further understand the pathogenesis of EAE, amniotic and allantoic fluids were collected from experimentally infected pregnant ewes at 30 (7 samples from each fluid), 35 (8 samples from each fluid), 40 (10 samples from each fluid) and 43 (6 amniotic fluids and 7 allantoic fluids) days post-infection to determine pathogen numbers and other markers of infection. Whilst experimentally infected ewes had characteristic placental lesions, only two amniotic and seven allantoic fluid samples were positive for *C. abortus* by Real-Time PCR. In contrast, all amniotic and allantoic fluids were positive for immunoglobulin. Immunoglobulins were generally detected earlier in allantoic fluid than in amniotic fluid and the numbers of samples containing immunoglobulins increased as infection progressed. IgG in amniotic and allantoic fluids was shown to be specific for *C. abortus*, and reacted with the major outer membrane proteins, polymorphic outer membrane protein and macrophage infectivity potentiator protein. A comparison of two-dimensional immunoblots using purified IgG from the allantoic fluid, amniotic fluid, ewe serum and foetal serum of a *C. abortus* infected animals at 40 days post infection indicated a pattern of reactivity intermediate between that of the ewe serum and the foetal serum. Results suggest that a maternal source of immunoglobulin is predominant at 30 days post-infection but that foetal derived antibodies may be contributed at a later stage.

Conclusion

In each case, proteomics confirms the expression of pathogen-specific genes during infection, and thus potential mechanisms of host-pathogen interactions. Further, results identify those antigens that specifically interact with the host immune response to provide novel vaccinogen and diagnostic candidates.

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Making a splash! Determining proteome dynamics in fish species

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Proteomics is conventionally described as the study of the full protein complement of a cell, tissue, body fluid or organism under defined conditions. Proteomics has rapidly developed into a sophisticated discipline that is not only concerned with the cataloguing of large numbers of proteins, but one which seeks to provide a functional link between expressed genes and phenotypic outcomes. The advances in proteomic technologies now permit the proteome to be probed to unprecedented levels and provide the ability to obtain quantitative data for large cohorts of proteins. However, unlike the genome, which is considered the blue-print of an organism, the proteome is not a pre-determined, static entity. Instead, the proteome is in constant flux, which can be influenced by environmental conditions, cellular function, developmental status and extracellular challenge. Even in a position of apparent steady-state, the protein complement of an animal cell is constantly changing, with new proteins being synthesised, and older proteins being degraded and recycled. Therefore, in order to take a holistic view of an animal system, it is vital that we can elucidate the dynamics of the proteome and monitor time-related adaptations. Moving from a static "snap-shot" of a system to dynamic processes presents a considerable challenge and requires the use of sophisticated analytical and bioinformatic strategies. This presentation will focus on recent developments in methods to explore proteomic dynamics and outline how we have applied these experimental approaches to the study of cellular systems, animals and fish.

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By the skin of the teeth: Proteomics of dental calculus to track disease in farm animals?

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There has been considerable interest in analysis of the salivary proteome of domestic animals as a tool to track animal health and disease (Lamy *et al*. 2009; Ang, Ching-Seng *et al*.2011). As archaeologists we are interested in a long-view of animal husbandry and health. Recent research has suggested that ancient dental calculus is a rich repository for microscopic remains (such as food debris, starch grains, and phytoliths) and even remains of DNA from microorganism of the oral microbiome (Adler *et al*., 2013). In this presentation we present recent findings of the potential for proteins entrapped calculus (for example from slaughter houses, skeletonised remains and archaeological teeth) to provide a long-term record of the salivary proteome.

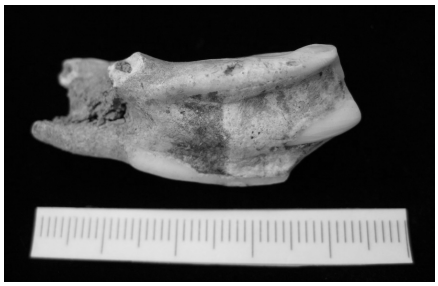

Figure 1 Medieval Cattle calculus, Dalheim Germany.

Material and methods Cattle and sheep calculus (Fig 1) were obtained from a Medieval Site of Dalheim Germany, the sample was estimated to be approximately 900 years old. Generation of tryptic peptides from tooth and dental calculus specimens was performed using a filter-aided sample preparation (FASP) protocol (Wiśniewski *et al*. 2009), modified for mineralized and degraded samples. Total protein extraction was performed on two ancient fauna calculus/cementum samples (F1 and F5), Samples were extracted at the Centre for Evolutionary Medicine (ZEM) at the University of Zürich. Sample extracts were then sequenced at the Functional Genomics Center Zürich (FGCZ).

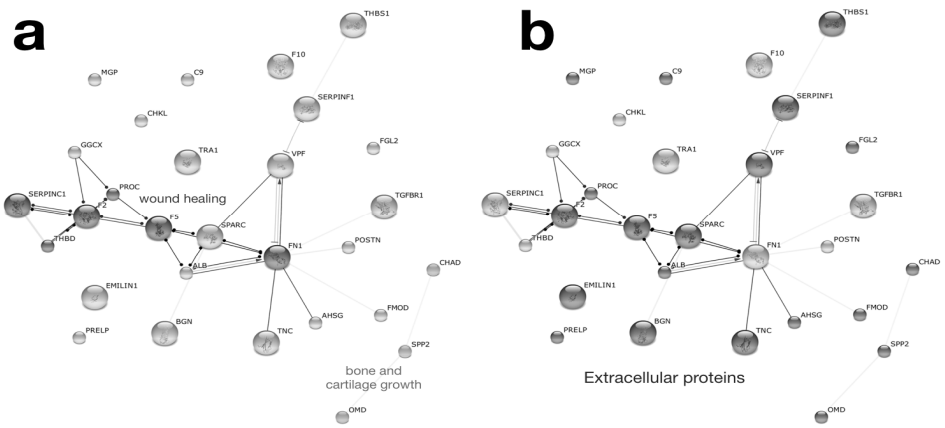

Figure 2: Dalheim cattle calculus STRING diagrams (REF): relatively few proteins are identified proteins (a) those that are, are linked to wound healing and osseous growth, the (b) majority being extracellular

Results After excluding contaminants and collagens (which generally show poor taxonomic discrimination), all proteins from the faunal samples were assigned to Eukaryota. For the cattle sample, 16 proteins were assigned to *Bos taurus* and 7 proteins were assigned to other taxa (mouse, rat, pig, rabbit, human); of the mis-assigned proteins five have no *Bos taurus* coverage in the UniProt KB database. For the sheep sample, five proteins were assigned to *Ovis aries,* while five were assigned to other taxa (goat, cow, pig, mouse, human); all five of the mis-assigned proteins have no *Ovis aries* coverage in the UniProt KB database. Analyses of the functional role of these proteins suggests that these are primarily linked to bone growth and wound healing (Fig 2a). Many are associated with osteogenesis, suggesting that the 'calculus' may at least in part be composed of an organised bony growth such as cementum; this is further supported by the absence of any microbial proteins, which would be more typical of dental calculus.

Conclusions The survival for almost 1,000 years of such a rich suite of proteins in dental calculus is remarkable. The data we can recover from these proteins less so. The absence of microbial proteins, the few proteins detect, and the surprisingly high abundance of proteins associated with osseous growth suggest that our sample appear to be under control of the animal, and appear to be analogous to cementum layer with perhaps some entrapped saliva.

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NFU's response to government's methods of tackling bTB in England

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Introduction

Bovine TB (bTB) is a chronic infectious disease which has several routes of transmission. Bovine TB is a devastating disease that resulted in the slaughter (in GB) of 34,897 cattle from January to November 2012, and continues to take a terrible toll on our farmers, our dairy and beef industry and on rural communities. The average cost of a TB breakdown is £34,000, of which £12,000 falls to the farmer. In England, Government spends £100m on bTB controls every year and will cost UK taxpayers £1bn over the next decade.

Badgers have been shown to be a significant source of infection. The Randomised Culling Trial showed that proactive culling reduced incidence of confirmed TB breakdowns by 31.5%. TB continues to be a serious problem in the South West and Midlands where TB is endemic in the wildlife populations; cattle control measures alone will not eradicate bTB.

Bovine TB Eradication Plan

Government is committed to a package of measures to control bTB in cattle, badgers and other animals. Cattle control remains the foundation of the eradication plan and we have seen measures strengthened in 2012 and further measures are planned for 2013. A risk-based approach has been taken to develop the eradication plan, which includes tailoring control strategies to areas of risk; voluntary risk based trading; improved advice to farmers and tackling TB in non-bovine species.

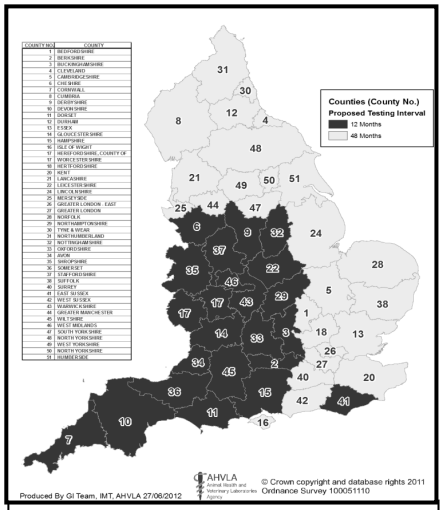

Figure 1: TB testing intervals, 2013

Figure one show's the new risk-based approach to surveillance; testing interval is counties and is more epidemiologically focused than the previous parish-based system. The system targets more surveillance on the 'edge' area and prevents wasting resource in non-endemic areas.

Defra has responsibility within Government for the badger control policy**.** Following two consultations it was announced that badger control would go ahead. Two pilot areas have been licenced and will test the effectiveness, humanness and safety of controlled shooting before a decision is made to roll out the policy more widely.

Natural England, the licensing body, has issued a licence to control badgers in West Somerset and West Gloucestershire. The National Farmers Union (NFU) is responsible for overseeing and coordinating the successful delivery of the badger control policy on the ground. Two farmer companies have been set up to coordinate the cull and with support from the NFU, are responsible for ensuring that the licence conditions set by Natural England are met. Culling operations will be paid for by industry and carried out by experienced, trained operators, following Defra's best practice guidance**.**

The Badger Vaccine Deployment Project (BVDP) has been using a licenced injectable vaccine since the summer of 2010 helping to build experience of large-scale vaccine deployment and provide training facilities for lay vaccinators. To encourage vaccination use within the pilot areas Defra have made £250,000 available to support non-participants and those within the surrounding area who keep susceptible livestock. Uptake has been limited due to the high costs of deployment and the lack of scientific evidence to show that vaccinating badgers reduces bTB in cattle. Many farmers are choosing instead to invest in biosecurity measures in an attempt to keep badgers out of farm buildings and feed stores. Further research is being carried out into cattle vaccination, which is currently prohibited by EU legislation, and an oral bait badger vaccine.

NFU response

The NFU supports cattle control measures as part of much wider package of measures; however, controls must be risk-based and give farming businesses the ability to continue to trade.

We fully support Government's policy to control badgers in areas where the disease level is persistent and high. Nobody wants to see badgers being culled but science and the experience of other countries shows that we cannot get on top of TB without tackling this disease in wildlife as well as in cattle. If a useable vaccine were available now we would be using it. However, a workable vaccine remains many years away. In a recent letter to the Secretary of State, EU Commissioner Tonio Borg said cattle vaccination is unlikely to be available for wider deployment until 2023, we cannot wait that long.

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Biotechnology applications in animal reproduction

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A major challenge for the global farming industry for the next 50 years is to increase the productivity of livestock species to address the food needs of the many societies around in our world. Thousands of years of domestication led to changes in the reproductive physiology of animals accelerated recently through programs of genetic improvement aimed at selection for specific (non-reproductive) productivity traits. Assisted Reproductive Technologies (ART) offer the potential for increased productivity through improved reproduction and can be summarised as providing the tool box to maximise the number of offspring produced by each female animal that are also fit for purpose.

There are three opportunities:

1. to increase total number of animals (in a global arena);
2. to maximise development of appropriate phenotypes;
3. to optimise sex bias to reflect animal usage.

For a technology to be useful it must full fill two criteria. It must work, by which I mean it must be useful, and it must be practical, in that the technology must be cost-effective in its application. For the former, any technology must build on strong scientific knowledge, while the latter should build on robust engineering and process methodology. In the exciting world of biotechnology applications in ART are at the forefront of scientific endeavour. This is driven largely by the rapid advance of knowledge and the development of innovative tools. Many ART are firmly embedded in the 'research' research phase of R&D but it is timely to look forward to when the progress into 'development'.

We all know of many well established ART including artificial insemination, embryo transfer, in vitro fertilisation – even nuclear transfer cloning - but what are the new applications and why are they exciting. Technical frontiers include the development of stem cells – primordial germ cells in birds, spermatogonial stem cells and induced pluripotential stem cells in mammals. In parallel the development of robust cell biobanks and improved in vitro preservation methods, perhaps even moving towards methods enabling recombination in vitro as part of an in vitro breeding programme. Other goals include extending the functional longevity of dairy cattle milk production and moving towards innovative strategies to alter, even control, sex bias – and there is a growing momentum that genetic engineering will become an applied technology in farm breeding; the time is right to exploit the genomics and DNA sequencing revaluation we are currently living in.

For the future, ART will continue to advance and complement the genomic-facilitate strategies including whole-genome selection programs, enhanced genetic prediction and early life breeding value predictions. Scientists, industrialists and regulators must work in concert to exploit the new ART in a society-friendly manner to increase animal productivity while enhancing animal welfare to reap maximum benefit for the farm animal sector.

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Biotechnology applications in animal health

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Biotechnology has revolutionised advances in biology over the last forty years especially since the development of the methodologies for manipulating DNA. Advancements and improvements of the various technologies in molecular biology have accelerated at an exponential rate. For example since the late 1970s until 2000 the Sanger method for sequencing DNA enabled complete genomes to be deciphered, initially from bacteriophages and latterly eukaryotic organisms including man, but this was expensive, time consuming and for large genomes required large sequencing facilities, new methodologies now allow a human genome to be sequenced in one hour. With respect to animal health one of the major impacts of biotechnology is in the control of infectious diseases. This has involved the development of more reliable and quicker diagnostic tests, to understanding the mechanisms of pathogenicity and the development of safer and more effective vaccines with the potential to eliminate several important diseases and to improve the health of animals and humans. With the growth and continual predicted growth in the global human population and the increase in meat based diets improvements in animal health and welfare is essential for food security; control of infectious diseases will continue to play a significant role in animal health and welfare.

Vaccination is one of the major successes of modern biology and is a crucial approach for the protection of animals and man from infectious diseases. Virtually all available animal vaccines were developed using empirical conventional methods. In most cases live attenuated vaccines offer the best protection and in ease of application involving large numbers of animals such as poultry. However, live attenuated vaccines also have a dark side in that they can revert to virulence or in some cases recombine with field isolates resulting in new pathogenic viruses. Modern biotechnological tools have not only led to a better understanding of the molecular mechanisms involved in pathogen replication/life cycle but have also opened up new opportunities for rationally designing safer, and more effective vaccines. A major advantage in using biotechnology for the generation of vaccines is for developing vaccines that can be differentiated from the causative pathogen; DIVA vaccines (differentiate infected from vaccinated animals). A major challenge associated with conventional vaccines, especially live attenuated vaccines, arises from the fact they are derived from the causative pathogen and therefore it is difficult and often impossible to determine whether a vaccinated animal has only seen the vaccine or whether a vaccinated animal may asymptomatically be a carrier of the pathogenic organism. An example of this is foot and mouth disease virus (FMDV), vaccination may protect against clinical signs but vaccinated animals cannot be transferred to vaccine free countries as they could be potential carriers of pathogenic FMDV. A DIVA vaccine will allow differentiation between an animal that has been vaccinated and the detection of virulent pathogen in vaccinated animals. The DIVA vaccines are marked in a way to differentiate them from the disease causing organisms. An added advantage of DIVA vaccines is that they are very useful for eradication programmes. Genes encoding immune stimulatory factors or pathogen-encoded proteins that down regulate immune responses can also be engineered into pathogens or inactivated making the vaccines more potent and to give longer lasting immunity.

As a result of the ease and increase in travel, changes in land use and changes in global weather patterns exotic pathogens have the capability to move to new geographical areas, often over very large distances, and in the case of vector borne diseases the ability to adapt to use new or alternative carrier species to occupy new ecological niches with the potential to cause diseases in animals that were traditionally absent from a particular geographic region. Additionally, changes in farming techniques to satisfy the requirements to produce more animals with better food conversion ratios and the use of inappropriate vaccines has increased the potential for the emergence of new endemic pathogens. In order to counteract these new threats to livestock it is imperative that we have efficient and rapid diagnostic tests, the ability to rapidly identify the genetic makeup of a pathogen and a means for rapidly developing safe, efficient and effective vaccines. The use of antibiotics/antivirals is not acceptable for use in livestock animals due to residues in food and more the fact that misuse or over use can lead to resistance not only in the target organism but can be passed onto human pathogens. Introduction and improvements in new technological methods in biotechnology directed towards animal health provides a means to identify and counteract challenges from new or re-emerging pathogens.

I will present information of how biotechnology has contributed to and continues to contribute towards improvements in animal health with concomitant effects on animal welfare and support of food security. Given the remit of The Pirbright Institute I will use examples from the institute's research portfolio on how recent advances in biotechnology have helped or have the potential to help improve animal health. This will include a broad outline of how new techniques in biotechnology have been applied or can be applied to animal health. I will present two examples, one based on FMDV and one on the poultry pathogen the avian coronavirus infectious bronchitis virus, on how biotechnology offers new ways in which to develop safer rationally attenuated vaccines offering different properties to traditional vaccines that would be very difficult if not impossible to produce by classical methods.

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Development of overground endoscopy for evaluating dynamic upper respiratory tract collapse in the athletic horse

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Dynamic collapse of the upper respiratory tract occurs when the soft tissue structure(s) are unable to withstand the high inspiratory pressures that occur during exercise and therefore collapse into the airway creating an obstruction to airflow. In the athletic horse this leads to abnormal respiratory noise and poor athletic performance. The majority of obstructions are 'dynamic' i.e. are only present during exercise. The reason for this lies with the dramatic increases in airflow and airway pressure changes that occur during exercise. The horse is an obligatory nasal breather and unlike other species, including man, is unable to switch from nasal to oral breathing during exercise in order to adapt to the movement of large volumes of air. Although obstructions to airflow can occur from the nostrils to the trachea, dynamic dysfunction at the level of the pharynx and larynx accounts for the majority of cases in athletic horses and numerous disorders have now been described in the literature.

Commonly horses are evaluated by endoscopy when stood still in the stable. However, several studies have now shown this to be unreliable in many cases. In order to obtain an accurate diagnosis endoscopy needs to be performed during exercise when the problem actually arises. Until recently, the only method available has been to perform an endoscopic examination during high-speed treadmill exercise. Unfortunately the cost, time implications and misconceptions regarding the safety of the technique mean that this is not always performed and many horses undergo surgery based on a history of abnormal noise and resting endoscopic findings. Although treadmill exercise was considered to be the gold standard technique for assessment of dynamic URT collapse, it is well known that treadmill exercise does not replicate exercise in the field, and therefore there is the potential for misdiagnosis with this technique. There are significant differences in heart rate, blood lactate, stride frequency and stride length between field exercise and treadmill exercise. It was clear that there was a need to develop a low-cost field based technique in order to enable more horses to have a definitive diagnosis prior to undergoing surgery.

In a joint venture between veterinary and engineering departments a portable telemetric endoscope was developed. The equipment is fitted entirely onto the horse and the images of the upper respiratory tract are transmitted to a hand-held screen for viewing. Endoscopic examination of the upper respiratory tract during field exercise has a number of advantages over the use of a high-speed treadmill. Firstly the exercise test can be conducted in the environment typically used for competition and horses may be examined in a manner appropriate to their discipline. In addition, the effects of the tack and rider are accounted for. Initial studies showed that the equipment was well tolerated by horses during ridden exercise in an arena and on the gallops and that diagnostic images could be produced. As a result of this early work several commercial systems have been developed. The technique is considered to be one of the most significant advances in equine sports medicine in the last decade. It is now in wide spread use throughout UK and internationally and has enabled a large number of horses to undergo exercising endoscopy. Furthermore it has enabled research to take place that would have been unlikely before its development. As with many new techniques however, it has thrown up as many questions as it has answers!

The technique is invaluable for the assessment of sport horses with abnormal noise. In particular it has shown how head and neck flexion can have a significant effect on the patency of the upper airway.

In racehorses the value of the technique depends on the presenting complaint and the gallop facilities available. For racehorses that make abnormal noise during normal training then performing the endoscopy on the trainers gallops is very useful. However many racehorses, particularly National Hunt horses, only have the problem during a race and do not make any noise during training at home. This is likely a reflection of the degree of fatigue reached during racing. In these horses, overground endoscopy on the trainers gallops should be interpreted with caution as a false negative is possible. It is best to try and recreate racing conditions as closely as possible and undertaking a racecourse gallop may be required. The technique has shown that upper airway obstructions are probably more prevalent than we had originally thought.

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Advances in equine genomics

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From genetics to genomics in the horse Genetics is defined as the branch of science that deals with heredity and the variation of inherited characteristics in living organisms. Since the first description of the action of genes by Crick in 1958, tremendous effort has been devoted to improving our understanding of the relationship between the genes, or gene variants, an animal carries (its genotype) and its visible characteristics (phenotype). Genetic mapping in the horse began in the 1970's and as increasing numbers of markers were discovered, their potential utility in parentage testing was recognised. During the 1990s, as reports began to accumulate on the identification of genes or chromosomal regions that influenced traits of economic importance in a range of livestock species, equine geneticists realised the potential utility of a genetic map of the horse in tackling common diseases. This realisation prompted the foundation of the Horse Genome Project, the initial goal of which was to identify genetic landmarks on each chromosome that could be used to establish points of reference with the human genome sequence, which had already begun to be sequenced. Whilst this enabled a number of candidate gene studies to be carried out, by 2005, it had become clear that a revision of strategy was necessary. Research had shown that coding sequences of genes, whilst highly conserved across species, typically made up a very small proportion of mammalian genomes and that the remaining 98% of the genome was also likely to be important in regulating gene function. Consequently, the Equine Genome Sequencing Consortium was founded and set about compiling the complete genome sequence of the horse. Sequencing began in February 2006 and already by January 2007 the first draft of the genome sequence was released. This sequence was subsequently updated in September of the same year (EquCab2). This process of map development led equine geneticists into the era of genomics.

Genomics, disease and the horse Genomics is defined as the scientific study of nucleotide sequencing, gene mapping, and the analysis of genome; new tools for conducting such analyses are constantly being developed and made available to advance this field. In the horse, approximately 1.5 million single-nucleotide polymorphisms (SNP) were identified during the assembly of the genome. A selection of these SNP markers were then used to produce a DNA microarray, the Illumina Equine SNP50 BeadChip ('50K SNP chip'), which contains over 50,000 SNPs and was released in 2007; in 2012 this chip was superseded by a chip with more than 65,000 SNPs. The potential for such dense SNP genotypes to be used in so-called genome-wide association studies (GWAS) to identify regions of the genome associated with quantitative traits, including disease, has already been demonstrated in human studies. To date, GWAS have been applied to the horse to map susceptibility loci for complex disease (e.g. osteochondrosis, recurrent exertional rhabdomyolysis) and loci associated with desirable traits (e.g. racing performance); such loci are referred to as quantitative trait loci (QTL). Assuming an associated region is identified and validated, fine-mapping using additional markers and possibly across-breed analysis, is used to search for closely linked markers and ultimately the causal variant. The identification of such causal variants can help to improve our understanding of the underlying biology, illuminating new biological pathways and in turn informing new treatments and management strategies. In the context of domestic animals, QTL can also be utilised in breeding strategies and this is referred to as marker assisted selection (MAS). When combined with traditional phenotypic records, the dense marker sets that are now available also have the potential to be used to generate genomic breeding values. Furthermore, genomic data can be informative with respect to a population's history and help us to understand the relationships between breeds, information that may be useful in a conservation context.

The future of genomics in the horse Rapid progress continues to be made in the field of genomics. The first human genome took roughly ten years to sequence, followed by a further three years of analysis, and cost close to 3 billion USD. In contrast, next-generation sequencing technologies now allow researchers to sequence multiple genomes in a single run, producing data in around one week, for a fraction of the cost. Whilst these technologies have their limitations they do have broad applicability in functional genomics research, for example in gene expression profiling and genome annotation. To date, next-generation sequencing has seen limited application in the horse but its use can be expected to increase. Currently, members of the equine genomics community are engaged in two major projects. The first is to develop a high density SNP array containing 670,000 SNPs, using the Affymetrix Axiom genotyping platform. The second is to develop a publically available tool containing the high density genotypes of many horses, providing a resource similar to the human HapMap.

There is rapid progress in the technology required to more deeply understand the relationship between genotype and phenotype. However, to deliver tangible benefits to the horse industry from using such methodologies, large cohorts of well-phenotyped horses are needed across a wide range of relevant breeds both for preliminary analysis and for subsequent validation work. Factors that are currently inhibiting the large scale collection of well-phenotyped samples include: cost, logistics, suspicion in the industry and a lack of agreement about disease phenotypes amongst practitioners. There is a great need for a more cost-effective approach to the study of disease and performance traits in the horse. One option is to collect samples in a more collaborative way through the establishment of a biobank, an organised collection of biological samples and associated data from large numbers of people or animals. Samples and data can then be made available to researchers to use in genetic and epidemiological studies of complex traits and diseases. The success of this approach would be dependent on a wide range of factors relating both to the equine industry and to the research community. These factors have been investigated as part of a feasibility study, commissioned by the British Equestrian Federation, to determine whether a UK equine biobank would enable the horse industry to better exploit new genomic technologies in the future.

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The equine placenta – a vital clue to neonatal problems

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For the mare owner there are few things as exciting and wondrous as the birth of a foal. However, as the onlookers are busy checking and assessing the new arrival little thought is spared for the placenta despite the fact that this temporary, but complex, organ has had responsibility for ensuring that the fetus is sufficiently developed and mature to make the transition from an intra- to an extra-uterine existence. If a foal is compromised or dies at or soon after birth the placenta may well be submitted to a pathology laboratory for examination. However, for most other births, apart from a cursory check for completeness the placenta is usually discarded unexamined. Yet there is much to be gained by a careful examination of this remarkable organ, with anomalies in both gross and microscopic structure providing clues to its functionality during fetal life. Some abnormalities may be are indicative of problems that influence neonatal health whereas others are suggestive of uterine pathology.

It is important to remember that the placenta at term represents the final stage of its development and throughout the 11 month gestation period it has adapted to meet the demands of the growing fetus. In early pregnancy a transitory yolk sac placenta exists, which bears little resemblance to the placenta seen at term. The yolk sac imbibes secretions from the mare's endometrial glands (histotroph), to provide essential nourishment for the embryo. However, the yolk sac soon becomes unable to fulfil the escalating nutritive requirements of the conceptus and it is gradually replaced by the allantochorion, to provide the definitive placenta which supports the fetus to term. The allantochorion forms an increasingly complex interdigitation with the maternal endometrium to produce the vast number of microcotyledons which function essentially to bring the fetal and maternal capillary beds into close proximity and thereby facilitate haemotrophic nutrition. Between this myriad of microcotyledons lie absorptive areas called areolae which facilitate the up-take of histotroph throughout gestation. In addition to nourishing the growing fetus the placenta also functions as an endocrine organ that secretes a variety of hormones to orchestrate essential events in both placental and fetal development.

Critical developmental windows exist *in utero* for the development of most fetal organs and body systems. Hence, incorrect or inferior placentation at any stage of gestation will have implications for the development and well-being of the fetus, either at the time of placental perturbation or later in gestation. Poor placentation also impacts overall growth of the fetus and low birthweight in many species is often associated with an increase in the incidence of problems in early life and disease in later life. In the mare the area of the allantochorion is correlated positively to foal birthweight and shortfalls in this parameter, occasioned either by poor development of the microcotyledons or a reduction in the area of functional endometrium in contact with the microcotyledons, invariably results in reduced foal birthweight. Although research has shown that placental area in the horse is influenced by maternal size, age, parity and genotype, other factors are also likely to play important roles. For example, the nutritional status of the pregnant ewe can modulate placental development and, although such a relationship has not been established convincingly in the horse, pregnant Thoroughbred mares that suffered severe weight loss occasioned by *Streptococcus equi* (Strangles) infection in mid-gestation showed alterations in placental and fetal development at term that correlated positively to the amount of weight lost previously.

Aberrations in the overall shape of the allantochorion and untoward changes in its linear dimensions can also reveal uterine abnormalities since the allantochorion provides a map of the uterus and it illustrates the amount of 'space' the fetus had to develop in. A recent study indicated that foals born with flexural limb deformities showed changes in their placentae that were indicative of uterine narrowing. However, it was unclear if these changes reflected uterine constriction as the underlying cause of the flexural deformities or were the consequence of diminished movements or mal-positioning of the fetus occasioned by the deformities.

Stretching, or even frank tearing, of the allantochorion gives an indication that it has been subjected to undue force during delivery. This can occur when the cervical star fails to rupture in a timely manner. Likewise, a complete cervical star with rupture of the allantochorion further back in the body region may imply that the placenta is thickened or it has separately prematurely. Both conditions have implications for fetal health.

The position, length, vasculature and degree of twisting of the umbilical cord is of particular importance since the cord carries all the fetal blood to and from the allantochorion. Hence, any shortfalls or variation in its morphology will impact directly levels of nutrients and oxygenated blood going to the fetus. Cord length is a good illustration of the impact cord morphology can have on the fetus; short cords predispose the foal to premature rupture of the cord during delivery and *intra-partum* anoxia (oxygen starvation), while long cords increase the risk of fetal strangulation, twisting of the cord with resulting obstruction of blood flow and necrosis of the placenta at the cervical pole. Even the under-researched amnion, the curious hippomane consisting of a concentric layers of allantoic debris and the usually well-hidden remnant of the yolk sac, can all provide valuable information about the foal's intrauterine existence.

Careful examination of the placenta at term provides a unique opportunity to learn more about the uterine existence of a foal and 'what went wrong' in individual animals with problems. More importantly, in a species that is bred primarily to perform as an athlete, continued research into the structure and function of this incredible organ will enable optimisation of mare management during gestation to, in turn, optimise fetal wellbeing *in utero* and the birth of a viable and robust foal with good athletic potential.

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The application of Preimplantation Factor to clinical equine reproduction

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Preimplantation factor (PIF) is a small peptide with wide reaching influences and potential. It is secreted by the viable embryo and foetus throughout gestation and influences both itself and its host. Research using IVF embryo culture media has shown that PIF may be first be discerned at the two cell stage and increasing concentrations of PIF can be identified in maternal circulation in line with advancing embryo development (Stamatkin *et al.*, 2011). Research investigating the properties and function of this peptide has implicated its role in promotion of embryonic development and modulation of peripheral and endometrial maternal innate immunity to create acceptance of the semi-allogenic conceptus. Furthermore the immune-modulatory mechanisms of PIF have been translated effectively to prevention and treatment of auto-immune conditions such as multiple sclerosis and juvenile diabetes mellitus, in mouse models (Weiss *et al.*, 2011).

How can this knowledge of PIF be of relevance to clinical equine reproduction?

Preimplantation factor can be identified in equine placental tissue, as in the human and mouse, inferring that it is secreted during equine pregnancy. It has been shown to bind to naïve equine peripheral macrophages in *in vitro* testing, similar to the situation in the human circulation, implying that PIF modulates the immune response through interaction with the innate immune system. Preimplantation factor also abrogates inflammation in equine endometrial tissue explants cultured in the presence of lipopolysaccharide. Although preliminary, these findings suggest that the role of PIF may be conserved between the horse, human, mouse and other mammals and paves the way for application of this peptide in equine medicine and reproduction.

Persistent mating induced endometritis is a significant cause of reduced fertility in equine breeding populations, with at least 15 % of Thoroughbred mares developing the condition (Zent, 1998). Recent investigations have been aimed at developing treatment strategies which aim to normalise the immune response to uterine challenge in mares susceptible to this condition (Christoffersen *et al.*, 2012). Preimplantation factor has been shown to modulate equine and human endometrial cytokine profiles and innate immune responses so may provide a novel treatment for equine endometritis. Development of a reliable, simple and reproducible assay to detect PIF in maternal serum will allow embryonic viability to be tested early in pregnancy and could be a useful resource for the stud veterinarian to follow up diagnosis of pregnancy. Similarly measurement of the peptide in embryo holding media may assist in grading and selection of embryos in embryo transfer procedures. The trophic effects on embryos cultured with PIF may also be exploited to advance the success of *in vitro* embryo production.

With further investigation there may be broad application for PIF in the advancement and efficiency of techniques in equine reproduction.

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The researcher and the user in the field – a two way street or a stop sign?

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Each year, much scientific research is performed with the ultimate intent of assisting equine reproduction. Some research disproves theorem that have been considered dogma for many years; other has far-reaching effects into the future for processes not yet perfected; while other has immediate value and impact for the practitioner and breeder. How rapidly does that new research make its way to the field though? What avenues are available for the practitioner and breeder to access that information? How much of the research ends up "on the shelf", or used by only a few for many years before finally becoming "mainstream"?

The converse is also true – what reproductive factors are the practitioner and breeder facing on a regular basis that would benefit from research but are never researched because the scientists are not aware of the issues? What channels of communication between "the field" and "the lab" are available? Do more need to be developed?

While there are scientific meetings where the scientists themselves can gather and discuss research, and there are similar meetings for veterinarians and for breeder groups, the opportunities for all three spheres of reproduction to meet and discuss on an even playing field are often at a minimum.

This session will briefly present an overview of some practical work with a review of some often overlooked research with practical application – some of it not so new – related to equine breeding and pregnancy. In addition it will stimulate subsequent discussion with the particular aim of developing a list of areas that would benefit from research and development of a better means of transmission between the industry groups. Some of the items being reviewed include the following, although this is not an all-inclusive list:

Pre-breeding evaluations
Use of a 1/10th dose of prostaglandin to promote oestrus while avoiding unpleasant side-effects in the mare;[1]
The importance of a cytology smear being prepared and read in conjunction with a uterine swab culture - and the lack of accuracy of the culture alone.[2]

The breeding process
Value of exogenous oestrogens to encourage receptivity and improve uterine blood flow.[3]
Identification of the delayed uterine clearance mare and suitable treatment - oxytocin, frequency, dose and type.
Use of agents for improving uterine biopsy scores or treatment of biofilm-producing organisms.[4,5]
Use of anti-inflammatory treatment for control of inflammatory response.[6]

Early pregnancy
Development of the "early pregnancy factor" test and the value thereof.
Nuclear transfer - "cloning" - value, scares and registry options.
Progestin supplementation - do we or don't we and why is there no research to tell us?
Use of colour doppler ultrasound for early pregnancy diagnosis.

Mid to late pregnancy
Placentitis detection and treatment;
Colour doppler ultrasound for fetal and placental evaluation;
Dogma - 340 days "my mare is due now" – a wide variation in "normal" gestational duration.[7]

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Schmallenberg virus: One year on

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First reports of an unusual condition affecting dairy cattle in a significant number of herds, characterised by an acute reduction in milk yield, diarrhoea and a transient fever were reported from continental Europe in Summer 2011. Next generation sequencing determined a novel virus provisionally named after the location samples were derived from as Schmallenberg Virus (SBV). This novel virus showed a close relationship to members within the genus *Orthobunyavirus* family *Bunyaviridae* (Hoffmann *et al*. 2012). In line with other Orthobunyaviruses research soon provided evidence to support the hypothesis that SBV is transmitted by *Culicoides* midges (de Regge *et al*., 2012). While a zoonotic risk could soon be discounted as low to negligible, there was evidence to suspect malformed calves or lambs as a consequence of prior exposure to SBV.

In England and Wales, the Animal Health and Veterinary Laboratories Agency (AHVLA) provides a subsidised diagnostic service through their regional network in order to deliver horizon scanning surveillance intelligence to Defra. Consequently, the first suspect fetuses were submitted and the prior incursion of SBV into GB demonstrated in January 2012, when it was identified by PCR in samples from malformed lambs from 4 sheep farms in Norfolk, Suffolk and East Sussex. Subsequently there was a steep rise in cases as the lambing season progressed and the calving season started. The precise period of susceptibility for cattle or sheep fetuses is not known and estimates have been made by analogy with Akabane virus. Consequently, SBV had probably entered GB before the end of September 2011. Earlier incursions of SBV could have occurred and would have been undetected clinically or if animals had not been at a susceptible stage of pregnancy when exposed to the virus.

Midge activity is heavily influenced by climate and reduces as the temperature falls. The decline in the number of reported cases from April 2012 can be attributed to the concurrent reduction in midge activity and the gradual end of the seasonal lambing period. However, cases did not cease completely (Steinbach *et al*. 2012) and it soon became evident that SBV was to stay both in Europe and in the UK.

Carrying out a serological survey using samples from sheep that were being collected for routine surveillance for brucellosis and samples submitted to the AHVLA it became clear that SBV was spreading rapidly across both England and Wales during the Summer of 2012. The survey also demonstrated that SBV infection had occurred in parts of England and Wales where acute clinical disease or malformed offspring had not been reported.
One year after the first detection it is evident that there are many scientific questions still unanswered and both national and European projects are underway to address some of the knowledge gaps.

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Influenza: dealing with an evolving menace

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Danger of highly pathogenic avian H5N1 virus

Human cases of highly pathogenic avian influenza (HPAI) H5N1 virus infections carry an alarming mortality rate of 50-60%, according to cumulative figures from the World Health Organisation. Despite its high death rate in humans, HPAI H5N1 virus infections are mainly confined to wild birds and poultry. However, owing to the inherent nature of the segmented RNA virus to mutate and undergo genetic reassortment, the danger of HPAI viruses gaining the ability to efficiently transmit horizontally between humans whilst retaining high virulence cannot be ignored. The 1918 influenza A (H1N1) pandemic that took place during the First World War, with a mortality rate of around 2% and total death toll of approximately 40 million people, is a sombre reminder of the devastating capability of influenza infections. A commonly cited complication of virulent influenza A infections in humans is the rapid development of hyperacute dysregulation of pro-inflammatory cytokines and chemokines, described as **hypercytokinemia** or **cytokine storm**, which is a self-destructive and often fatal syndrome despite supportive medical intervention (*1*). Prevention by vaccination, and treatment by anti-neuraminidase drugs are the mainstays of influenza management but they are not without major shortcomings, namely long lead vaccine production time and the development of drug resistance (*2*). A further strategy that is urgently needed to tackle future highly virulent influenza epidemics or pandemics is to develop therapeutic agents that target hypercytokinemia. However, simply blocking pro-inflammation alone does not improve mortality rates in HPAI H5N1 virus infections (*3*). There is therefore a great need to understand the **early host triggers** of influenza-induced hypercytokinemia, to enable the development of rational pharmaceutical interventions to maintain or restore a regulated pro-inflammatory response.

Contrasting innate resistance to HPAI H5N1 virus between humans and pigs

In contrast to humans, pigs appear largely refractory to contemporary Eurasian HPAI H5N1 virus infections and are highly resistant to developing any clinical effects. Experimental HPAI H5N1 virus challenge studies in pigs found no or only transient and mild clinical symptoms, and subsequent seroconversion (*4, 5*). Key innate immune cells that play important roles in the pathogenesis of influenza A infection are respiratory epithelial cells, macrophages (*6*), and, the more recently recognised, vascular endothelial cells (*7*).

Major differences in cellular response to HPAI H5N1 infection between human and pig cells

We recently found that primary respiratory epithelial cells and peripheral blood derived macrophages from both human and pig showed comparable susceptibility to initial infection (that included virus entry) with a clade 2.2.1 HPAI H5N1 (A/turkey/Turkey/1/05) and two moderately pathogenic H1N1 influenza viruses (human A/USSR/77 and classical swine A/sw/Iowa/15/30), but there were sharp contrasts in host innate immune response between the same cell types of each species. Human epithelial cells and macrophages mounted vigorous cytokine (TNF-α and IL6), and chemokine (CXCL9, CXCL10 and CXCL11) responses to H5N1 virus infection. However, the corresponding pig cells showed weak or no TNF-α and chemokine induction to the same infections. The apparent lack of a strong pro-inflammatory response in pig cells, corroborated by the absence of TNF-α induction in HPAI H5N1 virus challenged pigs, coincided with reduced release of infectious virus from infected pig epithelial cells. Consistent with these findings, viral RNA (sense and anti-sense) from each of the 8 viral segments was twice as abundant in human than pig epithelial cells, based on RPKM values from deep sequencing of total RNA from H5N1 virus infected cells.

Naturally resistant host species could provide key knowledge in the treatment of virulent influenza infection

A strategic approach to identify critical host factors or cellular responses that confer host resistance or susceptibility at the level of virus replication and pro-inflammation is to establish in detail the molecular differences in host innate response between susceptible (human) and resistant (pig) mammalian cells. One such mediators is the suppressor of cytokine signalling (SOCS) family of eight genes whose members are widely recognised to negatively regulate a range of cytokines (including interferons) and growth factors that signal through the JAK-STAT pathway (*8*). We hypothesise that in HPAI H5N1 influenza virus infection, members of the SOCS family are key regulators of pro-inflammation and play a major role in the prevention of acute pro-inflammation (*9*).

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Penside testing for avian influenza infections in poultry by lateral flow devices and isothermal amplification: Advantages and disadvantages

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Introduction Emergence of a spreading epizootic of H5N1 highly pathogenic avian influenza (HPAI) during the past decade has been accompanied by developments in diagnostic approaches that require critical evaluation and validation prior to acceptance. Laboratory-based diagnosis of AI outbreaks now includes validated RealTime (RRT)-PCR methods for rapid, highly sensitive and specific detection of infection (Hoffmann *et al* 2009, Slomka *et al* 2010). Lateral flow devices (LFDs) for AI detection have been developed by several commercial manufacturers for use in rapid penside testing, but with little (if any) initial validation with field specimens to demonstrate their fitness for purpose. As an alternative to RRT-PCR, the AI recombinase polymerase amplication (RPA) is emerging as a new isothermal amplification technology (Piepenburg *et al* 2006) for use in regional / remote laboratories and potentially in penside setings.

Materials and methods (i) AI LFD (Anigen): Swab (188) and feather (94) specimens were collected from poultry (46 chickens and 48 domestic ducks) during H5N1 HPAI outbreaks in Vietnam during 2009 (Slomka *et al* 2012). LFD sesnitivity and specificity were determined in comparison to AI RRT-PCR for both these species and different specimen types. Validated AI RRT-PCR served as the gold standard in being the most sensitive diagnostic method. For low pathogenicity (LP)AI infections, LFD sensitivity and specificity was addressed in a population of 240 and 224 swabs from ducks and turkeys respectively that had been experimentally-infected with H5N2 LPAI. (ii) AI RPA and LFD were compared to AI RRT-PCR for 94 of the Vietnamese H5N1 HPAI outbreak clinical specimens. AI RPA was also assessed in other groups of clinical specimens from LP and HPAI-infected poultry.

Results (i) AI LFD: For H5N1 HPAI infected and diseased chickens in Vietnam, sensitivity of the AI LFD was 32% for swabs (viral shedding) and 56% for feathers. For ducks from the Vietnamese H5N1 HPAI outbreaks, LFD sensitivity was 0% for swabs and 33% for feathers (Slomka *et al* 2012). Specificity was 100% in all clinical specimen groups. However, testing of swabs from H5N2 LPAI experimentally-infected turkeys and ducks revealed LFD sensitivities of 1% and 5% respectively. (ii) AI RPA: Ninety-four of the Vietnamese H5N1 HPAI clinical specimens were available for testing by AI RPA and enabled a comparison to be made to the LFD. For 59 chicken specimens, AI RPA and LFD had sensitivities of 65% and 48% respectively for swabs, and equal sensitivities of 88% for testing feathers. For 35 duck specimens, AI RPA and LFD had sensitivities of 33% and 0% respectively for swabs, and 60% and 53% respectively for testing feathers. Interestingly, AI RPA had a sensitivity of 79% compared to AI RRT-PCR in testing 14 swabs from H5N2 LPAI experimentally-infected ducks, and detected AI in swabs collected from Nepalese H9N2 (LPAI) outbreaks.

Conclusions The above validation experiments are representative of the diverse manifestations of AI infections in different poultry species caused by different AI subtypes of differing pathogenicities. AI RRT-PCR data was interpreted quantitatively to show that the LFD frequently detects AI shedding in high titre swabs that are obtained from H5N1 HPAI-infected and diseased chickens. LFD failure to detect AI in swabs (0% sensitivity) from infected ducks reflected the lower shedding titre in this species (Slomka *et al* 2012). However, HPAI infection is systemic, and high viral titres in the feathers of both chickens and ducks was reflected in a higher sensitivity for AI LFD compared to the corresponding swabs. These findings in Vietnamese H5N1 HPAI outbreaks indicated that swabs are not appropriate for successful LFD detection of AI in ducks, but feathers were identified as a sample type for the most sensitive detection of AI by LFD in H5N1 HPAI infected poultry of both species (Slomka *et al* 2012). For LPAI infections in experimentally-infected turkeys and ducks, low shedding titres resulted in very low AI LFD sensitivity in testing swabs. Therefore LFDs would be inappropriate for investigating LPAI outbreaks, while feathers are irrelevant to testing non-systemic LPAI infections.
AI RPA demonstrated a greater sensitivity than AI LFD in the Vietnamese H5N1 HPAI specimen group and has the potential for acceptance for penside use. Its sensitivity deficiencies compared to AI RRT-PCR may be compensated by collecting a sufficient number of clinical specimens from suspect infected flocks. AI RPA also has an advantage over AI LFDs in being able to detect the lower shedding titre observed in LPAI infected poultry. Rigorous validation of any new diagnostic technologies remains imperative to prove a sufficient "fitness for purpose" which is crucial for confirming notifiable infectious disease.

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Molecular diagnostics and future prospects: making the tricky, routine and easy in the lab, but can we go outside now?

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Introduction Rapid and accurate detection of pathogens in clinical or environmental samples is at the cornerstone of diagnosis of disease. In the last 20 years there has been a move to enhance diagnostic capability using more conventional diagnostic tests such as ELISA, serology and pathogen cultivation and isolation by using molecular diagnostics as an adjunct to these tests rather than replacing them. Molecular diagnostics involve the detection of specific nucleic acids and frequently the amplification of these sequences in order to allow the products to be easily detected. As such the design of molecular tests has been greatly accelerated by advances in genome sequencing technologies that have provided the base information (the specific pathogen sequences) for the design of these molecular tests.

What should a molecular diagnostic assay do? As with all diagnostic tests, molecular based assays must fulfil certain criteria in order to be implemented successfully and accepted by the end user as being reliable. The assays must be sensitive in that they must detect pathogens in clinical samples at least to the level of performance that traditional methods do. They must be specific in that they must not misidentify closely related (or not so closely related sequences) as indicative of the presence of the pathogen. The tests must be repeatable and robust in that they should be able to be performed by different appropriately trained individuals producing the same results for the same samples. For the most part the variability between test results should be low (%CV of <10% is acceptable) and should relate to quantitation only i.e. the diagnostic measures should remain invariant.

Current Molecular Diagnostics The **polymerase chain reaction** (PCR) is the most commonly used and dominating technology applied to molecular diagnostics both in the clinical and veterinary field. Its power lies in its simplicity of design and application requiring a basic understanding of clean room techniques. The specificity of the assay relies on the hybridisation of short stretches of synthetic DNA (oligonucleotides) complementary to the target sequence and amplification of the sequences between these oligonucleotides using the enzymatic activity of *Taq* polymerase. PCR assays are easy to design as there are many software packages freely available on the Internet. **Isothermal amplification methods** are also coming to prominence principally due to the ease with which these methods can be applied in that they do not require a programmable heating block, being the heart of the PCR machine, but simply a constant source of heat. Nucleic Acid Sequence Based Amplification (**NASBA**) first published in 1991 was one of the first methods to gain prominence. An isothermal amplification method that appears to offer many of the advantages over others available is loop mediated isothermal amplification or **LAMP**. This method employs the strand displacement activity of *Bst* polymerase (or equivalent) and four specific primers that hybridise to six different regions of the target DNA. Despite the apparent complexity of the molecules produced (cauliflower-like with multiple loops) they are essentially composed of repeats of the initial amplification molecule and as a consequence undergo DNA melting at a consistent and reproducible temperature in much the same way PCR products do. Despite the use of this technology in many diagnostic assays a major limitation of the technology appears to be its apparent lack of tolerance to sequence variation in the primer binding sites. An ingenious strategy for DNA amplification has been adopted in a process known as recombinase polymerase amplification or **RPA**. This isothermal amplification method is similar to PCR in that only two opposing primers are employed but where heating is used to melt the template and subsequent products globally, RPA uses recombinase-primer complexes to scan the double stranded DNA, facilitating strand exchange at cognate sites (specific site melting in other words). The technology in our hands has been applied to the amplification of bacterial and viral targets where there has been a degree of success. However, the optimisation of assays requires an empirical approach. Both RPA and LAMP can be detected in real-time using intercalating dyes or more simply at the end of the reactions using tagged primers (FITC, biotin) on lateral flow devices (LFD).

Future Prospects PCR will continue to dominate but has and will become increasingly more rapid so that it can compete with more rapid isothermal methods. Commercial manufacturer's are now claiming "cycling times to result" of 25 minutes with some, such as AB producing specific FAST machines, recognising that *Taq* polymerase is not working at a maximum rate in conventional machines. In well equipped laboratories the rate limiting step is shifting from the detection method used to the method of extraction. One of the most exciting prospects for molecular testing is to take these diagnostics to the infected animals rather than taking the samples from these animals to the central testing laboratory. The principal of **pen-side testing** has been established using RT-PCR methods and combined extraction/real-time PCR machines. However the cost of these machines and consumables where they are most needed in the world is prohibitive and this is clearly problematic for commercial companies producing these devices. As such the AHVLA has developed a suite of molecular amplification methods employing LAMP and RPA with a view to incorporating these into a simple and cheap plastic module in order to facilitate amplification and detection and critically prevent cross-contamination between samples. The problem of the availability of rapid and reliable **extraction methods** still remains for more recalcitrant templates.
Of a particular concern is not only the consideration of what a is gained but also what is lost by using pen-side testing for those collecting epidemiological data or responsible for control of disease (centralised laboratories). This is a pressing issue particular in light of the availability of veterinary diagnostics via the Internet.

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A walk through epidemiology

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Epidemiology is the population study of the distribution, determinants and control of diseases. Over the past 20 – 30 years there has been an upsurge in the use of epidemiology to study infectious and non-infectious diseases of animals, including a specific focus on animal welfare. Initial work used fairly simple statistical tools and study designs that were either observational or experimental (clinical trials) in nature. Studies have become more complex as computer power has increased to handle large datasets and software improved, so that non-experts can use more sophisticated tools to analyse unbalanced and clustered data. Mathematical models, the theory of which was developed in the early 1900s, have become mainstream now that computer simulations are feasible with fast computing power. The gap between mathematical and statistical analysis is closing with many tools in common between the two disciplines; particularly that of inference (prediction). Most recently, economic and social sciences (attitudes and perceptions of the stakeholders with an interest in animal disease) have been added to the 'ologies' that are included in epidemiology.

In parallel with the changes in content of epidemiology, epidemiology itself depends on an accurate case definition of disease and covariates. Simple case definitions can be made for some diseases but for others case definitions are complex. For example, as molecular tools are developing the potential for molecular epidemiology has arisen where analysis of strains of a pathogen is used to study the course of a disease in a population. In many areas an accurate case definition is currently unavailable. For example, in animal welfare there is a focus on defining a positive welfare state and identifying welfare indicators that truly identify whether an animal is in good welfare. These are currently in the research phase and require validating and refining before they can be used in epidemiological studies as a measure of good welfare.

The aim of this presentation is to use examples of epidemiological studies to illustrate the developments above and to consider the current strengths and limitations of epidemiology and where future work could be targeted.

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Developments in Dublin – veterinary education from Newman to the flipped Classroom

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The early days of veterinary education in Ireland were marked by "The Veterinary College" being passed somewhat erratically between the Government Department of Agriculture and two Universities. Notwithstanding, the dominant influence of University College Dublin is clear, both in terms of its founding fathers and the latter-day presence of the veterinary school on the main campus of Ireland's largest University. John Henry Newman was a key figure in the development of University College Dublin. In his discourses on "The Idea of a University" he held that "An academical system without the personal influence of teachers upon pupils, is an arctic winter; it will create an ice-bound, petrified, cast-iron University, and nothing else." We could also add the students' influence on each other as an essential ingredient in the recipe for a successful University Education. The Veterinary School in Dublin moved into its current premises, on the main UCD campus at Belfield, just over 10 years ago. After a long period marked by enthusiasm and dedication among students and academics, but stagnation in terms of the suitability of facilities, this move opened up an appetising menu of possibilities. During this time there has been a move from offering a single core programme in veterinary medicine towards a "cradle-to-grave" approach to veterinary education. The school now offers graduate and undergraduate entry pathways to veterinary medicine, a veterinary nursing degree, a professional doctorate for clinical specialists in training, and a growing portfolio of blended-learning opportunities for practicing professionals, as well as thematic and structured PhD programmes.A continuing interesting challenge we meet in our school on a daily basis is that of cultural harmonization. The potential for cultural clashes is multi-dimensional – veterinary *vs* vet nursing students, local students *vs* international, academics *vs* practically-inclined etc. Part of the solution lies in communication – and invariably, as more channels of communication become available, the more they are ignored.One of the opportunities afforded by educating veterinary practitioners and veterinary nurses in the same school is that of offering teamwork education. In terms of clinical education of veterinary students, our model of assessment *via* OSCEs and CPEs has served us well. However, this year we have moved to a more diversified model of assessment. Some of the clinical disciplines have adapted slightly faster than others in this respect. Our Herd Health/Farm Animal Clinical Studies group, for example, has completely reworked their module to include more herd-health problem-solving activities, and fundamental clinical procedures. These changes were made in response to a survey of employers engaged in farm animal practice. This discipline has also altered their assessments to include direct observation of practical skills (DOPS), meaning that the high-stakes end-of-year clinical exam is removed. The use of online, electronic or blended learning, however, will mean much more than simply sharing of resources. The chorus of academic staff members complaining about the declining attendance at lectures as a result of "The notes/Powerpoints" being available on the VLE is likely to be as familiar as those same academic staff complaining about the requirement to have the timetables finalized months in advance, and about the competing time requirements of clinical work/research and teaching.

An obvious remedy is to reserve the precious face-to-face meeting time of academics and students for truly interactive activities. Modern ICT makes innovative collaborative learning (WIKIs, discussion forums etc.) available electronically as well, but there is still a dimension of human interaction that cannot be captured electronically. The notion of "flipping the classroom" is gaining traction at all levels of education. we not at the stage now when the shackles imposed by space and time constraints can be loosened We to well to remember, as Newman stated, that the essence of a University Education requires the up close and personal interaction of students with both peers and academic staff. In veterinary education, where we have relatively high staff: student ratios as compared with other disciplines, but also a heavy load of activities (clinical duties, research) which compete with teaching, loosening the strings of fixing lectures in space and time, as well as limiting these to the essentials, can create space for the more fruitful two-way interactions discussed. These principles are already working admirably in the Dublin school our online/blended learning programmes for practitioners – it only remains for us to further capitalize on them for veterinary students in the core curriculum.

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From there to here: reflections on the evolution of the BVMS programme 2007 – 2013, University of Glasgow

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The School of Veterinary Medicine at the University of Glasgow will launch a new professional veterinary programme in 2013.
"There" was the traditional "ology" based programme structure with sequential phases of basic sciences, paraclinical sciences and clinical instruction with a substantial number of course specific assessments. "Here" is a vertically integrated, spiral curriculum creating an outcomes based educational programme overtly addressing the competencies required by the accrediting professional bodies (RCVS/EAEVE/AVMA) of new veterinary graduates, whilst providing a university science based education to students.

The transformation from one to the other has been through a process of evolution of the existing programme taking the educational experience towards the requirements of the new programme and a final revolution to fully realise the ambition. This process has engendered in staff both excitement at the potential outcome and stress in relation to undertaking the change. The experience of managing the change has highlighted potential reasons for curricular change being a source of stress and reasons for the failure of ambition to create change.

Why change:
External drivers:
Undoubtedly the biggest driver is the development of and requirements of outcomes focused accreditation
Stakeholder consultation.
Staff awareness of developments in health care education.
Internal drivers:
Staff desire to deliver optimum education and recognising change essential to maintain reputation.
Perception of competition in recruitment in relation to student quality

History of change:
Glasgow reputation for innovation in education:
Lecture free final year 1980
Team teaching integrating subjects (Combined Integrated Course – pathology, medicine and surgery)
Two curricular change processes in last 12 years that have stalled

Obstacles to change:
Significant variation in the degree of group buy in drivers for change
Distribution of group buy into the drivers for change, in particular ownership of the outcomes
Traditional structure of separately managed courses aligned to staff groups
No tradition of a programme of education thus no leader of programme
Lack of programme management tools for managing programme outcomes and content

The "hidden changes" associated with curriculum change
For a veterinary school what is taught and how it is taught defines its staff
Changing the education mission influences the staff required, the skills staff require, job descriptions, promotion criteria
Thus for an established school with an established programme changing the curriculum will change the working environment for established individuals

Creating the environment for change:
Ensuring a leadership priority
Gaining confidence in being able to cope with and implement change by identifying and undertaking specific changes
Develop the momentum for change by increasing staff exposure to the drivers
Increase the evidence if necessary introduce independent, external perspective
Be confident that the decision making structures can enact change effectively
Create ownership of changes by distributing responsibility

Institutional outcome of educational programme change
The requirements of the professional curriculum define a veterinary school and changing an established curriculum will redefine significant aspects of the school.

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Development of a custom genotyping panel for dairy and beef cattle breeding and research

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Introduction Genomic information, in the form of microsatellites, has been used in parentage verification in cattle (and other species) for several decades. However, genotyping platforms exploiting single nucleotide polymorphism (SNP) markers are now more frequently used since they are more amenable to automation and therefore are lower cost. However changing technology for parentage verification usually implies that back-pedigree would have to be re-genotyped using the SNP technology thereby incurring a cost. Furthermore, genomic technologies are now being exploited in dairy cattle breeding programs and will be soon exploited in beef breeding programs. However, the larger the number of SNPs to be genotyped the greater the cost thereby squeezing the cost:benefit of genotyping strategies. The objective of this study was to develop a low-cost international dairy and beef cattle custom SNP genotyping platform that will 1) facilitate imputation or prediction of a larger number of SNPs for genomic selection, 2) be able to accurately compare offspring genotyped using the SNP panel with parents genotyped using microsatellites, 3) screen for known lethal recessives and major gene effects and 4) provide a vehicle to genotype a large number of animals for SNPs of interest in research projects. The chosen SNPs will be added to the commercially available Illumina low density (LD) genotyping platform which contains 6,909 SNPs; to limit cost for producers the total number of SNPs on the custom chip was limited to 19,000.

Material and methods Illumina high density (HD) genotypes (777,962 SNPs) were available on 3,124 dairy and beef animals. A threshold of additional SNPs per chromosome to be added to the LD panel was proportional to chromosome length. The most informative SNPs were sequentially chosen, within chromosome, based on a dual objective of maximising the minor allele frequency weighted across breeds, and minimising the maximum level of linkage disequilibrium between each candidate SNP and the SNPs already chosen. Imputation from the original LD genotyping platform, the custom genotype platform, and the commercial available Bovine50 Beadchip (54,001 SNPs) to the Illumina high density platform was undertaken in dairy and beef cattle using BEAGLE (Browning and Browning, 2007).
A total of 3,744 SNPs from the HD genotyping platform flanking the 12 parentage verification microsatellites from over 9,000 *Bos Taurus*, *Bos indicus* (and their crosses) cattle representing 40 beef and dairy breeds were phased with BEAGLE (Browning and Browning, 2007). SNP haplotypes that were unique to a specific microsatellite allele across multiple breeds were identified from a reference population of 1,200 to 4000 animals (not all animals had information on all 12 microsatellites). Microsatellite alleles in a validation population of 8,740 animals were subsequently imputed using the identified SNP haplotypes. An additional 1,873 SNPs were added which are part of on-going research in Ireland on the role of mutations in the genes of the somatotropic axis on performance. Finally, known causative SNPs 1) within lethal recessive genes, 2) that cause congenital disorders, or 3) were within major genes of interest were also added to the custom chip.

Results The mean accuracy of imputation to high density from the commercial available LD, the custom SNP chip described here, and the commercially available Bovine50 beadchip was 0.95, 0.97 and 0.99, respectively; moreover if the sire was genotyped on the higher density genotype platform then the respective accuracy of imputation was 0.97, 0.98 and 0.99. The accuracy of the microsatellite imputation in the validation animals, determined by comparing the imputed microsatellite allele to the parental microsatellite alleles was 95.6%. In 68.3% of the validation animals, all microsatellite alleles were correctly imputed. Microsatellite genotyping errors, however, also exist and are thought to be between 1% and 5% (Bonin *et al*., 2004; Weller *et al*., 2004). Mutations in 56 genes were included in the custom SNP panel, which included the four common lethal recessives in cattle (CVM, BLAD, DUMPS, Brachyspina), 35 common congenital disorders including Arthrogryposis and Hypotrichosis, and 17 genes with known major effects on quantitative traits in dairy and beef cattle including Myostatin, DGAT1 and A1/A2 β-casein.

Conclusions This custom SNP genotyping panel, known as the International Dairy & Beef 19 (IDB19), contains 19,000 SNPs to aid in more accurate imputation to greater SNP density for genomic selection, imputation to microsatellites for parentage verification against back-pedigree that do not have SNP information, screen for lethal recessive mutations, genetic defects and major genes as well as providing genotype information on SNPs of research interest which, when linked to phenotypes, can be used to elucidate the genomic architecture of phenotypic performance. The list of SNPs included in the panel is freely available and the panel is under annual development. Animals are currently being genotyped with this new panel in Ireland.

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Effects of antibiotic resistance in foodborne bacteria on human health

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Antibiotic use has brought huge changes to the world, especially in expectations of survival of children into adulthood, and underpins all areas of modern medicine. The theme of World Health Day, 2011, was “antimicrobial resistance: no action today and no cure tomorrow”. However, the demise of antibiotic drug discovery, research and development brings the spectre of untreatable infections. Recently, at the World Economic Forum 2013 in Davos, antibiotic resistance and the lack of new drugs was added to the Global Risks Register. To prevent a crisis of untreatable bacterial infections, urgent action is needed and so Antibiotic Action was launched in 2011 to educate all about the need for discovery, research and development of new antibacterial drugs. In this talk I will give an overview of the scale and context of antibiotic resistance and how it with the paucity of antibiotics to treat some infections impact upon human health. I will also briefly overview the contributory factors that select and disseminate antibiotic resistant bacteria. One concern is that antibiotic use in animals for food production could create an environment for antibiotic resistant bacteria to be selected and transferred through the food chain, such that if these bacteria cause an infection in humans therapy could be compromised. I will briefly give examples of antibiotic resistance in bacteria isolated from animals to illustrate that the selection of antibiotic resistant bacteria is influenced by the antibiotic used and what therapeutic options remain if these bacteria cause infection requiring treatment in humans.

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Practical antibiotic stewardship in the UK poultry industry

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Introduction Consumers continue to voice concern over the use and abuse of antibiotics in animals. The poultry industry is not immune to these criticisms and indeed is often accused of contributing considerably to human drug resistance and the development of superbugs. These concerns are understandable but the criticisms often lack factual evidence or focus due to a combination of misunderstanding and possible misinterpretation.

Antibiotic use in poultry Antibiotic use to control serious poultry health diseases and reduce the impact of food safety issues is necessary and justifiable. However, the industry must not ignore these issues, both in the court of public opinion, but also in making serious attempts to reduce any contribution made to human health issues associated with the treatment of disease in poultry.
The main areas of concern aimed at the poultry industry include some or all of the following assumptions:

1. Antibiotics are constantly fed to poultry in a totally uncontrolled manner.
2. The use of antibiotics in poultry leads to the production of antibiotic resistant super bugs, damaging to human health.
3. Such antibiotic use is also bound to lead to the presence of harmful residues in the meat and eggs we eat.
4. Antibiotics contribute to poor animal welfare by allowing birds to survive in less than optimal conditions.
5. Antibiotics are being used for growth promotion and rapid growth rather than to treat disease.

In fact the true situation is far less alarming. Antibiotics have an important role in managing human and animal health and have done for over 50 years. It is a fact of life that the use of antibiotics in animals will lead to the development of resistant organisms in those treated animals – it is a form of natural selection or selection pressure. In much the same way, antibiotic use in humans will contribute to the development of resistant organisms in the human population. Anything that can be done to reduce the use of antibiotics in animal production will reduce the likelihood of antibiotic resistance development, as indeed it will in human medicine too. The poultry industry is well aware of the role played by husbandry, management, nutrition and environmental control in the incidence, severity and magnitude of disease challenges. The role of effective biosecurity and the use of structured vaccination programmes will reduce dependency on antibiotic use.

Veterinary diagnosis and targeted therapy One of the most important aspects of ensuring targeted therapy is accurate and prompt diagnosis of disease challenges. This requires effective communication between producers and their veterinarians with rapid identification of signs of ill health and professional investigation as to the cause of any morbidity or mortality. This in turn requires access to specialist poultry veterinarians, and a range of rapid and effective diagnostic tools. Structured monitoring plans can then be devised to allow early identification of health issues and help assess the relative contribution of infectious, management and environmental factors. This can help prevent any dependency on unnecessary medication strategies and should be incorporated into the practical implementation of effective Veterinary Health and Welfare Plans. Where antibiotic use is considered necessary under veterinary direction the aim should be to use this targeted therapy early in the disease process (based on pre-treatment sensitivity testing) and more accurate diagnosis will allow therapy aimed specifically at the organism being treated enabling use of narrow spectrum antibiotics.

Responsible use of medicines Responsible use of medicines guidelines have been produced by a number of industry bodies in many countries over recent years to promote judicious use. One of the leaders in this process has been the Responsible Use of Medicines in Agriculture (RUMA) alliance in the UK. This alliance was established in 1998 as a cooperative and independent body encouraging best practice in the use of antimicrobials in farmed livestock. Their guidelines for the responsible use of antimicrobials in poultry production, produced in consultation with the poultry industry, serve as a useful template for others to follow. These guidelines include the storage, use, dosage, withdrawl period observance, recording and disposal of antibiotics and their importance within an overall veterinary health plan and farm assurance system.

Conclusions There will be continued pressure on the reduction of antibiotic use in animals, especially those where medical authorities would like to see certain classes of antibiotic reserved only for human use (e.g. fluoroquinolones and cephalosporins). To safeguard the availability of necessary and effective antibiotic therapy for poultry and other animals all those involved in the supply, procurement and usage of these products should act responsibility in their use. This will help to ensure the health and welfare of poultry flocks whilst reducing the likelihood of the development of antibiotic resistance, reduce the likelihood of unwanted residues in foodstuffs and prolong the ability to use antibiotics effectively.
These aims can best be achieved by:

- Reduced dependence on antibiotics to control disease, with use under strict veterinary guidance
- Employing best practice in all aspects of disease biosecurity, hygiene procedures and terminal cleansing and disinfection
- Continued availability and targeted use of effective vaccines
- Consideration of the role of alternative products i.e. non-antibiotic products to control disease
- Monitoring antibiotic use and the development of resistance against specific pathogens

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Practical antibiotic stewardship in the UK dairy industry

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The healthcare of dairy cattle has changed greatly in the last decade, as has the structure of the dairy industry. The small family run dairy farm, where the vet was often only called to diagnose and treat sick or injured animals, has increasingly given way to large commercial farms, often now milking in excess of 500 cows. On these units regular systematic health and welfare monitoring, disease prevention, and the use of Herd Health Plans have become the norm. Health plans are now a central pillar of the industry standard Red Tractor Farm Assurance Dairy Scheme. In addition to this, all animals have a unique identification number and all medicine use is recorded so that there is an audit trail from point of manufacture to administration.

Antibiotics / antimicrobials have been used in UK agriculture for almost as long as they have been available in human medicine; however the way we use them has changed over time. For example the mastitis Five Point Plan to control mastitis devised in the 1960s by the National Institute for Research into Dairying stipulated that every cow should receive intramammary antibiotics at the end of lactation. Today we might employ a rather more evidence based decision making process before administering such treatments.

Some in society ask why we need antibiotics in livestock production. Yet their availability and responsible use impact on animal welfare, productivity and efficiency of production, food security and food safety. Great care is taken by the industry to ensure such products don't enter the food chain.

Antimicrobial resistance (AMR) is a growing concern world-wide both in medicine and veterinary medicine and we in the dairy industry must act responsibly. Whilst it is rare to encounter clinical situations where multi-resistance bacteria are causing disease on farm, potentially harmful multi-resistant pathogens are occasionally detected. For example meticillin-resistant *Staphylococcus aureus* in bulk tank milk Paterson *et al.* (2012).

The ultimate responsibility for antibiotic stewardship on farms rests with the veterinary profession as it is only veterinary surgeons that have the right to prescribe such medications; however milk producers, their other advisors and those controlling every step in the milk supply chain also have a degree of responsibility, as do those responsible for the production, promotion and supply of veterinary medicines.

This paper explores the current statutory, and voluntary, measures taken to promote responsible use of antimicrobials, the possible legislative changes that may be seen in the future and their impact on the veterinary profession and the dairy industry. We are already seeing changes to regulations that should have a positive impact such as the ban on advertising antimicrobials to livestock keepers, but other changes could be more problematic; for example separating prescribing from dispensing.

The veterinary profession supported by organisations such as the *British Cattle Veterinary Association* and *British Veterinary Association*, and farmers supported by the *National Farmers Union*, *Dairy UK* and industry wide groups such as the *Responsible Use of Medicines in Agriculture Alliance* are all working hard to further promote responsible use of medicines and practical antibiotic stewardship. Nevertheless there remains a need for better education primarily of veterinary surgeons and farmers, to promote best practice and disease prevention, but also of the public and consumers at large to promote a better understanding of all aspects of livestock food production.

Compared to some other sectors of livestock production, the UK dairy industry uses relatively little antimicrobials but there is still a need to Reduce, Refine and Replace wherever possible to minimise the risk of AMR. Where further change is needed one can speculate where the primary drivers will come from; EU legislation, RCVS Practice Standards Scheme, Professional Code of Conduct, prescriptive or even restrictive formularies, antimicrobial audits and benchmarking are all possibilities for the future. Already major milk buyers and the leading retailers are taking a very close interest in medicine usage on dairy farms.

Whatever rules and regulations are put in place, education of individuals responsible for the critical control points, primarily veterinary surgeons and farmers holds the key to responsible use across the industry. The author of this paper is currently working with industry stakeholders to promote this approach.

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Inflammatory gene expression in the bovine mammary organ: Systemic effects and regulation

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Regulation of the inflammatory response to potential mastitic agents in the bovine mammary tissue is key to determining whether the infection will be cleared without pathology or associated systemic effects. In any inflammatory response, dozens of newly synthesised proteins are required for activation of local phagocytic and microbicidal systems, recruitment and activation of fresh immune cell populations, clearance of infection, tissue repair and resolution of inflammation. Thousands of genes are involved in the implementation and regulation of these activities. We began by examining expression of key acute phase proteins, pathogen recognition receptors, inflammatory cytokines and chemokines as well as families of antimicrobial peptides in mammary tissues obtained from animals challenged with mastitic organisms. In a model of subclinical mastitis, we found increased expression of members of each of the key families of inflammatory molecules. We found detectable expression of inflammatory genes in liver tissue and also in non-challenged mammary tissue from these animals, indicating the systemic effects of mild, local inflammation. Of particular interest was expression in infected and neighbouring mammary tissue of acute phase protein genes, normally thought to be expressed exclusively in the liver. We also detected significant expression of cathelicidin and beta defensin genes in infected mammary tissue. We found that bovine epithelial cells could produce significant levels of several cathelicidins and defensins. We propose that local expression of these anti-microbial peptides mediate important immunoregulatory funcitons as well as microbicidal activity in mammary tissue.

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Using shotgun and targeted proteomics to investigate bovine host response to mastitis pathogens

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Technological advances in mass spectrometry have greatly improved accuracy and speed of analyses of proteins and biochemical pathways. These proteome technologies have transformed research and diagnostic methods in all biomedical fields, and in food and farm animal sciences proteomics is particularly important for investigating and monitor specific marker proteins and peptides within complex food matrices. Likewise, specific diagnostic markers associated with compromised welfare, or with early infections can be monitored to improve welfare in large industrial settings of current livestock industry. The combination of discovery based LC-MS/MS methods and the more hypothesis-based targeted mass spectrometry method commonly referred to as selected reaction monitoring or SRM, provide a powerful approach for investigating farm animal biology.

This talk will present our work with using both shotgun and targeted proteome methods to investigate bovine host response to mastitis pathogens.

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Streptococcus uberis: searching the genome for determinants of virulence

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Introduction Improvements in milking hygiene, routine dry cow therapy and culling of persistently infected animals have significantly reduced mastitis caused by *Streptococcus agalactiae* and *Staphylococcus aureus*, however the same is not the case for infections caused by pathogens from environmental sources, namely *Escherichia coli* and *Streptococcus uberis*. *S. uberis* is an opportunistic pathogen of dairy cattle responsible a significant proportion of clinical cases of mastitis in the UK and remains one of the major causative agents of bovine mastitis worldwide. This pathogen impacts negatively on animal welfare and the economics of milk production.

S. uberis has been shown in experimental infection models to colonise the bovine mammary gland rapidly, induce neutrophil diapedesis and cause an acute local inflammation during which milk becomes denatured and the mammary gland is distended and swollen. *S. uberis* persists within the mammary gland in the presence of neutrophils and other host defences which seem to offer little control (at least to virulent strains), however, the infection remains localised to the mammary gland and the bacterium is largely found in the secretion present within the lumen of the gland. Although a common mammary pathogen, *S. uberis* is often found as part of the normal, commensal micro-flora at other body sites and is not commonly associated with other disease states.

The ubiquitous nature of this organism and its ability to cause acute disease in a defined organ of a limited range of target species indicates a level of specificity in the interaction between host and pathogen. Acquisition of the full genomic sequence of *S. uberis* has enabled a series of studies in which mutagenesis has been used to alter expression of genes to determine their role in pathogenesis. This review will present the outcome from a one such study (funded by BBSRC and Defra) and discuss this in the context of our current knowledge of the interactions between *S. uberis* and the dairy cow that result in infection and disease.

Review: The role of the regulatory gene, *vru*, in virulence of S. uberis in the lactating dairy cow The regulatory gene, *vru*, of *S. uberis* encodes a standalone response regulator protein with similarity to Mga of Group A *Streptococcus*. Experimental intramammary challenge of dairy cattle with a mutant of *S. uberis* carrying an inactivating lesion in *vru* showed reduced ability to colonise the mammary gland and an inability to induce clinical signs of mastitis. Analysis of transcriptional differences of gene expression in the mutant was determined by microarray analysis. This identified a variety of coding sequences with altered expression, including both known and putative virulence determinants (*lbp* (sub0145), *sclB* (sub1095), *pauA* (sub1785) and *hasA* (sub1696)). The transcriptional data relating to *lbp*, *pauA* and *sclB*, were validated with respect to their effect on protein expression. In the absence of Vru, neither PauA nor Lbp were detectable and SclB was shown to be present at a significantly lower level. These data were consistent with their comparative transcriptional levels detected in the presence/absence of Vru, thus indicating that transcriptional analysis was reflected in protein expression.

The role of the transamidase sortase A (SrtA) in anchoring essential virulence determinants of S. uberis Sortase A (SrtA) has been shown to be responsible for the covalent attachment of proteins to the Gram positive bacterial cell wall. Anchoring is effected on secreted proteins containing a specific amino acid motif located towards their C-terminus. The motif for SrtA in Gram positive bacteria often incorporates the sequence LPXTG. Due to their location on the outer bacterial surface, such proteins are available to play a role in host pathogen interactions during the establishment and persistence of infection. Comparison of cell walls from *S. uberis* and an isogenic mutant strain unable to produce lacking SrtA revealed that the mutant failed to anchor nine proteins at its surface. Analysis of these sequences implied the presence of two possible anchoring motifs for *S. uberis*, the classical LPXTG and an additional hexa-peptide motif, LPXXXD. Bioinformatic analysis of the genome sequence revealed one further sequence likely to encode a SrtA anchored protein.

Unlike the wild-type strain, the SrtA mutant was only able to infect the bovine mammary gland in a transient fashion. Subsequently, mutants that each failed to express one of the ten sortase anchored proteins (sub0135, sub0145, sub0207, sub0241, sub0826, sub0888, sub1095, sub1154, sub1370, and sub1730) were isolated and their virulence determined in a similar challenge model. This revealed those lacking sub0145 (Lbp), sub1095 (SclB) and sub1154 (a protease with similarity to the C5a peptidase of other streptococci) were attenuated in cattle. The remaining mutant strains were able to induce clinical mastitis at a similar frequency to the wild type. These data demonstrate that at least three sortase anchored proteins appear to play an important role in pathogenesis of *S. uberis* infection within the lactating bovine mammary gland.

Discussion Of the proteins encoded by genes shown to be regulated by Vru, only Lbp (sub0145) and SclB (sub1095) were shown to impact virulence; the absence of other coding sequences encoding putative virulence determinants that were down-regulated in this strain have been shown not to alter virulence. Similarly, the reduced virulence of the mutant lacking SrtA was probably, in part attributable to the failure to anchor Lbp (sub0145) and SclB (sub1095) and putatrive C5a peptidase (sub1154) at the bacterial surface. In conclusion, this study has identified 3 proteins located on the surface of *S. uberis* that appear the play an important role in pathogenesis. In two instances, the role of these proteins was indicated from two distinct routes. The precise biochemical function of these proteins during pathogenesis is yet to be determined.

The data presented in this review has been presented in the following publications: Egan, *et al* (2012). Microbiol. 158:1581; Leigh, *et al* (2010). Vet. Res. 41:63 and Egan, *et al* (2010). J Prot. Res. 9:1088.

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