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The effect of herbage allowance on the day-time preference of dairy cows to be indoors or at pasture

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Introduction There are several factors that influence dairy cow motivation for access to pasture (Charlton et al., 2011). In light of increasing interest in continuously housed dairy systems it is important to understand these factors in order to ensure that both welfare and production are not being compromised. The aim of this study was to determine whether herbage allowance (3000±200kg DM ha^{-1}-high or 1800±200kg DM ha^{-1}-low) had an influence on dairy cow preference to be indoors or at pasture.

Material and methods The preference of mid-late lactation Holstein dairy cows (n=16) for high/low pasture or being indoors (free-stall housing with mattresses; 1.5/cow) was tested during July-September 2011. Cows were randomly allocated into two groups of 8; each group of 8 was further divided into two groups of four, and tested during two study periods for 6 weeks each. In order to test cow motivation for access to pasture, each treatment was offered at two distances (38m-near or 254m-far). A Latin square was used to allocate each group to the order of low/high herbage and near/far distances. Cows were given 8 day training periods with access to both herbage allowances (high vs. low) followed by 5 days of measurement for each treatment. During measurements cows had free access to either being indoors or at pasture with a low herbage allowance or being indoors or at pasture with a high herbage allowance. Cows had *ad libitum* access to a Total Mixed Ration (TMR) (40.7% DM, 12% MJ of ME/kg of DM, 17.5% CP, 35.6% NDF, 23.4% starch & sugar, and 4% oil) indoors via Calan gates allowing individual cow TMR intake to be recorded. Overall time spent outdoors and behavioural activity during daylight hours was recorded. Behavioural activity during daylight hours was analyzed using a two-way Analysis of variance in GenStat (13th edition; Lawes Agricultural Trust Co. Ltd., Rothamsted, UK) in order to determine effects of herbage allowance and distance. The model was created to find a treatment effect, a distance effect, a treatment x distance effect, and blocked by cow group.

Results Cows spent a greater percentage of their time at pasture than indoors during daylight hours when given access to the near pasture (73.7% vs. 28.8%, $F_{1,9}$=21.94, p=.001), and this was not influenced by herbage allowance ($F_{1,9}$=0.36, p=0.564). Additionally cows that had access to high herbage grazed more (18.4% vs. 11.1%, $F_{1,9}$=5.82, p=0.039) than cows that had access to low herbage, and this was not influenced by distance to pasture ($F_{1,9}$=4.13, p=0.073). Lastly, TMR intake was not affected by distance to pasture ($F_{1,9}$=0.39, p=0.546) or herbage allowance ($F_{1,9}$=0.21, p=0.660).

Table 1 Effect of herbage allowance (3000±200kgDM-high or 1800±200kgDM-low) and distance to pasture (38m-near or 254m-far) on behavioural activity during daylight hours.

Behavioural Activity During Daylight Hours	Herbage		P value	Distance		P value	s.e.m	P value herbage x distance
	high	low		near	far			
Time spent on pasture (%)	54.1	48.3	P=0.564	73.7	28.8	**P=0.001**	6.78	P=0.972
Time spent indoors (%)	35.7	39.4	P=0.619	23.9	51.2	**P=0.004**	5.06	P=0.857
Time spent on track (%)	10.2	12.3	P=0.562	2.5	20.0	**P<0.001**	2.42	P=0.637
Time spent lying (%)	44.2	44.2	P=0.985	52.1	36.3	**P<0.001**	1.91	P=0.549
Time spent standing (%)	51.7	50.8	P=0.794	44.5	58.0	**P=0.002**	2.24	P=0.462
Time spent walking (%)	4.1	4.9	P=0.452	3.4	5.6	P=0.068	0.75	P=0.476
Time spent eating TMR (%)	14.8	17.8	P=0.190	13.0	19.6	**P=0.013**	1.50	P=0.987
Time spent grazing (%)	18.4	11.1	**P=0.039**	17.8	11.6	P=0.073	2.15	P=0.763
Time spent drinking (%)	1.2	1.3	P=0.655	0.8	1.6	**P=0.026**	0.22	P=0.079
Time spent ruminating (%)	26.5	27.5	P=0.565	31.0	23.0	**P=0.001**	1.19	P=0.468
Time spent idling (%)	39.2	42.3	P=0.395	37.3	44.1	P=0.086	2.50	P=0.439
Avg. daily TMR intake (kg)	46.8	47.3	P=0.660	47.4	46.7	P=.546	0.71	P=0.081

Conclusions These results support previous findings that distance influences day-time preference for pasture, but suggest that herbage allowance may not be a driving factor in dairy cow preference to be at pasture. Additionally TMR intake is maintained irrespective of distance to pasture or herbage allowance.

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002

The behaviour of continuously housed cows given free choice to access an outside environment

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Introduction A "sustainable intensification" in food production is needed to meet future requirements for an increased population (Foresight 2011). Grazing-based systems for milk production limit annual milk yields compared to feeding nutritionally balanced total mixed rations (TMR). To achieve dietary inputs required to sustain high levels of milk production and allow tighter control over management inputs more cows in future may be managed in continuous housing systems (CHS). Although there is no indication that animal behaviour in CHS as measured by conventional means is compromised (Haskell 2005) there is concern that a shift to more intensive systems of dairy cow management may limit the ability of the cow to express normal behaviour. The purpose of this study was to investigate the behaviour of continuously housed cows given free choice to access an outside environment while fed a TMR indoors, and some of the factors influencing their choice of indoor versus outdoor environments.

Material and methods The study was conducted with an average of 27 high yielding lactating Holstein dairy cows. Cows were housed in one of two systems in separate buildings: cubicles or a straw yard. Between the morning (06:00h) and afternoon (16:00h) milking cows were allowed a free choice of either their indoor housing or a pasture. The pasture provided no significant grazing and was located within 50m of the indoor feed barrier where the same TMR was fed *ad libitum* to cows in both systems. The free choice routine was implemented ten days before observations of behaviour began, which were taken using an instantaneous scan sample at 12:00 and 14:00 h on 58 days between 06th April and 25th August 2011. Observation days and times were chosen so there were no external influences over cow behaviour from routine management operations. Numbers of cows indoors and outdoors, cow activity, temperature and weather conditions were noted at each observation. Observations were categorised into site (cubicles or straw), external temperature (<17°C, >17 to 20°C, >20 to 23°C, >23 to 27°C or >27°C) and quartiles by date of the observation period and were analysed statistically using the GLM procedure of SPSS 17.0 and a model testing effects of sampling time, site, temperature, and quartile of measurement on the proportion of cows outside and other activities.

Results and Discussion During the study access to pasture was provided for cows in two different housing systems while all their nutrient requirements were met by an indoor fed TMR. Sampling time did not affect (P = 0.825) behaviour patterns. There was an effect of housing type on environment choice, with 55% of cubicle and 71% of straw yard housed cows (P<0.001) preferring outside activities overall. This level of outside activity is higher than that observed by Legrand *et al* who reported that less than one-third of cows chose to go outside between morning and evening milkings and Charlton *et al* who observed that less than 10% of cows chose to spend time outdoors. Legrand *et al* observed that behaviour was influenced by daytime temperature and in the current study temperature tended to have an influence on behaviour (P<0.082), where 77% of cows engaged in outside activity at <17°C falling to 51% of cows at >27°C (Tukey HSD P<0.05). Average inside temperature was recorded as 18.6°C for cubicles and 20.4°C for straw yard (P<0.001). External temperatures were the same for both sites and this smaller differential between external and internal temperatures for the straw yard may have influenced activity choice. The seasonal timing of observations appeared to have a strong influence over behaviour where 79, 71, 61 and 40% of cows in quartile 1,2,3 & 4 respectively engaged in outside activity. This effect was confounded with changes in temperature (Figure 1), but the effect of quartile was highly significant (P<0.001) with outside temperature included in the statistical model as a covariate (P<0.02). There was no clear influence of weather on behaviour (P = 0.364).

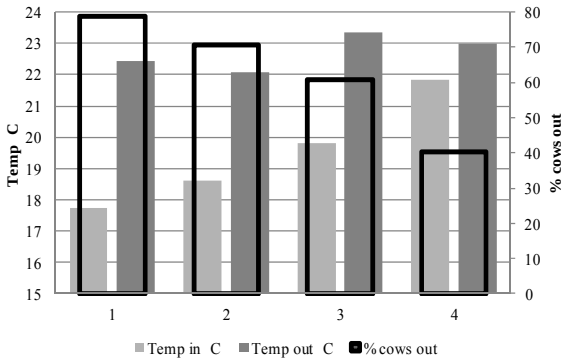

Figure 1 Average internal and external temperatures (°C) and cow outside activity (%) across study quartiles.

Conclusions The current study indicated, on average, that when given a free choice, over 55% of cows engaged in outside activities. The cubicle housing provided cooler internal temperatures and more indoor activity was observed with this housing system. As external temperatures increased housing preference shifted to indoors with only 51% of cows choosing to remain outside at temperatures >27°C. This suggests that if housing conditions could be optimised to suit the cows requirements then CHS would have less impact on normal behaviour. The study indicated that when attempting to quantify cow behaviour in terms of housing preference, the type of housing, season and temperature during the period of observation will have an influence cow behaviour.

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003

What do handling temperament tests tell us about home pen activity in beef steers?

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Introduction Ever since territorial sticklebacks were found to be more aggressive to predators (Huntingford, 1976), the study of animal personality has been concerned with the consistency of responses across contexts. Accelerometer-based activity monitors (often called pedometers) can be used to record behaviour in cattle by calculating lying times, number of steps taken and general activity levels. As such, it is possible to monitor the activity of animals in the home pen continuously and unobtrusively. Crush score (CS), as measure of the animal's reactivity to handling, is assessed by rating the animal's activity when confined in a crush. Flight speed (FS) is the speed at which the animal leaves the crush after handling. Both are often found to be correlated with average daily gain and so perhaps these animals spend more energy responding to stimuli in general. However, it is unknown if these handling temperament tests are indicative of a heightened level of activity in the home pen. In this study, we investigated the relationship between home pen activity and the temperament measures CS and FS in 69 housed cross-bred beef steers.

Material and methods 69 Aberdeen Angus x Limousin steers with an average age of 474±16 days were housed in four groups, balanced for weight for the trial period of 57 days. Each group was housed in an identical pen and fed a barley-based diet *ad libitum* with or without a yeast-based probiotic and a partial glycerol supplement. Diet had no effect on temperament measure or activity. The activity monitors were IceTag Pros (IceRobotics Ltd, South Queensferry, Edinburgh, UK). The animals were CS and FS tested four times at two week intervals during the trial. To obtain a CS score the same observer rated each animal on a scale from 1-6 based on the animal's reaction to being held in a crush with 1 indicating a calm animal and 6 indicating an animal which is dangerous to approach. FS was measured in ms^{-1} using a light gate over a 4m distance upon exiting the crush. Within each pen, half the animals carried IceTags from Days 1-14 and Days 29-42 and the other half were tagged on Days 15-28 and Days 43-53. Any days with partial or interrupted data, e.g. weigh days or tag removal days, were removed from the dataset, as were the first two tagged days of each period to allow for the time required to habituate to wearing the devices. We calculated the average daily step count, lying time and standing time (STEP-COUNT, LIE-TIME & STND-TIME) using IceTagAnalyser. We also calculated the mean length of a lying bout (LBOUT) and standing bout (SBOUT) using a Fortran script, and the average daily number of lying bouts (DNLB). MotionIndex™, a proprietary measure of IceRobotics which describes the average acceleration recorded over a given period and is a proxy measure of energy expenditure was examined as a daily average (MI) and average steps taken per minute standing (STEP/MIN). Repeatability of traits was calculated using a variance components method and quadratic regression analyses as in Müller & Von Keyserlingk (2006) were applied to describe the relationship between FS_{mean}, CS_{mean} and activity in Minitab(15^{th} Edition, Minitab Inc, 2006)

Results CS and FS were repeatable at 0.36 and 0.48 respectively. Descriptive statistics for all traits and the results of the quadratic regressions are shown in ***Table 1***. The strongest of these was the relationship between STEP-COUNT and FS (R^2=0.14, P=0.02), and similarly MI and FS, which were positive. The relationship was quadratic with a high effect of lower FS values on STEP-COUNT There were no lame animals. Between the activity traits there was a negative linear correlation between SBOUT & DNLB (R^2 = 0.40, P<0.001). Animals with shorter lying and standing bout lengths tended to take more steps per standing bout (SBOUT R^2=0.16, P<0.001, LBOUT R^2=0.16, P<0.001). Short standing bouts were associated with short lying bouts (R^2=0.25, P<0.001).

Table 1 Characteristics of standard and novel activity traits in beef steers and relationships with temperament measures.

Trait	STEP-COUNT	LIE-TIME (mins)	STND-TIME (mins)	MI	DNLB	LBOUT (mins)	SBOUT (mins)	STEP /MIN
Mean ± S.D.	1144 ±294	881.2 ±81	554.9 ±79.9	3543 ±1056	13.28 ±3.42	71.23 ±12.94	46.45 ±12.34	2.062 ±0.44
Repeatability	0.29	0.48	0.51	0.16	0.47	0.67	0.70	0.26
Relationship with CS_{mean}	P=0.704	R^2=0.06 P=0.016	R^2=0.04 P=0.029	P=0.723	P=0.726	P=0.523	R^2=0.02 P=0.092	R^2=0.03 P=0.066
Relationship with FS_{mean}	R^2=0.14 P=0.020	P=0.441	P=0.484	R^2=0.13 P=0.041	P=0.650	R^2=0.01 P=0.224	P=0.565	R^2=0.06 P=0.049

Conclusions This is the first study to link temperament and home pen activity in cattle, showing that animals with a low FS tend to take fewer steps in general. The long-term measurement of activity traits may help to explain the link between temperament measured in short-lived handling tests and traits measured over the course of months, such as average daily gain. More complex models of activity may enable researchers to identify reactive animals without subjecting them to handling stress.

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004

The temporal changes in behaviour of beef bulls during exposure to *Ostertagia ostertagi* and subsequent recovery from infection

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Introduction Health challenges can cause losses in performance and impair animal welfare, especially in the case of a subclinical infection as these often go undetected. The early detection of health challenges will improve animal welfare, treatment effectiveness, and reduce costs. As behaviour is known to be affected by the health status of an animal, an early diagnosis by monitoring changes in animal behaviour to indicate the early onset of disease may be achieved (González *et al.,* 2008). In this study, the temporal changes in behaviour that could be used as health challenge indicators during a parasitic (*Ostertagia ostertagi*) infection in beef cattle were quantified. The rate of change in the recovery of behaviour after the challenge was interrupted was also investigated. The hypotheses for this study were that: (i) behavior would change gradually with progression of the infection and (ii) it would quickly recover to pre-challenge levels after the infection was interrupted by anthelmintic treatment.

Material and methods Twenty-six Holstein-Friesian cross beef bulls of approximately 3 months of age were randomly allocated to one of four groups: two control groups of 6 and two parasitized groups (P and PI) consisting of 7 animals each. *O. ostertagi* L3 larvae were given to the parasite treatment bulls by gavage as a trickle dose spread over the first three weeks, 100,000 L3 on Days 0, 7 and 14. The control groups received a water gavage on these days. On Day 32, the interrupted parasite treatment (PI) and one of the control groups were drenched with an anthelmintic. Bulls were weighed twice a week and faecal egg counts (FEC) were taken on the same days starting from Day 0. Blood samples, to measure pepsinogen concentration, were taken once a week. An activity sensor (Icetag) was fitted to the front leg of each bull to record activity and posture. The experiment was concluded on Day 44 post first infection. Lying and standing bouts were identified according to Tolkamp *et al.* (2010). A water meter was used to monitor group water intake. As there were no differences between the two control groups, these were combined during analysis. The results were analysed using a repeated measures ANOVA, after transformation of the raw data if these were not normally distributed.

Results There were positive FEC from Day 17 onwards in parasitized bulls; peak FEC was 638 eggs/g on Day 42. Parasitized animals had elevated pepsinogen levels by Day 21 which remained elevated up to Day 44. Average daily weight gain differed (P<0.001) between treatments, with the P treatment showing the lowest daily gain (0.365 kg/day, SE 0.20), followed by PI animals (0.56kg/day, SE 0.20) compared to the control groups (1.29 kg/day, SE 0.07) (Figure 1). From Day 21, the average duration of lying (P<0.001) and standing (P=0.013) bouts were longer for the parasitized animals (Figure 2) as was the total daily time spent standing (P=0.03). The frequency of lying and standing bouts (P<0.001) was lower in parasitized than in control bulls. Overall activity as measured by the number of steps taken on a daily basis, showed a treatment by time interaction (P<0.001) due to a lower activity from Day 21 for the P and PI animals. The posture and activity of PI animals returned to control levels within 5 days of receiving the anthelmintic.

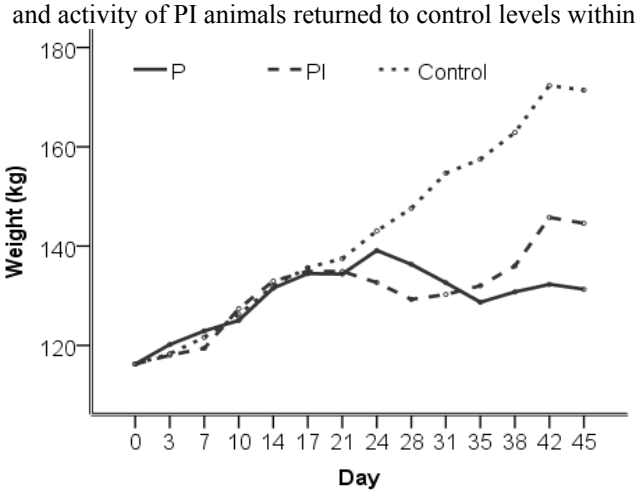

Figure 1 Live weight (in kg) for parasitized (P), interrupted (P), parasite treatment (PI) and an uninfected control.

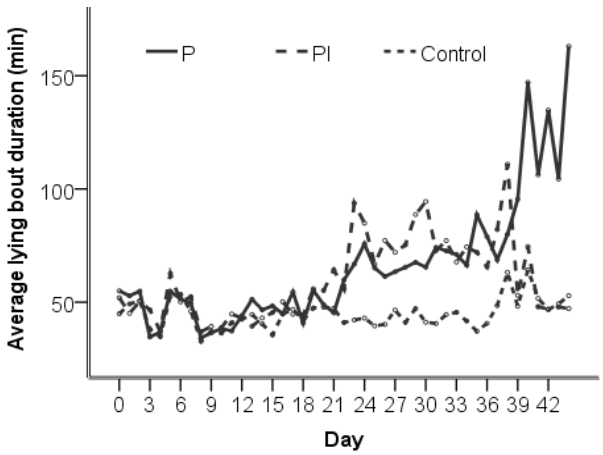

Figure 2 Average lying bout duration (in min) for parasitized interrupted parasite treatment (PI) and an uninfected control.

Conclusion Persistent behavioural changes in posture and activity following infection were rapidly reversed after the challenged bulls were treated with an anthelmintic. The performance changes due to parasitism, such as in weight, were also reversed by anthelmintic treatement but at a slower rate. Within the duration of the trial, there was no natural reduction in the level of parasitic infection.

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005

The topical anaesthesia welfare revolution on wool sheep farms in Australia: can xylazine assist?

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Introduction Mulesing is the surgical removal of perineal skin from lambs to provide life-long protection against the risk of 'breech strike' caused by *Lucilia cuprina*, the sheep blowfly inadvertently introduced well after the skin 'wrinkle' phenotype was widely dispersed in the Merino population in Australia. The 'Mules operation' is now only done by accredited contractors, but until recently it was performed without analgesia causing significant global welfare concerns. However estimates are that 2million sheep would die annually of flystrike if the operation were banned. The breeding of sheep less susceptible to flystrike is continuing but in the interim, a farmer applied spray-on formulation containing two topical anaesthetics (TA) administered in the immediate post-operative period was introduced in late 2005 and made available on permit by APVMA (the Australian agency for drug registration) for sale through veterinarians (Trisolfen®, Bayer Animal Health, Australia). Research confirms this intervention significantly reduces wound pain-related behavior scores for the first 8hrs (Lomax *et al*, 2008) and up to 24hrs following mulesing (unpublished observations) compared with placebo gel treated (P=0.03), and untreated mulesed lambs (P<0.001). It is also efficacious for lamb castration (Lomax *et al*, 2010). TA has been readily adopted by Australian Merino producers with estimates that 60-70% of mulesed lambs received treatment in 2011 when the product achieved registration. However should lambs also receive analgesia prior to the marking procedure? We have been examining the efficacy of low dose intramuscular xylazine treatment in lambs prior to the spray-on topical anaesthesia administered at surgery as this has been shown previously to provide analgesia in treated lambs (Grant and Upton, 2004).

Material and methods Female Merino weaned lambs (n = 44) aged 6-8months and run on pasture on a commercial wool property were randomly allocated into 3 treatment groups:
Control (n=6) sham mulesed by hand manipulation of the breech;
Untreated (n = 16) mulesed and receiving TA at surgery only; plus
Treated (n=22) mulesed and receiving TA at surgery plus IM xylazine (0.1mg/kg) administered 15-20mins prior to mulesing.
Wound pain was assessed by two independent observers recording either central cognition or peripheral nerve reflex responses to the mulesing procedure, using a Numerical Rating Scale (NRS) with ratings designated as 0, 1, 2 or 3. The NRS responses between groups was analysed by an ordinal logistic regression proportional odds model as previously published (Lomax *et al*, 2008).

Results Although both the 'Untreated' and 'Treated' groups displayed significantly higher NRS scores that the 'Control' or sham mulesed animals (Figure 1), the xylazine 'Treated' animals did have significantly reduced pain scores compared to the 'Untreated' group (P < 0.001).

Conclusion Mulesing is a painful procedure reluctantly performed by many wool producers to improve the life-time welfare of their Merino sheep. TA considerably reduces the welfare burden of the procedure and has been enthusiastically adopted by a majority of producers prepared to pay between AUD0.05-0.90 for better sheep welfare. Adoption of TA has occurred voluntarily and in the absence of direct financial returns as there is rarely a premium paid for wool from farms using TA for mulesing. Our studies with xylazine in addition to TA appears to offer a superior welfare outcome than TA alone, although many issues including the safe use of injectibles on farms (Windsor *et al*, 2005) need to be addressed prior to wider application of this approach. However at a meeting of many sheep industry, welfare and other stakeholders at APVMA in November 2011, there was consensus for continuation of this research on xylazine.

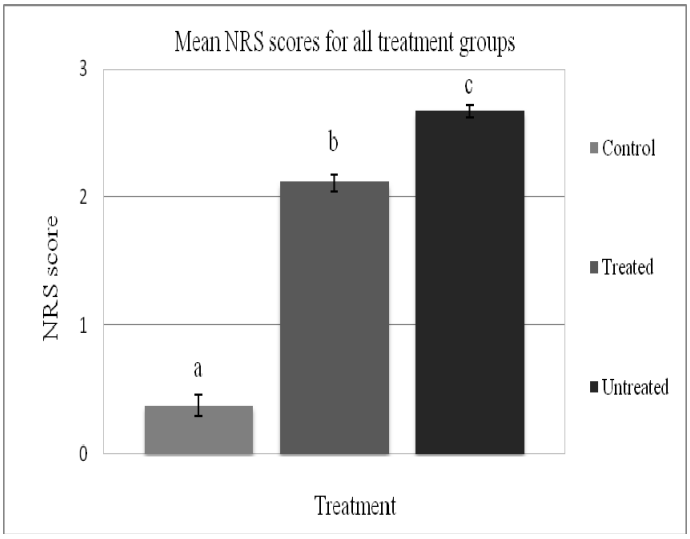

Figure 1 NRS scores for 3 groups of lambs at mulesing

Acknowledgements This work was funded by the Australian Research Council and conducted under approval of the University of Sydney Animal Ethics Committee.

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006

The effect of radio on the welfare and behaviour of sows and their piglets in farrowing crates

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Introduction There are few studies investigating audio enrichment of pigs and there are contrasting findings reported. Cloutier *et al*. (2000) reported that playing music to piglets during stressful events had no significant effect, whereas de Jonge *et al*. (2008) suggested that music could stimulate play behaviour and consequently decrease aggression, which is beneficial to welfare. This study investigated what effect playing radio during the daytime would have on the behaviour of sows and their piglets during the 3 weeks spent in a farrowing crate and if it could improve their welfare. The study also investigated the potential of radio acting as a buffer to negative noise and having a positive effect on sow reaction response towards humans.

Material and methods 32 sows and their litters were housed (4 sows per room), in either a control (C - without radio) or enriched environment (R - with radio tuned to a pop music/chat station). Behaviour observations were carried out on 16 sows and their litters once per week (8 per treatment/ 2 per room) for 10 h (07:00 to11:00 h and 13:00 to 19:00 h) over 3 consecutive weeks using CCTV cameras. Behaviour and posture of sows and the behaviour of piglets were recorded using 5 minute instantaneous scans. Additionally, the total number of aggressive events (biting, head knocks, pushing) and play (scamper, head toss, chase without bite) were recorded. A sow reaction response to a person (KL dressed as farm staff) entering the room was tested twice a week over 3 weeks on all 32 sows (4 per room), based on the method of Hellbrügge *et al*. (2009). Briefly, this is a 5 point scale, with the highest score indicating the greatest sow response to the observer. For statistical analysis, sow behaviour was calculated as a percentage of observations for each day (out of 122 scans), as was piglet behaviour (number of scans dependent on litter size) and was analysed by week using Mann-Whitney U-test for Radio vs Control. Piglet play and aggression and average week score for sow response were normal in distribution and analysed using a repeated measures ANOVA with a Greenhouse-Geisser correction, blocked by room.

Results Piglets from litters given the Radio enrichment treatment consistently played more than those on the Control treatment ($P<0.001$), although play significantly increased for both treatments during the study ($P<0.001$, shown in Figure 1). Piglets from both treatments spent most of their time in the covered creep area (49.7 vs. 51.4 ± 5.46, radio vs control, mean per cent observations with SED), with similar levels of lying (20.0 vs. 20.9 ± 5.46, R vs. C) and movement (9.5 vs. 11.9 ± 1.11, R vs. C). Sows from the Radio treatment showed significantly less sitting (2.6 vs. 5.0 ± 0.96, R vs. C, $P<0.05$) and tended to show less abnormal behaviour (0.6 vs. 2.3 ± 0.78, R vs. C, $P=0.06$) compared with the Control sows, but these were at low levels of behavioural performance. The most marked difference was the increased level of nursing seen in the R sows during the observation period compared with the C group (40.8 vs. 33.5 ± 2.54, R vs. C, $P<0.01$) that was associated with increased suckling seen in their piglets. R Sows consistently showed a reduced response to an observer compared with C sows for all 3 weeks studied ($P< 0.05$ - Figure 2).

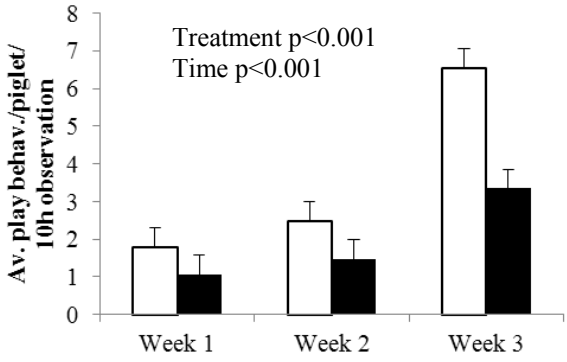

Figure 1 Play behaviour for piglets given enrichment using a radio or control treatment (mean + SED)

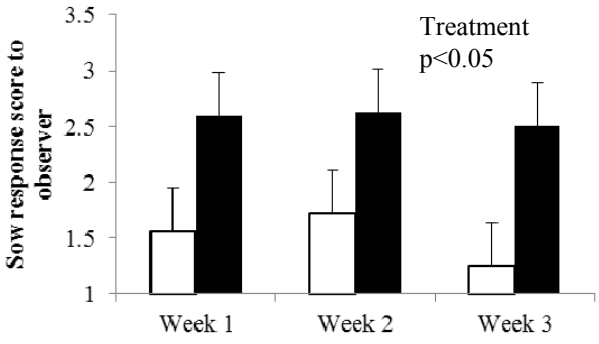

Figure 2. Responsiveness of sows to an observer after enrichment using a radio or control treatment (means + SED)

Discussion Playing a radio in a farrowing room appeared to be beneficial to the welfare of both the sows, indicated by reduced sitting and abnormal behaviour (both signs of poor well-being), and the welfare of piglets, as indicated by their increased play. Audio enrichment may provide a stimulus that reduces the boredom of confinement conditions, reducing the sow reactivity to a human and may also stimulate the higher activity levels of piglets playing. This study only observed the sows for 10 hours of daylight, but the increased nursing in the Radio sows compared to the Control sows was quite marked, perhaps stimulated by increased piglet activity levels, and may have production implications. Further studies would include measures of piglet performance and a 24 h observation.

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007

Evaluation of nest design and nesting substrate options for the PigSAFE free farrowing pen

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Introduction The farrowing crate is the most widely used housing system for sows during parturition and lactation, but raises significant concerns about sow welfare, particularly in relation to the restriction of nest building behaviour. In typical commercial systems, this arises from both restriction of space and absence of nesting substrate (Jarvis *et al*., 2002). The adoption of free farrowing systems which permit expression of nest building behaviour has been prevented by concerns about piglet survival in the absence of the physical protection provided by the crate and the management benefits which it offers. A prototype, free-farrowing pen (PigSAFE – Piglet and Sow Alternative Farrowing Environment) has been developed in an attempt to reconcile these conflicting concerns. The design was based on an extensive review and analysis of the biological needs of the animals (Baxter *et al.*, 2011), which identified that some features of design and management required further research to determine the optimal choices. In this experiment, the possible benefits of a more enclosed and quieter nest area, and the amount of substrate provided for nesting behaviour, were evaluated.

Material and methods Twelve farrowing pens, in 3 rooms of 4 pens, were constructed to the same basic PigSAFE design, with a nest area with straw and piglet protection features, a heated creep, a slatted dunging area and lockable sow feeder, in a total pen area of 7.7m^2. Two nest designs, and two levels of straw provision prior to farrowing were tested in a 2 x 2 factorial design with 25 litter replicates over a one year period. Half of the pens were fitted with noise suppression material lining the nest walls and a sound insulated roof (QUIET), with the remaining pens serving as controls (CONT). Digital recordings of piglet screams were used to test the sound attenuation of insulation material at different points in the pen. A 10 decibel reduction in noise, reducing acoustical energy by 90%, effectively made noise in the QUIET pens sound half as loud. The substrate treatment compared a minimum amount of substrate (MIN), comprising 2 kg of long stemmed straw provided at entry on day -5 and replenished as necessary to maintain this level until day +2 after farrowing, with a more generous straw allowance (MAX) of 4 kg. Continuous video recordings were made over the farrowing period and standard production records taken on sows and piglets. Data were analysed by two-way analysis of variance using the GLM procedure in Minitab 15, with covariates applied where appropriate.

Results Neither nest design or substrate level affected the farrowing location and orientation of the sows. Only 1 sow farrowed outside the nest and 69% of all piglets were born with the sow facing the nest entrance and with the birth site and udder in close proximity to the heated creep. High litter sizes and limited fostering possibilities challenged piglet survival. There was no main effect of treatments, or interaction between treatments, on total piglet mortality (stillborn pigs plus those subsequently dying before weaning) or on mortality of liveborn piglets (Table 1). Sow feed intake, lactation weight loss and litter weight at weaning were also unaffected by treatment. The higher level of straw posed problems for manure management, with significant amounts entering the liquid manure storage channel under the dunging area.

Table 1 Performance of litters in a free farrowing pen with different nest design and substrate provision

	Nest treatment		Substrate treatment			Significance	
	QUIET	CONT	MIN	MAX	SEM	Nest	Substr
Total litter size	14.4	14.7	14.3	14.8	0.46	0.38	0.46
Total mortality (%) †	18.2	21.2	21.5	17.9	1.61	0.19	0.12
Stillborn (%)†	5.0	5.2	5.3	4.8	0.99	0.87	0.71
Liveborn mortality (%) ‡	14.0	17.0	17.3	13.8	1.52	0.16	0.11

†total litter size as covariate (P<0.001) ‡Born alive as covariate (P<0.001)

Conclusions The overall design was successful in promoting farrowing in the desired location, irrespective of nest construction and substrate level. In the absence of a significant improvement in piglet survival, the management advantages of an open pen for better visibility and ease of working, and of lower substrate level for better manure management, suggest these to be better commercial recommendations. However, more detailed analysis of sow behaviour during the nesting and parturition period is in progress to confirm this conclusion.

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008

Assessment of kinematic gait characteristics of pigs reared on three different floor types with a single-plane stereophotogrammetric motion capture method

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Introduction Lameness is a common cause of lost productivity for the pig industry worldwide, owing to premature culling in the breeding herd and reduced growth performance in the finishing herd. It is also a significant threat to animal welfare. Lameness may arise from poor conformation, lesions in hoof or integument and other disorders in the musculoskeletal and nervous systems. Common methods to identify unsound animals employ subjective scoring systems. However, these require assessors with accurate diagnostic abilities and time for individual gait assessment, giving concerns about the reliability of visual gait analysis. The objective of this study was to develop a method for stereophotogrammetric capture of the sagittal-plane motion of pigs to assess development of kinematic gait characteristics when reared on either fully-slatted, part-slatted/solid concrete or deep straw-bedded concrete floors.

Material and methods 12 entire male and 12 female growing pigs (36kg liveweight at entry) were divided into groups of eight pigs and housed in one of three pens with a different floor type as described above, five weeks prior to motion capture. Pigs were trained for a period of two weeks to follow a target along a standard solid-floored runway and obtain a treat when movement was acceptable (i.e. regular, continuous and straight). After two training weeks, pigs were regularly subjected to application of reflective markers at anatomical landmarks and walking gait was filmed by 3D infrared cameras. At least two acceptable sequences of three to four strides in each direction were captured on each occasion. Pigs were weighed weekly and subjectively scored for conformation and lameness. When pigs reached approximately 90 kg they were slaughtered and the major articulations of all four legs inspected and scored macroscopically for signs of degenerative joint disease (DJD; i.e. thinning, erosions, ulceration in articular cartilage). The effect of age, weight increase and floor, and the intra- and inter-pig-variability of seven variables of gait (knee, hind pastern, elbow, front pastern angles, step length front, step length hind and walking speed) were investigated. Variation of gait within and between pigs was tested using the General Linear Model (GLM) command in an Analysis of Variance with pig as a factor. Differences between treatments within week were analysed by GLMs with significantly correlated parameters as covariates. Changes over time between two selected measurement points (weeks 5 and 12) were analysed using paired t-tests.

Results The individual pig effect on angular parameters was significant in all cases, indicating consistency of gait. The pig effect on step length of both front and hind pair of legs was significant in week 5, when walking speed also showed a significant pig effect, but these parameters were not consistent in week 12. The age effect on angular parameters was significant and positive for maximum knee angle and elbow angle (Table 1). The range of motion (RoM) at the knee joint was the same in week 5 and 12, whereas the range of motion (RoM) at the elbow joint was reduced in week 12 compared to week 5. Pig liveweight was negatively correlated with minimum front pastern angle in week 12 ($r=-0.641$, $p=0.01$), suggesting that digital flexor tendons are subject to increased passive stretching in heavier pigs during the stance phase of the gait cycle. RoM at the elbow joint was significantly smaller for pigs from part-slatted flooring compared to the group on full slats in week 5, but there were no other significant floor differences. In front legs where DJD lesions were present in the carpal joint, both maximum ($p=0.009$) and minimum ($p=0.016$) elbow angle was decreased.

Table 1 Effect of age on angular and linear gait parameters

Gait parameter	Week 5	Week 12	SED	p-value
Max knee angle (Degrees of flexion)	84.3	90.8	1.75	0.003
Max hind pastern angle (Angle pastern to floor)	112.5	115.1	1.39	0.092
Max elbow angle (Degrees of flexion)	85.7	95.6	1.91	0.000
Max front pastern angle (Angle pastern to floor)	135.6	134.7	1.79	0.609
Range of motion at knee joint	42.8	42.6	1.15	0.843
Range of motion at elbow joint	41.9	37.7	0.9	0.000
Step length front (mm)	327	367	10	0.001
Step length hind (mm)	336	369	16	0.060

Conclusions The method developed in this study is reliable in the detection of pig-specific gait patterns. Maximal sagittal flexion in the major joints of front and hind legs increases with time. Range of motion in the elbow was reduced over time, suggesting a loss of straightness in the front leg. Time spent on different floor types resulted in few differences in gait parameters when tested on standard flooring to study possible effects on musculoskeletal soundness. Conformational deficiency and the presence of DJD lesions may be reflected in differences in gait parameters.

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009

Effect of forage type and an extruded linseed supplement on methane production by lactating dairy cows

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Introduction There is currently considerable interest in developing management practices to reduce methane emissions attributable to milk production. Numerous dietary strategies are known to reduce dairy cow methane production or methane yield (methane per unit feed dry matter intake [DMI]). As regards carbohydrate type, previous studies have shown that replacing fibre with starch and replacing grass silage (GS) with maize silage (MS) reduces methane yield (Mills *et al*., 2001; Reynolds *et al*., 2010). In addition, a variety of supplemental dietary lipids reduce methane excretion in cattle (Beauchemin *et al*., 2008), with oils containing longer chain polyunsaturated fatty acids shown to be particularly effective (Martin *et al*., 2008). The objective of the present study was to determine if an extruded linseed supplement (ELS) will reduce methane production in lactating dairy cows, and if the response was affected by dietary forage type (MS versus GS).

Method Four mid-lactation Holstein-Friesian dairy cows were fed *ad libitum* total mixed rations consisting of a 50:50 mixture (dry matter [DM] basis) of forage:concentrate, with the forage comprised of either 25:75 or 75:25 MS:GS (DM basis). Concentrates were formulated without and with ELS at 50g/kg diet DM (264 g oil/kg DM) in a 2 x 2 factorial experiment. Diets were formulated to be isonitrogenous (16% crude protein). Cows were randomly assigned to treatments within a 4 x 4 Latin Square design with 4 week periods. Measurements of respiratory exchange and milk composition were obtained over 4 days in the last week of each period. Milk yield and DMI were recorded daily. Average rates for each cow and period were statistically analyzed using mixed model procedures for fixed effects of period and treatment and random effects of cow, with period as a repeated effect. Treatment by period interactions were not significant and were removed from the model used for the final analysis of data.

Results Feed DMI tended to by higher for MS than for GS, but was not affected by ELS (Table 1). Dietary forage type and ELS had no significant effects on milk yield or milk composition. methane production, methane yield (L/kg DMI), or methane per kg of milk. There were no significant interactions between forage type and ELS.

Table 1 Effect of extruded linseed supplementation (ELS) and dietary forage type on DMI, milk yield, milk composition, and methane production by lactating dairy cows.

	Treatment				s.e.m	*P*		
	MS	MS-ELS	GS	GS-ELS		Forage	ELS	Forage*ELS
DMI, kg/d	20.3	21.2	19.2	19.7	1.1	0.094	0.310	0.712
Milk yield, kg/d	36.1	37.4	35.7	35.4	1.13	0.358	0.709	0.519
CH_4, L/d	598	580	567	553	35.0	0.274	0.520	0.939
CH_4, L/kg DMI	31.7	30.2	29.3	28.6	2.08	0.312	0.554	0.814
CH_4, L/kg milk	16.5	15.5	16.1	15.7	1.09	0.878	0.391	0.719
Milk fat, g/kg	33.0	33.6	38.9	34.1	3.4	0.223	0.400	0.300
Milk protein, g/kg	31.6	32.1	32.3	31.8	0.5	0.609	0.955	0.200

Conclusions Contrary to previous reports, dietary forage type had no affect on methane yield. In the present study starch concentration of the MS diets was higher (200 vs 153g/kg DM), and NDF concentration lower (361 vs 383 g/kg DM), than the GS diets. However, the GS fed was from a sward of Italian ryegrass with a high D value (74.6%), whilst in previous studies in our laboratory GS from perennial ryegrass was fed (Reynolds *et al*., 2010). Thus in addition to carbohydrate content, carbohydrate quality can also be important in determining methane yield. The ELS inclusion rate used in the present study was twice that recommended in commercial practice, but the amount of supplemental oil provided did not measurably affect methane production or yield. In previous reports an ELS similar to the ELS fed in the present study significantly reduced methane production and yield, but the dietary inclusion rate was approximately 4 times higher than in the present study (Martin *et al*., 2008). In conclusion, feeding approximately 1 kg of ELS to lactating dairy cows had no effect on methane production.

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010

Development and preliminary testing of an animal mounted sensor system to estimate methane emission from cattle

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Introduction Methane (CH_4) is the second most important greenhouse gas (GHG). Enteric fermentation in ruminant livestock is one of the major sources of CH_4. Many factors influence ruminant CH_4 production, including level of intake, type and quality of feeds, genetics and environmental temperature (Shibata and Terada, 2010). To identify appropriate feeding, management and breeding strategies for mitigation of CH_4 emission from ruminants, it is important to identify non-invasive methods that can accurately estimate CH_4 emissions particularly from grazing animals. The aim of this work was to develop and test a direct measurement system to estimate respiratory and eructed CH_4 using sensor technology mounted on halters.

Material and methods The initial approach identified sensor modules that were both sensitive enough to measure eructed CH4 concentration, and sufficiently compact and convenient to be mounted on-animal for lengthy periods. The initial sensor modules chosen had a lower detectable limit of around 100ppm CH_4. The respiratory gas-capture design incorporated a multi-hole sample tube adjacent to, and mounted above, the nasal area. The sample tube carried the sample gas stream back to the sensor modules using a miniature air pump. The whole system was self-powered using battery packs. Sensor modules were linked wirelessly to a personal computer. A pilot experiment was conducted on 2 steers, located in two individual respiratory chambers, and CH_4 output was measured continuously for up to 24h. The halter-mounted sensor system recorded CH_4 concentration measures for each steer along with the relevant time stamp every 5 seconds. Chamber data were recorded every 4 min. The time recorded in the chamber was matched with that of the sensor data. Sensor data were then summarised as spot (time matched), mean and median (for each previous 4 minute time interval). Because of the time lag between sensor and chamber records, autocorrelation and cross-correlation coefficients were estimated for different lags (up to 40 min) and corresponding plots assessed to investigate the association between chamber and collar data. Due to perceived issues with measurement during animal feeding activity, data were truncated to night records only, to assess what the effect would be on cross-correlations.

Results The magnitudes of autocorrelation coefficients declined with increasing lags. From lag 0 to 40 minutes, the estimates ranged from 0.91 to 0.72 and 0.58 to 0.42 for chamber 1 and collar 1 median data respectively. All estimates were different to zero ($P<0.05$) for both chamber and collar data. The estimates of cross-correlation coefficients between collar (spot, median and mean) and chamber CH_4 data also decreased with increasing lags (Figure 1). The estimates of cross-correlation coefficients between chamber 1 and median collar 1 ranged from 0.45 to 0.32 from lag 0 to 40 minutes. Spot and mean data also showed similar trends. The data for chamber 2 comprised a discontinuous time-series, hence only cross-correlation of chamber data with spot collar data with no lag (0.34) was relevant. All estimates were different to zero ($P<0.05$). Estimates of cross-correlation at lag 0 between chamber 1 and spot, mean and median collar data increased when a subset of rolling mean data (segment size of 5) were considered (cross-correlation coefficients 0.73, 0.79 and 0.80 respectively). Chamber 1 and median collar data comparison is presented in Figure 2.

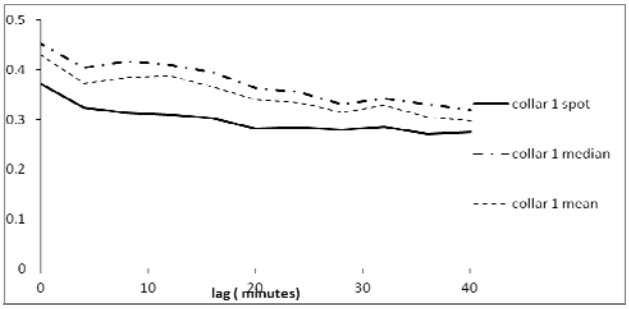

Figure 1 Cross-correlation coefficients between sensor (spot, median and mean) and chamber 1 CH_4 at different time delays

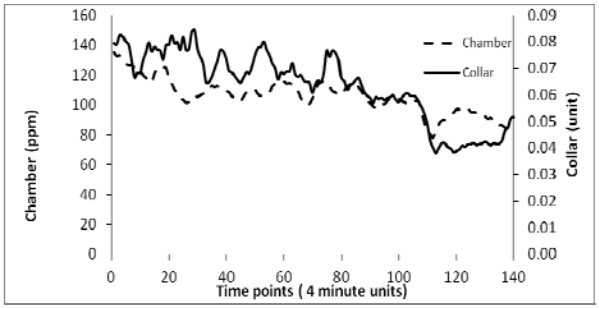

Figure 2 Comparison of 5 segment rolling mean chamber 1 and collar median for data sub-set

Conclusions This preliminary trial showed statistically significant evidence of association between the sensor and chamber data for CH_4 concentration, albeit in an elevated CH_4 environment. Feasibility of automatic wireless transmission of data from animal to data collection device was established. Further trials need to be undertaken to establish the accuracy of data collected by collar sensors, and whether the inclusion of other gas measures could assist in quantifying methane production rate in an uncontrolled environment

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011

Genetic improvement of Australian Angus cattle for lower methane emission

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Introduction Methane is a potent greenhouse gas produced by cattle and sheep as a natural by-product of the fermentative digestion of plant material in their rumen. Breeding beef cattle with naturally lower methane emissions is an attractive option to reduce emissions in extensive grazing systems. Methane yield (MY), defined as the amount of methane produced per unit of feed consumed, is used to compare animals for the amount of methane they produce relative to their feed intake. This report presents results for methane test traits for young Angus cattle, and for differences between sires in the mean MY of their progeny that provides preliminary evidence for genetic variation in MY.

Material and methods Methane test traits were measured on N=280 yearling-age animals tested in four groups, being Angus heifers in 2010 (N=73) and bulls in 2011 (N=70) from a northern research herd, and N=62 Angus bulls in 2010 and N=75 Angus bulls in 2011 from a southern research herd in New South Wales, Australia. The animals were adapted to the test ration for 10 days before being trucked to the methane test facilities. There, in individual pens inside an animal house, each animal had methane production measured while being fed a fixed daily allowance of a roughage diet (ca. 9 MJ ME/kg DM). The amount offered to each animal was calculated to provide 1.2-times the estimated energy requirement for maintenance based on the animal's liveweight at the end of the initial adaptation period. This was done to minimise day-to-day variation in daily methane production and to avoid 'level of feeding' effects. In 2010, methane production was measured over 5 x 24h consecutive periods using the SF_6 tracer dilution method. In 2011, methane production was measured over 2 x 24h consecutive periods in 10 individual respiratory chambers. In 2010, animals were tested as cohorts of 32 animals, and in 2011 as cohorts of 40 split into 4 groups of 10. Care was taken to ensure sires were equally represented in each cohort. Traits recorded were feed intake as DM intake (DMI; kg/d), initial liveweight (WT; kg), methane production (MP; L/d), and MY (L/kg DMI). For each group, fixed effects of sire and cohort were fitted in a general linear model, with age and weight at start of measurement fitted as covariates. The interaction of sire and cohort was not significant and not included.

Results There was variation in all test traits recorded, with a greater than 3-fold range in MY being observed (Table 1). Methane production was phenotypically correlated with DMI ($r=0.35$; different to zero at $P<0.05$) and with animal WT ($r=0.44$; $P<0.05$) reflecting that the animals with lower feed intake, and smaller, lighter animals, had the lowest MP. However, these animals might be regarded less productive and less profitable in traditional farming enterprises. Unlike MP, MY was largely phenotypically independent of DMI and animal WT (correlations being -0.29 and -0.13 respectively, both $P<0.05$). Should these relationships hold at a genetic level, they indicate that selection for low MY need not favour smaller animals and lower feed intake. Rather it should be possible to breed animals of a desired size that produce less methane from the feed they consume. There was a large range in the mean for MY of progeny of the sires of the cattle tested. Compared to the sires whose progeny had the lowest average MY in each test cohort, the sires whose progeny had the highest average MY, had average MY that were 24%, 24%, 16% and 19% higher across the four groups.

Table 1 Means and descriptive statistics for the N=280 young Angus bulls and heifers

Trait	Mean	Std Dev	Maximum	Minimum
Age at test (days)	670	67.9	774	537
Pre-test animal WT (kg)	480	76.4	670	318
Feed intake (DMI; kg/d)	7.6	1.0	9.5	4.8
Methane production (MP; L/d)	235	52.7	516	70.3
Methane yield (MY; L/kg DMI)	31.1	6.7	57.7	14.6

Conclusions Our results show that there are cattle that naturally produce less methane relative to their feed intake (that is, have a lower MY). Differences between sires in MY by their progeny provides preliminary evidence that there exists genetic variation in MY and demonstrate that sires exist that can be used in cattle breeding to produce progeny with naturally lower methane emissions.

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012

Gastrointestinal parasitism *per se* has no effect on methane output by lactating ewes

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Introduction Ruminants lose from 2 to 12% of their gross energy intake in the form of methane and methane release is affected by feed intake level (Johnson and Johnson, 1995). Factors that influence intake, such as disease, are likely to affect methane output as well. Here we assess the effects on methane release of both parasitized and restrictedly fed lactating ewes to test the hypothesis that methane output is not affected by parasitism *per se*.

Material and methods Twin-bearing individually housed Mule ewes were divided into three groups of 16 ewes on day$_{-39}$ (day$_0$ is parturition) with similar mean body weight (BW, 68.2±1.1 kg) and condition score (2.5±0.06), and drenched to remove existing worm burdens. From day$_{-32}$ onwards, two groups of ewes were *ad libitum* fed pelleted lucernce and either sham infected with water (C) or dosed with 10,000 infective *Teladorsagia circumcincta* larvae every Mon-Wed-Fri (P). A third group of ewes (R) were as C ewes during pregnancy (C/R) but fed restrictedly at 80% of achieved C ewes intake during lactation. Ewes were fed at ~7.30 am and ~15.00 pm. Fresh feed intake was measured twice weekly from day$_{-28}$ until day$_{36}$. Ewe and litter BW were assessed weekly; faecal egg counts (FEC, in eggs per gram (epg) fresh faeces) were taken post drench and at lambing from all ewes, and then weekly from P-ewes only. FEC were log-transformed for analysis, and reported as back-transformed means and lower and upper SE range. Staggered lambing allowed for four rounds of housing in one of six methane chambers for six days from day$_{30}$, with two ewes per treatment per chamber, individually housed with their lambs to achieve two treatment replicates per round. Methane output (g/h) was measured for the last three days in chamber; the last 24 h data were used for the present analysis. Feed intake and hourly methane release were analysed via repeated measures ANOVA; ewe and litter BW at birth, BW gain and daily methane output were analysed using ANOVA.

Results Treatment did not interact with time for feed intake (P>0.20), which averaged 3.2 and 2.9 kg/day during late pregnancy for C/R and P ewes, respectively (s.e.d. 0.15 kg/day; P=0.058) and 4.8, 4.4 and 3.7 kg/day during lactation for C, P and R ewes, respectively (s.e.d. 0.10 kg/day; P<0.001). Body weight gain during late pregnancy of C/R and P ewes averaged 272 and 228 g/day, respectively (s.e.d. 31 g/day, P=0.112), their BW at birth averaged 70.3 and 67.7 kg, respectively (s.e.d. 1.39 kg; P=0.086) but litter BW at birth was not affected (averaging 9.1±0.2 kg). During lactation, BW change for C, P and R ewes averaged -69, -162 and -252 g/day (s.e.d. 37 g/day; P<0.001) and for their litters 718, 669 and 654 g/day, (s.e.d. 24 g/day; P=0.033), respectively. Ewe FEC averaged 101 (86 to 119) and 0 (0 to 1) epg at day$_{-39}$ and day$_{-25}$, respectively. Post lambing FEC of C, P and R ewes averaged 2 (1 to 3), 26 (15 to 43) and 5 (3 to 7) epg, respectively (P<0.001). FEC of P ewes gradually increased to 86 (55 to 134) epg by day$_{27}$.

Figure 1 shows that the average hourly methane output of sets of two C, P and R ewes significantly interacted with time (P<0.001). From 07.00 h to 19.00 h, average methane output was higher for C ewes (4.99 g/h) than for both P (4.43 g/h) and R ewes (4.19 g/h, s.e.d. 0.24 g/h; P=0.010). However, from 19.00 h to 07.00 h, average methane output was higher for C (4.09 g/h) and P (3.79 g/h) ewes than for R ewes (2.55 g/h, s.e.d. 0.26 g/h; P<0.001). Total daily methane output averaged 109, 98 and 81 g per two C, P and R ewes, respectively (s.e.d. 5.50; P<0.001). However, scaled by feed intake, which averaged 11.1, 10.3 and 8.5 kg/day for two C, P and R ewes, respectively (s.e.d. 0.21; P<0.001), these were similar at 9.86, 9.56 and 9.49 g/kg fresh feed, respectively (s.e.d. 0.51; P=0.752). The average 9.64 g methane produced per kg lucerne intake equates to an estimated loss of ~3.3% of lucerne gross energy content.

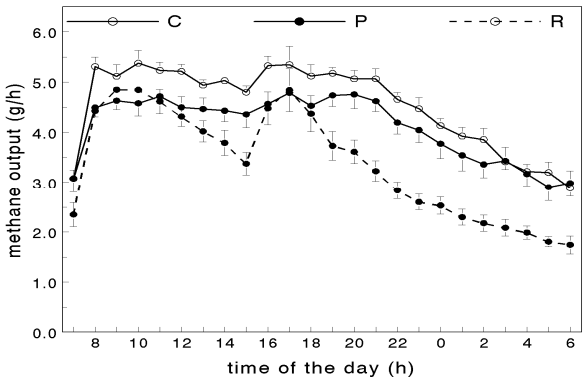

Figure 1 Hourly methane output of sets of two ewes on day 35 of lactation fed lucerne pellets. C: sham infected, *ad libitum*, P: parasitized, *ad libitum*, R: sham infected, fed at 80% of C ewes.

Conclusion The data support our earlier observation that anorexia in response to parasitism occurs in the periparturient ewe, even on high quality foods (Zaralis *et al*., 2009). The relatively low FEC of the P-ewes are likely the combined outcome of high protein intake (which is known to affect host resistance to nematodes) and faecal dilution. Both are affected by the observed high feed intakes and that may have resulted in relatively short digesta retention times, which could explain the relatively low methane output per kg feed intake. Our data show that the reduced level of methane output in parasitized animals is a direct result of parasite-induced anorexia, which supports the thesis that parasitism *per se* is unlikely to affect methane release per kg of feed consumed.

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013

Greenhouse gas (GHG) life-cycle assessment of mammalian rendered products in the UK

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Introduction As a result of the Bovine Spongiform Encephalopathy epidemic, meat and bone meal (MBM) was banned from inclusion in farm animal diets, and animal by-products (ABP) were classified by risk under European legislation ((EC) No 1774/2002) into category 1, 2 and 3 materials, which are usually processed by rendering. As a result the feed industry is now heavily reliant on imported oils and protein meals which carry a high environmental burden. Following a recent risk assessment, the EU is currently re-considering the use of non-ruminant processed animal protein (PAP) derived from category 3 ABP in non-ruminant diets. This could potentially reduce our reliance on imported oils and protein meals and reduce the environmental burden of animal production. The objective of the study was to quantify the GHG life cycle emissions of mammalian rendered products.

Material and methods Two product systems (S1 and S2) and three functional units were defined. Product systems S1 and S2 represented the rendering of category 1+2 and category 3 ABP respectively. The three functional units were 1 kg category 1+2 mammalian rendered fat (MRF1), 1 kg category 3 MRF (MRF3) and 1 kg category 3 PAP (Figure 1). Product system S1 was expanded to replace British electricity with electricity produced by combustion of category 1+2 MBM in fluidised bed combustion (FBC) power plants. In product system S2, GHG emissions were allocated based on mass. Both product systems used natural gas and MRF1 as fuels in the rendering process. Primary and secondary data was used to calculate GHG emissions. Primary data on the quantity of ABP processed, rendered product yields, fuel, electricity, water, chemical use and wastewater treatment was collected from UK rendering plants that processed 0.3-0.4 of ABP between 2006 and 2008. Secondary data included published literature and inventory databases. Simapro® 7.2 was used for system modelling and calculation of results. Climate change was assessed using the Greenhouse Gas protocol 1.00 impact assessment methods. As disposal of ABP represents a cost to the producer they are considered to be wastes in LCA methodology, which do not carry any of the environmental burdens associated with their production. Similarly, energy derived from MRF1 is considered to be biogenic and therefore does not contribute to net GHG emissions.

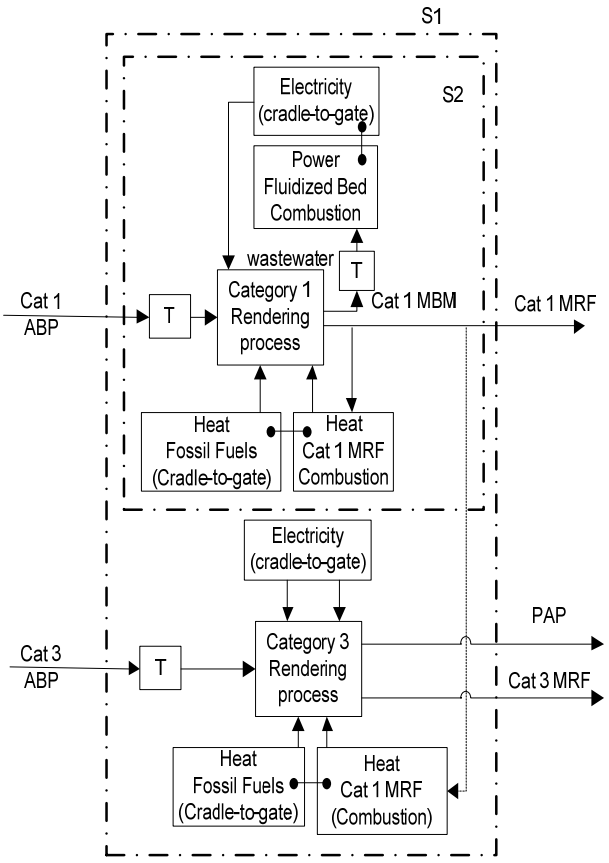

Figure 1 System boundaries for the production of categories 1+2 and 3 rendered products.

Results Rendering of category 1 material produced 0.13 kg MRF1 and 0.27 kg MBM/kg ABP processed, whereas rendering of category 3 materials produced 0.24 kg MRF3 and 0.33 kg PAP/kg ABP processed. Thermal energy use was 2646 and 1357 MJ/kg ABP processed, and electricity use was 260 and 375 MJ/kg ABP processed for category 1 and 3 materials respectively. On average 0.75 of thermal energy was derived from MRF1 with the remainder being derived from natural gas. Using this scenario, net GHG emissions were -0.77, 0.15 and 0.15 kg CO_2e/kg for MRF1, MRF3 and PAP respectively.

Conclusion As thermal energy is used to evaporate water, differences in rendered product yields and energy use between S1 and S2 reflect differences in the composition of ABP processed. Negative GHG emissions for MRF1 arise from the avoidance of British electricity by burning MBM in FBC power plants. The GHG emissions for MRF3 and PAP were considerably lower than those for palm oil (0.61 kg CO_2e/kg) and soya-bean meal (0.50 kg CO_2e/kg) derived from the Ecoinvent database (excluding land use change). It is estimated that at current UK production levels, the use of MRF3 and PAP as direct replacements for palm oil and soya-bean meal in current applications could reduce GHG emissions by 67,700 tonnes/year.

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014

Ruminant feed production in the United Kingdom in 1990 and 2010

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Introduction The UK Government has set a target of an 80% reduction in the emissions of greenhouse gases (GHG) by the year 2050 compared to the baseline of 1990 (Office of Public Sector Information, 2011). This target represents a major challenge to the livestock sector. Currently GHG inventories are derived from statistics of livestock populations and emission factors (EF). There are uncertainties in determining EF and proxy estimates relating to efficiency of production, for example kg feed per kg animal product or the balance of different types of feed in ruminant diets, might provide indirect indications of possible changes in GHG per unit of livestock product. The objective of this paper is to estimate the production of ruminant feeds in the UK relative to the UK ruminant population in 1990 and 2010.

Material and methods UK national statistics (DEFRA, 2011abc) and authors' estimates of mean dry matter (DM) concentrations were used to estimate the production of hay, silage and the industrial production of concentrates (compounds and blends) for the cattle and sheep populations of the UK in 1990 and 2010.

Results Estimated production of hay decreased between 1990 and 2010 from 3.8 to 2.3 million tonnes DM whilst grass silage increased from 12.1 to 13.2 million tonnes DM. Arable silage (mainly maize and whole-crop wheat) increased markedly in the same period from 0.4 to almost 2.0 million tonnes DM (Table 1). Total conserved forage increased from 16.3 to 17.5 million tonnes DM between 1990 and 2010. Total conserved forage DM per livestock unit (LSU) increased by 35% between 1990 and 2010, reflecting decreased populations of dairy cows and breeding sheep (McDonnell *et al*., 2012) over the period. Concentrate production increased from 4.3 million tonnes DM in 1990 to 4.9 million tonnes DM in 2010 and increased by 44% per LSU in the same period. There were increases in average annual milk yield per cow, carcase weight of beef cattle and carcase weight of lambs, of 42%, 21% and 7%, respectively (DEFRA, 2011d), which reflected the increased inputs of conserved forage and concentrate feed per LSU over this period. In the absence of statistical data on the contribution of grazed pasture to ruminant diets it is not possible to conclude whether or not there was a major shift in the balance of ruminant feeds between 1990 and 2010. However, the marked reduction in fertiliser nitrogen applied to grassland in the period (McDonnell *et al*., 2012) suggests that grazed pasture yields were unlikely to have increased. There was also a 6% decrease in the total area of permanent and temporary grassland and sole-right rough grazing in the UK over the period 1990 to 2010.

Table 1 Estimated production of ruminant animal feeds in the UK: 1990 and 2010

	1990		2010	
	FM*	DM	FM*	DM
	million tonnes			
Hay**	4.5	3.8	2.7	2.3
Grass silage	46.5	12.1	41.8	13.2
Arable silage	1.3	0.39	6.2	1.9
Total conserved forage	-	16.3	-	17.4
Concentrates	4.2	4.3	5.6	4.9

* Fresh matter. ** Includes artificially dried fodder.

Conclusions Estimated UK production of conserved forage and concentrate DM increased between 1990 and 2010 despite a reduction in the ruminant population during the period. Increased production of conserved forages and concentrates reflected increased average output per animal. There may also have been less reliance on grazed pasture in 2010 than in 1990.

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015

Quantifying the environmental benefits of using home grown protein sources as alternatives to soyabean meal in pig production through life cycle assessment

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Introduction The UK's most important source of protein in pig diets is soya bean meal (SBM), which is primarily imported from Brazil and Argentina. There are increasing concerns about the environmental impact associated with soya production and its long term sustainability, particularly in relation to deforestation and conversion of rangelands to croplands. In order to promote sustainable pig farming and reduce the environmental impact of the UK pig industry, there is a need to find viable home-grown protein sources as an alternative to SBM in pig diets. This study compares the environmental impact arising from using typical SBM-based pig diets with diets where the major protein source is either faba beans or peas.

Material and methods Life Cycle Analysis (LCA) methodology was used to estimate the global warming potential (GWP), acidification and eutrophication per kg live weight gain (LWG). It was assumed that maximum inclusion rates for peas and faba beans are 30% and 20% replacing ~55% and 100% of SBM in grower and finisher diets, respectively. A pig growth model (Wellock *et al*., 2004) was used to determine the amount of starter, grower and finisher diet required to grow pigs from 20 to 120 kg,. With the exception of SBM and pure amino acids, a rotation was designed that incorporated all crops required to produce the diets. It has been assumed that the slurry produced by the pig is returned to the crops. The N requirement of the crops has been determined from RB209 (Defra, 2011), and thus the soil type, previous crop and climate are taken into account when determining the N required for crop growth. The fertiliser requirement is adjusted for the N supplied in the slurry. The calculation of the GWP for the crop growth is based on IPCC (2006) methodology. The energy requirements of the additional processes (including crop operations, preparation of feed, fuels use) have been sourced from the literature. The calculations of acidification and eutrophication were based on standard methodologies. The UK import approximately 53% and 45% of their SBM / soya either directly or indirectly from Argentina and Brazil respectively (Gerber *et al*., 2010). Due to deforestation and conversion of rangelands to croplands, the land use change (LUC) associated emissions in Brazil and Argentina are 7.69 and 0.93 kg $CO_{2\text{-eq}}$ / kg soya, respectively (Gerber *et al*., 2010). As SBM and rapeseed meal are co-products, allocation of environmental effects are based on economic allocation (Cederberg *et al*, 2000).

Results In the absence of LUC, GWP was the lowest for the pea diet; however, there was only 5% difference between the pea and the SBM diet (Table 1). However, the SBM diet has an appreciable higher GWP / kg LWG produced with LUC inclusion. With respect to the eutrophication potential, pea and SBM diets are similar, with the faba bean diet being 7.7% higher than the pea diet. The acidification potential of the pea diet is the lowest, with the faba bean and SBM based diets being respectively 25% and 50% higher. The largest contributors to GWP per kg pig arise from crop growth (23%) and slurry management (24%).

Table 1 The environmental impact of pea, bean and SBM based grower and finisher diets on pig production

	GWP (No LUC) (kg CO_2-eq / kg LWG)	GWP (LUC) (kg CO_2-eq / kg LWG)	Eutrophication (kg PO_4 / kg LWG)	Acidifcation (kg SO_4 / kg LWG)
Pea	1.61	1.85	0.130	0.012
Bean	1.66	1.86	0.140	0.015
Soya	1.69	2.37	0.131	0.018

Conclusions In agreement with our earlier simulations (Stephen *et al*., 2009), using peas and beans as alternatives for SBM has relatively small impacts on GWP per kg LWG in the absence of including LUC. However, the current LCA results support the view that if LUC is included, home grown peas and faba beans as alternatives to SBM can reduce GWP of grower and finisher pigs by ~20%. Variation in LUC arises from country of soya origin and relative proportions of the crop planted on land that has recently been converted to croplands from either forests or rangelands. Consequently, the higher the proportion of SBM originating from sustainable sources, the lower the environmental benefits of using peas or faba beans. In addition, results will also be sensitive to the relative prices of SBM and soya oil as they were used to determine the economic allocation, global feedstuff prices, and the drive for alternative sources of energy.

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016

Statistical indicators of livestock efficiency related to greenhouse gas emissions

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Introduction Uncertainties in measuring greenhouse gas emissions (GHG) from livestock focuses attention on the utility of proxy or indirect indicators of livestock efficiency such as fertiliser use or livestock feed conversion ratios. National statistics from surveys and other sources may be used to populate proxies and to indicate trends which can assist the development of government policies, research and advice to producers. However, insufficient data may limit the utility of this approach. The current paper comprises a summary of some statistical indicators of UK national livestock efficiency. Gaps in current statistical information are indicated.

Material and methods National livestock statistics were used to investigate the extent to which they might be of value in indicating trends in efficiency and hence in GHG per unit of product. The major sources of statistical data were the June Agricultural Survey of registered holdings, the British Survey of Fertiliser Practice, monthly surveys of animal feed production by feed mills and the British Cattle Movement Scheme's Cattle Tracing System (DEFRA, 2011. Details of the methodology and sources of information are in DEFRA (2011). Annual statistics were obtained, where possible, from the above sources for the period 1990 to 2010. The starting year of 1990 was chosen because it was the baseline year against which the 2050 target for the UK net carbon account was determined in the Climate Change Act, 2008 (Office of Public Sector Information, 2011).

Results Between 1990 and 2010 the total number of breeding dairy cattle, breeding sheep and breeding pigs in England decreased by 42% (from 2.0 million), 31% (from 9.4 million) and 44% (from 0.6 million), respectively. The reductions in cattle and sheep populations accounted for much of the decrease in estimated methane emissions from agriculture over the period (MacCarthy *et al*. 2011). The average level of fertiliser nitrogen applied to grassland in England and Wales decreased from 132 kg N/ha in 1990 to 62 kg N/ha in 2010 which accounted for most of the decrease in estimated nitrous oxide emissions over this period. (MacCarthy *et al*. 2011). Total factor productivity (the volume of agricultural output per unit of input) doubled in the UK between 1990 and 2010. Of the major inputs to agriculture, only animal feed increased (by 17%) over the period. Production of concentrate (compound and blended) feed per cow and milk yield per cow both increased substantially over this period and there was no change in the ratio of milk produced per unit of compound and blended feed. An increase in the longevity of breeding females is desirable because fewer replacements are required annually and there was an increase in the median age of dairy females over 30 months of 0.8 years and in beef females over 30 months of 1.3 years in the period 2001 - 2010. The proportion of female cattle which calved for the first time in 2010 was 0.29 and 0.19 for dairy and beef cattle, respectively, but no data are available to indicate trends. Information is needed on calving interval, age at first calving and live weight of beef and dairy cattle. The mean carcase weights of prime cattle, lambs and pigs increased progressively between 1990 and 2010, from 289 to 348 kg/head for prime cattle, from 17.7 to 18.9 kg/head for lambs and from 65 to 78 kg/head for pigs. Median age at slaughter of beef cattle (under 4 years old) remained virtually unchanged between 2006 and 2010. There was little change in the monthly marketing pattern of lambs and pigs between 1990 and 2010. The national lambing percentage was similar in 2010 to that in 1990 (115%). Mean feed conversion ratio of finishing pigs increased from 2.70 in 1990 to 2.94 in 2010. Pigs marketed per sow increased from 0.35 per week in 1990 to 0.40 per week in 2010. Post-weaning piglet mortality decreased from 8% in 2006 to 5% in 2010, but there was no reduction in pre-weaning piglet mortality which averaged 11.6% in 1990 and 12.6% in 2010. There was no change between 2001 and 2010 in the average feed conversion ratio of poultry meat production. Information is needed on mortality and efficiency of feed use by broilers and laying hens in different systems of production.

Conclusions Between 1990 and 2010 mean carcase weights of cattle, lambs and pigs increased together with longevity in breeding cattle. Pigs marketed per sow per week also showed a small increase in this period. However there is need for more information to assist in determining national trends in livestock efficiency which might indicate changes in GHG per unit of product and to identify those measures which are likely to be most effective in reducing GHG emissions per unit of livestock output.

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017

Do finishing pigs emit enteric methane like sheep?

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Introduction Animal agriculture is responsible for 8 to 11% of total global greenhouse gas (GHG) emissions when assessed on the basis of IPCC (2006) accounting,, but with lifecycle analysis, its contribution increases to 18% of total global emissions. Monogastric animals contribute a comparatively small proportion of the total methane emitted by livestock, however, whilst pigs are responsible for the emission of a small proportion of enteric methane/ head / year, with one billion hogs in the world, overall contribution becomes fairly high. The purpose of this work was to quantify the enteric methane production from finishing pigs fed with a basal diet and compare these results to ruminant emission, in Brazilian conditions.

Material and methods This study was carried out in accordance with the Commission for Ethics in Experimentation with Animals of Centre of Nuclear Energy in Agriculture, University of Sao Paulo. Ten finishing male pigs (70 ± 2.5 kg body weight (BW)) and seventy sheep (22 ± 2.0 kg BW) were weighed and randomly housed in individual pens. Pigs were fed with 2 Kg/day of basal diet (corn and soybean meal), and sheep with 2.5% of BW (hay and concentrate with corn and soybean meal) twice daily at 08:00 and 16:00 h. After five days, the animals were taken to open-circuit chambers for quantitative measurement of CH_4 emissions. One chamber was used for monitoring the system (no animal in). There were 48 hour runs in chambers as previously described by Abdalla *et al*. (2011). The chambers were equipped with one inlet 5-cm i.d. orifice in the front and one outlet 5-cm i.d. orifice in the rear. An exhaust pump was connected to the rear orifice of the chamber to remove the inner air at a flow rate of 1 mL/min (measured using an anemometer). The outlet air was sampled into a 5 L balloon (coated with aluminum film) at 100 mL/min by using a peristaltic pump. A house-hold fan was placed inside the chamber, for circulating air, to keep temperature levels comfortable for the animal. Temperature (23 ± 2°C) and humidity (87 ± 4%) were measured at regular intervals. Methane in the outgoing sampled air was quantified using a gas chromatograph. Gas sample (1.0 mL) was injected into GC equipped with a Shincarbon ST 100/120 micro packed column. Temperatures of column, injector, and flame ionization detectors were 60, 200, and 240ºC, respectively. Methane concentration in the collected gas was determined by external calibration using an analytical curve prepared with pure CH_4. The amount of methane produced by the animal in the chamber was calculated as the product of the amount of air drawn through the chambers, corrected to standard temperature and pressure (STP), and the concentration of methane in the air leaving the chamber, corrected for the concentration of methane in the ambient air. The data were analyzed using GLM of SAS package (SAS Institute, 2001) and the analysis of variance and treatment means were compared by Tukey test ($P<0.05$).

Results The results show that the pigs emit enteric methane, at 10-20% the rate of sheep depending upon the means of data expression. ($P <0.001$).

Table 1 Average body weight (kg) and methane emission (L/day; L/Kg BW; and L/kg DMI) from pigs and sheep measured in open-circuit chambers

	ABW[1] (kg)	CH_4 (L/d)	CH_4 (L/kg BW)	CH_4 (L/kg DMI)
Pigs	61.50	4.83_b	0.08_b	2.54_a
Sheep	27.28	21.88_a	0.83_a	25.53_b
P	-	<0.001	<0.001	<0.001

[1] Average body weight;

Conclusions The results imply that the methane emission from global pig production could be high, and, therefore, it is necessary to study new techniques to control the emission of this atmospheric gas pollutant from pigs.

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018

Prediction of intramuscular fat from beef ultrasound scans

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Introduction Many studies have found that intramuscular fat (IMF) content of beef is associated with eating quality. Ultrasound scanning has provided promising results to predict IMF in live animals (Aass *et al.*, 2006, Aass *et al.*, 2009) and some scanning machines are now equipped with the means to automatically predict IMF. The objective of this study was to assess the accuracy of predicting IMF using parameters collected from a time sequence of ultrasound images taken at the lumbar region of beef cattle pre-slaughter.

Methods Beef cattle were ultrasound scanned pre-slaughter, at an average age of 542d. Animals were from a two-breed rotational cross of Limousin with Aberdeen Angus and were either steers (n = 58) or heifers (n = 40). A time sequence of 10 ultrasound images (taken approx 125 milliseconds apart) was collected for each animal longitudinally at the 12th rib. Following slaughter, samples from the cranial end of the *Longissimus lumborum* were removed and fatty acid analysis was carried out (Teye *et al.*, 2006), providing a measure of total intramuscular fat percentage (IMF) per animal (mean 3.2%, range 1.4 - 6.6%). Ultrasound files, each 720 columns by 576 rows in size, were used to compute texture statistics of greyscale values from within the muscle region of each image sequence - identified by eye as the region between columns 106 and 505, rows 121 and 195 of each image (Figure 1). Summary statistics of the mean ($\hat{\mu}$), standard deviation ($\hat{\sigma}$), and autocorrelations at a range of lags ($\hat{\rho}_{lmn}$) were defined as in Figure 2 for each animal. Lags of 1 to 3 in each direction were considered, therefore, the range of lags tested included $\hat{\rho}_{100}$, $\hat{\rho}_{200}$, $\hat{\rho}_{300}$, $\hat{\rho}_{010}$, $\hat{\rho}_{020}$, $\hat{\rho}_{030}$, $\hat{\rho}_{001}$, $\hat{\rho}_{002}$ and $\hat{\rho}_{003}$. The best combination of parameters to predict IMF was tested using best subsets linear regression in Minitab.

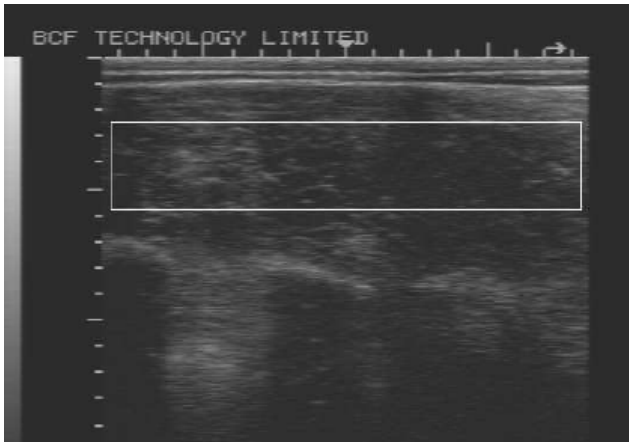

Figure 1 Region of interest (white box) in scan

$$\hat{\mu} = \frac{1}{N}\sum_{ijk} X_{ijk}$$

$$\hat{\sigma} = \sqrt{\frac{1}{N}\sum_{ijk}(X_{ijk} - \hat{\mu})^2}$$

$$\hat{\rho}_{lmn} = \frac{1}{N\hat{\sigma}^2}\sum_{ijk}(X_{ijk} - \hat{\mu})(X_{i+l,j+m,k+n} - \hat{\mu})$$

where X_{ijk} denotes the pixel value in column i, row j, for image k in the sequence for an animal, and N denotes the number of terms in the summation, i.e.(505-105) × (195-120) × 10 = 300,000.

Figure 2 Definition of texture parameters used

Results The best prediction of IMF was found to be: log(IMF) = 1.81 + 0.0167$\hat{\mu}$ - 0.0667$\hat{\sigma}$ + 0.0483$\hat{\rho}_{300}$ - 0.0365$\hat{\rho}_{010}$ + 0.0227$\hat{\rho}_{030}$ + 0.0991$\hat{\rho}_{002}$ - 0.0683$\hat{\rho}_{003}$; with an R^2 of 48.4% and a standard error of prediction of 0.220.

Conclusion These results suggest that IMF can be predicted with moderate accuracy using a time series of ultrasound images. Results were similar in accuracy to those found in lean cattle by Aass *et al.* (2006), although lower in accuracy than the latest model proposed by Aass *et al.* (2009). However, unlike these studies, the current prediction does not include ultrasound fat or muscle depths, so can predict IMF independently of these traits. This would be important if included in a selection programme designed to maintain IMF whilst optimising subcutaneous fat and muscle. These results should be validated on an independent data set.

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Compensatory growth in early and late maturing steers: Effects on beef colour and sensory characteristics

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Introduction Compensatory growth (CG) is the ability of an animal to undergo accelerated growth after a period of restricted feed intake (Hornick *et al*., 2000). The CG phenomenon allows the manipulation of feed supply and may enhance the efficiency of beef production. Data relating to the imposition of CG on meat quality for cattle are equivocal and there is little comparative information for breeds of cattle of differing in mature size used in beef production in Western Europe. The objective was to examine the effect of CG on sensory aspects of *M. longissimus dorsi* (LD) from Aberdeen Angus × Holstein Friesian (AA) and Belgian Blue × Holstein Friesian (BB) steers.

Material and methods Castrated spring-born male progeny of Holstein-Friesian dams, sired by either AA (n = 23) or BB bulls (n = 23) were assigned within genotype to either a high energy control diet (concentrates *ad libitum* and 10 kg of grass silage per head daily) for 299 days (H-H) or an energy restricted diet (grass silage *ad libitum* plus 0.5 kg of concentrate per head daily) for 99 days followed by *ad libitum* access to the control diet for 200 days (L-H). Mean initial weights were 295 (s.d. 30.0) and 287 (s.d. 48.6) kg for AA and BB, respectively. Cattle were slaughtered on day 300. Post-mortem (48h), colour of LD (4 hour bloom at 0^{o}C) and fat were measured using a Hunterlab UltraScan XE colorimeter while sensory analysis was carried out by a trained panel on 14-day aged LD. Data were analysed using mixed model ANOVA. Genotype, feeding treatment (H-H or L-H) and their interaction were included as fixed effects and sire of the animal was included as a random effect in the statistical model used.

Results There were few interactions between main effects and there was no effect of genotype or CG on fat colour variables (data not shown). Mean carcass weight was 354 and 369 kg ($P < 0.05$) for AA and BB, respectively and 373 and 350 kg ($P < 0.05$) for H-H and L-H, respectively. Muscle data are summarised in Table 1. Muscle lightness and hue were lower, and redness and chroma were higher for AA compared to BB ($P < 0.05$). Muscle from H-H animals was redder and had higher chroma than muscle from L-H animals ($P < 0.05$). Muscle from AA animals was considered more juicy, greasy, sweet, dairy and more preferred, but less bitter, acidic, cardboard, and vegetable than muscle from BB animals ($P < 0.05$). Muscle from H-H animals was more preferred than muscle for L-H animals ($P < 0.05$).

Table 1 *M. longissimus dorsi* colour and. sensory characteristics

Trait	Genotype[1] (G)		Feeding Treatment[2] (F)			P	
	AA	BB	H-H	L-H	s.e.d	G	F
Muscle colour							
Lightness	35.18	37.37	36.60	36.25	0.479	0.001	0.90
Redness	15.09	14.19	15.04	14.24	0.321	0.01	0.02
Hue	29.62	32.60	30.84	31.38	0.448	<.0001	0.24
Chroma	17.50	16.94	17.63	16.82	0.348	0.13	0.03
Sensory attributes[3]							
Tenderness	4.58	4.21	4.60	4.19	0.225	0.12	0.08
Juiciness	5.23	4.95	5.12	5.06	0.107	0.02	0.60
Beef flavour	4.60	4.23	4.43	4.40	0.078	0<.0001	0.72
Flavour[4]							
Greasy	18.00	11.31	14.51	14.81	1.315	0<.0001	0.82
Bitter	1.40	1.52	1.43	1.49	0.042	0.007	0.17
Sweet	16.95	11.91	14.71	14.16	1.332	0.001	0.68
Acidic	5.65	8.59	6.88	7.36	0.602	0<.0001	0.43
Cardboard	13.02	17.29	14.32	15.98	1.079	0.0007	0.14
Vegetable	12.25	14.24	13.31	13.18	0.742	0.01	0.86
Dairy	24.40	16.34	21.76	18.97	1.461	0<.0001	0.07
Overall liking	51.93	42.41	49.15	45.20	1.688	0<.0001	0.03

[1] AA = Aberdeen Angus; BB = Belgian Blue. [2] H-H = *ab libitum* access to feed throughout the study; L-H = Restricted feeding for 99 days followed by *ad libitum* access to feed until slaughter. [3] Eight point scale. [4] One hundred line scale.

Conclusions Under the conditions of this study CG had a small impact on LD quality compared to the effect of genotype. The preference for muscle from AA reflected the greater tenderness and difference in flavour characteristics when compared to BB.

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Effect of the Texel muscling quantitative trait locus (TM-QTL) on spine characteristics in purebred Texel lambs

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Introduction The largest phenotypic effects of the Texel muscling quantitative trait locus (TM-QTL) on loin muscling have been seen in progeny that inherit a single copy of the TM-QTL (TM) allele from their sire (superscript S) and the wildtype (+) from the dam (superscript D) ($TM^S/+^D$) (Macfarlane *et al*. 2009). These heterozygous lambs with a paternal copy of TM-QTL show ~ 9% increase in loin muscle depth and weight (Macfarlane *et al*. 2010). Recently, spine characteristics of the thoracolumbar (thoracic plus lumbar) region in sheep have been reported to show significant inter-breed/cross variation (Donaldson *et al*. 2011); further unpublished results show that intra-breed variation of these spine characteristics in Texels is also common. As the loin muscle runs the length of the thoracolumbar region of the spinal column it is of practical relevance to assess if there is any association with variation in spine characteristics and the TM-QTL.

Material and methods CT scans (topograms) of purebred Texel lambs (59 entire males, 83 females), of known TM-QTL genotypic classes ($+^S/+^D$ n=39; $+^S/TM^D$ n=17; $TM^S/+^D$ n=52; TM^S/TM^D n=34), were used to quantify spine characteristics. Spine traits measured directly from the scans included counts of vertebrae in the thoracic and lumbar regions (SPV_{THOR} and SPV_{LUM} respectively) and length (cm) of the thoracic and lumbar spine region (SPL_{THOR} and SPL_{LUM} respectively). These measures were then used to calculate the average length (cm) of individual vertebrae in the thoracic and lumbar regions (VL_{THOR} (SPL_{THOR}/SPV_{THOR}) and VL_{LUM} (SPL_{LUM}/SPV_{LUM}) respectively). The results from the two spine regions were also combined to give the number of thoracolumbar vertebrae (SPV_{T+L}), the length (cm) of the thoracolumbar region (SPL_{T+L}) and the average length (cm) of individual vertebrae across the thoracolumbar region (VL_{T+L} (SPL_{T+L}/SPV_{T+L})). Data were analysed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of genotype on the traits measured. Fixed effects fitted in the model for length traits (SPL_{THOR}, SPL_{LUM}, SPL_{T+L}, VL_{THOR}, VL_{LUM}, VL_{T+L}) were sex, dam age, rearing rank and genotype with live weight (covariate). The model for count traits (SPV_{THOR}, SPV_{LUM}, SPV_{T+L}) included only fixed effect of TM-QTL genotype as all other effects were shown to be non-significant.

Results Lambs with genotype $+^S/TM^D$ and TM^S/TM^D had significantly more SPV_{THOR} but significantly less SPV_{LUM} compared to those with genotype $+^S/+^D$ and $TM^S/+^D$, but there were no significant genotype effects for SPV_{T+L}. In terms of the length traits, SPL_{LUM} was significantly higher in $+^S/+^D$ and $TM^S/+^D$ lambs and traits SPL_{T+L} and VL_{T+L} were significantly higher in $TM^S/+^D$ compared to $+^S/TM^D$ lambs (Table 1).

Table 1 Least-squares means (and standard errors) for spine characteristics in Texel lambs by TM-QTL genotype

Trait	$+^S/+^D$		$+^S/TM^D$		$TM^S/+^D$		TM^S/TM^D	
SPV_{THOR}	12.8^b	(0.06)	13.0^a	(0.08)	12.7^b	(0.05)	13.0^a	(0.06)
SPV_{LUM}	6.26^a	(0.06)	6.00^b	(0.10)	6.31^a	(0.05)	6.03^b	(0.07)
SPV_{T+L}	19.1^a	(0.05)	19.0^a	(0.08)	19.0^a	(0.05)	19.0^a	(0.06)
SPL_{THOR}	26.3^a	(0.43)	26.4^a	(0.48)	26.4^a	(0.41)	26.5^a	(0.42)
SPL_{LUM}	18.5^a	(0.36)	17.6^b	(0.40)	18.6^a	(0.34)	18.0^b	(0.35)
SPL_{T+L}	$44.8^{a,b}$	(0.48)	44.1^b	(0.52)	45.0^a	(0.45)	$44.5^{a,b}$	(0.46)
VL_{THOR}	2.07^a	(0.03)	2.05^a	(0.03)	2.09^a	(0.03)	2.06^a	(0.03)
VL_{LUM}	2.94^a	(0.03)	2.91^a	(0.03)	2.92^a	(0.03)	2.94^a	(0.03)
VL_{T+L}	2.35^a	(0.02)	2.30^b	(0.03)	2.35^a	(0.02)	$2.33^{a,b}$	(0.02)

Means within row sharing a common character in their superscript are not significantly different ($P > 0.05$)

Conclusion While significant differences appear between the genotypes in traits SPV_{THOR} and SPV_{LUM}, non-significant differences in SPV_{T+L} imply that the reduction of vertebrae in one region may be balanced by additional vertebrae in the other. On the other hand, the length of the lumbar (SPL_{LUM}) and thoracolumbar (SPL_{T+L}) spine regions and the individual vertebrae across the thoracolumbar section (VL_{T+L}) show significant differences between genotype classes. In genotypes where a copy of the TM-QTL is inherited from the dam ($+^S/TM^D$ and TM^S/TM^D) SPL_{LUM} appears shorter compared with the wild type or where the TM-QTL is inherited from the sire ($+^S/+^D$ and $TM^S/+^D$). The largest phenotypic differences for SPL_{T+L} and VL_{T+L} are observed between the heterozygotes with ~ 1% and 2.2% decrease, for the traits respectively, in $+^S/TM^D$ lambs. The reduction in length observed for SPL_{LUM}, SPL_{T+L} and VL_{T+L} in lambs inheriting the TM-QTL from the dam (particularly in the heterozygote ($+^S/TM^D$)) could have an unfavourable impact on meat production. Interestingly, lambs inheriting TM-QTL paternally ($TM^S/+^D$) have increased loin muscle depth and weight compared to the wildtype but the spine characteristics measured in this study are not significantly different from the wildtype.

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Changes in caspase 3/7 activity and shear force during *post mortem* conditioning of the *longissimus* muscle of growing gilts after 7 days treatment with beta-adrenergic agonist or growth hormone

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Introduction The extent and rate of tenderisation does not occur equally in all carcasses, resulting in variable meat quality, and thus, the final toughness of meat depends in part on the degree of alteration of the structural components of muscle and associated proteins post-mortem (Kemp *et al*. 2006). It has recently been recognised that caspases are involved in skeletal muscle development and remodelling, with its expression being essential for normal muscle differentiation during myogenesis. Caspases are upregulated in conditions such as sarcopenia, muscular dystrophies and activated early in pathological events associated with hypoxia/ischemia (e.g. apoptosis), including the hypoxic conditions that occur post-mortem. Given this unique relationship between skeletal muscle and caspases, it is conceivable that caspases could be involved in post-mortem proteolysis and meat tenderisation (Kemp *et al*., 2010; Herrera-Mendez *et al*., 2006). The objective of this study was to evaluate the effect of a beta-adrenergic agonist (Ractopamine^{TM}) and growth hormone (Reporcin^{TM}) on muscle caspase activity and meat tenderness in growing gilts after a 7day treatment period.

Material and methods Forty five (45) White Duroc x (Landrance x Large White) gilts weighing about 85(±5)kg were sourced from PIC (Alpha Building, Nantwich, Cheshire), acclimatized to the feed and environment for 5days, before being allocated to one of three treatment groups. The Control (n=12) group were fed a standard commercial diet (high energy (14MJ/kg), high protein (16.7% CP)) *ad-libitum*, the β-adrenergic agonist (BA, n=12) group were also fed *ad-libitum* the standard commercial diet containing Ractopamine^{TM} (10mg/kg) and the growth hormone (GH, n=12) group were fed the commercial diet *ad-libitum* and administered Reporcin^{TM} (10mg) by intramuscular injection on days 0, 2, 4, and 6. After slaughter, the carcass was stored at 4^{o}C and samples of *Longissimus dorsi* (LD) muscle were collected at 0, 2, 4, 24 and 48hrs post-mortem, snap frozen in liquid nitrogen and stored at -80^{o}C prior to assay for caspase 3/7 activity as described by Kemp *et al*. (2006). After 48 hrs, chops from the same LD muscle were 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 (Genstat) by ANOVA or repeated measures ANOVA and *Post Hoc* Dunnett's test.

Results Although there were no significant effects of treatment on caspase 3/7 activity (Table 1), there was a significant decrease in activity with time post-mortem, such that the activities at 0 and 2hrs were significantly higher than all other time points (P<0.001). As an index of the relative level of caspase 3/7 activity remaining after 24hr the ratio of 24 to 0hr caspase activity was assessed, although this was not significantly different the BA treated chops had numerically higher values. There was a trend (P<0.1) for an effect of treatment on shear force measurements, BA treated chops being tougher. When all the chops were examined, irrespective of treatment, there was no significant correlation between 24:0hr caspase 3/7 activity ratio and 8 day shear force (P= 0.12) (n=36).

Table 1 Caspase 3/7 Activity post-mortem and shear force of LD muscles of gilts treated with BA or GH for 7 days

Measurement	Control	BA	GH		SED	P-value
Caspase 3/7 Activity (Activity/microg protein)						
0hr	12.49	12.58	13.06	Time	0.825	<0.001
2hr	11.45	12.23	11.74			
4hr	9.58	9.36	8.91	Treatment	0.639	0.554
24hr	3.51	5.21	3.30			
48hr	1.55	1.99	1.16	Time x Treatment	1.429	0.980
24hr:0hr ratio	0.30	0.42	0.25		0.080	0.114
Shear Force (Kg)						
5day	5.24	5.79	5.32	Time	0.187	0.439
8day	5.28	5.55	5.08	Treatment	0.229	0.09
				Time x Treatment	0.324	0.784

Conclusions Caspase 3/7 activity in LD significantly dropped with time post-mortem, and BA treated gilts tended to have a higher level of caspase 3/7 activity remaining after 24hr post-mortem and there was a trend for them to yield chops of higher shear force. It would be interesting to test the effects of these growth promoters over a longer time period of administration, since the treatment period in this study was just 7 days, rather than the 28 day period used commercially.

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Diet and gender effects on lamb meat quality

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Introduction Lambs finished on pure stands of chicory *Cichorium intybus* grow faster, finish earlier and have lower levels of parasitism due to the plant's anthalmintic properties (Athanasiadou *et al*., 2007). Houdijk *et al*. (2011) found chicory had minor effects on lamb sensory quality. The aim of the current research was to determine the effects of diet and gender on instrumental measures of lamb meat quality and to determine the correlation between instrumental parameters and trained sensory panel scores.

Material and methods 60 Texel-cross lambs (34 castrated males and 26 females) were finished on either grass or chicory over 193d on the same farm. Lambs were slaughtered and commercially processed on the same day. Carcasses were hip-suspended at 2°C for 24 h before ~200mm of *M. Longissimus lumborum* (*LL*) was removed from the left side. The posterior half was sent to Bristol University, aged an additional 9d at 2°C then prepared for sensory analysis as outlined by Houdijk *et al*., (2011), while the anterior half was prepared into two ~5cm parts. Ultimate pH (pH_{ult}) was measured using a calibrated Testo 205 pH meter. CIELAB Lightness (L*), redness (a*) and yellowness (b*) was measured after allowing 45min blooming using a calibrated Minolta CR-410. One quarter of *LL* (aged 1d) was cooked on a clam-shell grill to an internal temperature of 71°C. A 25mm x 10mm x 20mm slice of *LL* was prepared immediately after cooking so the fibre axis ran longitudinally. Peak force (PF) was determined by shearing cores with a 25mm bevel-edged blade on a Lloyd textural analyser set to a cross head speed of 500mm/min (Shackelford *et al*., 2004). The remaining quarter of *LL* was vacuum packaged and aged a further 9d at 2°C after which the PF procedure was repeated to determine 10d PF. PF difference = 1d PF – 10d PF. A paired t-test was used to determine the aging effect on *LL* PF (SAS Inst. Inc., Cary, NC). A 2 x 2 factorial design with gender (female [F] and castrated male [M]) and diet (chicory and grass) was analysed using REML. Hot carcass weight (HCW) was tested as a covariate but was included for a* only. No significant interactions between fixed effects and HCW were found. Simple correlations between instrumental traits and sensory scores were also performed in SAS.

Results Least squares means for diet and gender are shown in Table 1. HCW was higher for lambs finished on chicory than on grass. *LL* from chicory-fed lambs had a higher pH_{ult} than grass fed lambs. An additional 9d aging improved *LL* tenderness by 31.4 ± 2.59N, $p < 0.001$). *LL* from castrates was lighter and less red than females. Male lamb *LL* was tougher than female lamb *LL* at 10d aging.

Table 1 Effects of diet and gender on instrumental meat quality of *M. longissimus lumborum* from commercially finished lambs.

Trait	Diet				Gender			
	Chicory (n = 30)	Grass (n = 30)	S.E.D.	Effect[a]	M (n = 34)	F (n = 26)	S.E.D.	Effect[a]
HCW (kg)	22.0	20.1	0.30	<0.001	21.3	20.8	0.30	0.15
pH_{ult}	5.59	5.51	0.01	<0.001	5.55	5.55	0.01	0.81
Lightness (L*)	40.86	40.63	0.44	0.60	41.49	39.99	0.44	0.001
Redness (a*)	21.52	21.87	0.43	0.43	21.31	22.08	0.34	0.03
Yellowness (b*)	7.10	7.25	0.16	0.34	7.15	7.19	0.16	0.82
1d PF (N)	89.41	81.58	5.09	0.13	89.37	81.62	5.15	0.14
10d PF (N)	47.89	49.67	3.28	0.59	52.47	45.08	3.31	0.03
PF difference (N)	41.33	31.91	5.13	0.07	36.91	36.32	5.19	0.91

Note: a* was corrected for HCW *LL* redness increased by 0.35 units per kg ($p = 0.02$). [a] Significance of effects (p value)

LL pH_{ult} significantly ($p \leq 0.05$) correlated to PF difference (0.31), juiciness (0.31) and lamb flavour (0.30). Lightness significantly correlated to yellowness (0.43) and kidney flavour (-0.37). Redness was also significantly correlated to yellowness (0.59). 1d PF was significantly correlated to 10d PF (0.34), PF difference (0.79), lamb texture (-0.27) and grassy flavour (-0.29). 10d PF was correlated to lamb texture (-0.52). The relationship between 10d PF and sensory lamb texture was characterized by the following equation: Sensory lamb texture = $-709.13 - 0.019$ (10d PF) + 256.89 (pH_{ult}) – 23.08 (pH_{ult}^2), with $r = 0.6$ ($p < 0.001$) and RSD = 0.41.

Conclusions Lambs finished on chicory had higher HCW and pH_{ult} than those finished on grass. A gender effect was present in *LL* PF after aging for an additional 9d. Correlations between 1d PF and 10d PF and between instrumental and sensory parameters were generally low. However, including pH_{ult} dramatically improved the correlation between 10d PF and sensory lamb texture.

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Effects of using peas and faba beans to replace soyabean meal on carcass quality in pigs

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Introduction The UK pig industry relies heavily on imported soyabean meal (SBM) and there is a need to find viable home-grown protein sources as an alternative to SBM in pig diets. Peas and faba beans are potentially an alternative home-grown protein sources for pig diets. However, the pig industry will only consider peas and faba beans as a viable protein source if there are no detrimental effects on performance and carcass quality. Here, we assess the effect of peas and faba bean dietary inclusion on pig carcass characteristics.

Material and methods Details of the experimental diets, trial design and effects on performance are presented in Smith *et al*. (2012). In summary, pea variety Prophet or the faba bean variety Fuego were included in finisher pig diets at 75, 150, 225 and 300 g/kg, and together with one common control diet containing SBM at 120 g/kg resulted in 9 feeding treatments that were imposed on 4 pens of 4 pigs per feeding treatment. All diets were balanced for net energy and standardised ileal digestible lysine contents. Pigs were slaughtered at a commercial slaughter house where hot weight, cold weight and fat depth (P2) were recorded. Lean percentage (% Lean= 66.5-0.95 x P2 + 0.068 x cold carcass weight) and killing-out percentage (KO% = hot carcass weight/liveweight x 100) were calculated for each pig. Backfat samples were taken from entire male pigs for analysis of skatole and indole, which are associated with pork 'boar' taint (Annor-Frempong *et al*. 1997); the latter data were $\log_{10}$ transformed prior to analysis, and reported as backtransformed mean with lower and upper backtransformed standard error ranges. REML with contrast statements was used to locate treatment effects arising from pulse inclusion *per se*, pulse type, and linear or quadratic pulse inclusion level effects. The random model included group for KO%, skatole and indole concentration, and group nested in season for P2 and % Lean. Where significant, sex was used as a covariate.

Results Table 1 shows that there were no significant contrasts for P2, % Lean, KO% and backfat skatole levels. However there was a significant overall linear reduction in concentration of indole in backfat with increasing pulse inclsuion.

Table 1 Effect of feeding treatment on P2, % Lean, KO% and backfat skatole and indole levels.

Feeding treatment with pulse inclusion levels (g/kg)		P2 (mm)	Lean (%)	KO (%)	Skatole (µg/g backfat)	Indole (µg/g backfat)
SBM	-	11.9	60.3	78.0	0.08 (0.05-0.15)	0.04 (0.03-0.06)
Faba bean	7.5	11.7	60.5	79.4	0.11 (0.06-0.17)	0.04 (0.03-0.06)
	15	12.3	59.9	78.8	0.11 (0.06-0.18)	0.05 (0.04-0.07)
	22.5	11.8	60.3	77.8	0.07 (0.04-0.11)	0.03 (0.02-0.03)
	30	11.1	60.8	75.9	0.08 (0.05-0.13)	0.03 (0.03-0.04)
Pea	7.5	11.1	60.8	76.0	0.14 (0.08-0.24)	0.04 (0.03-0.05)
	15	11.3	60.8	78.0	0.04 (0.02-0.06)	0.03 (0.02-0.04)
	22.5	11.3	60.7	77.1	0.04 (0.02-0.06)	0.02 (0.02-0.03)
	30	11.9	60.4	77.3	0.09 (0.05-0.15)	0.03 (0.02-0.03)
SEM		0.6	0.5	1.5	-	-
P-value (Contrasts)						
Control vs Pulse *per se*		0.49	0.59	0.77	0.80	0.28
Bean vs Pea		0.19	0.25	0.42	0.42	0.11
Linear inclusion level effect		0.51	0.64	0.46	0.49	0.05
Quadratic inclusion level effect		0.99	0.98	0.56	0.61	0.92

Conclusions The results indicate that inclusion of peas and faba beans in pig diets, at the expense of SBM, has no effect on carcass quality or skatole/indole concentration in backfat. Additionally, the mean P2 values were not significantly higher than the 12mm upper limit for premium carcass payment (Kyriazakis & Whittemore, 2006). Furthermore, the mean skatole concentrations were below the currently accepted threshold levels of 0.2 µg/g backfat for 'boar' taint detection (Lundström *et al* 2009).

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Nutritional value of diets for growing / finishing pigs containing high levels of home grown legumes compared with one based on soyabean meal 2. Carcass quality

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Introduction If the use of home-grown legumes is to be increased in the UK, then the pig industry must be convinced that there will be no negative impacts on growth performance and carcass quality arising from including these raw materials in compound diets for growing / finishing pigs when compared with more conventional diets based on imported soyabean meal. Paper 1 in this series (White *et al*., 2012) confirmed that performance was unaffected by including peas and beans into diets that were formulated to be balanced for energy and nutrients. The current paper examines the effects on carcass quality including back fat skatole and indole measurements as indicators of 'taint' in pigmeat.

Material and methods Details of experimental diets and trial design as well as conduct of the experiment are presented in White *et al*. (2012). Five grower and five finisher diets (one containing 140 and 120g hipro soyabean meal respectively (O) and four others containing 300g of white-flowered peas Prophet (P), spring coloured-flowered faba beans Fuego (F), white-flowered faba beans Tattoo (T) or winter coloured-flowered faba beans Wizard (W) / kg) were formulated. Once an animal had reached a minimum 95kg liveweight, it was transported to the University of Nottingham experimental abattoir without a pre-slaughter starvation period and slaughtered by electrical stunning followed by exsanguination. The whole carcass was scalded and dehaired. As there is interest in gut fill and how this might be influenced by dietary treatment, both the small and large intestines were carefully excised immediately post-slaughter, weighed full, gut contents removed and then weighed empty. Hot P2 probe measurements were undertaken 45min post-slaughter. Carcasses were then split and stored in chilled rooms at +4C for 24hrs. A number of carcass measurements were then taken, including those that allow the calculation of total carcass lean content. Shoulder backfat was sampled for indole and skatole analysis conducted at the University of Bristol.

Results There was no effect of treatment on the ratio of empty to full intestine weights with the range being 0.51 – 0.54 (small; P=0.982) and 0.35 – 0.37 (large; P=0.924). Similarly there was no effect of treatment (P=0.178) on killing out % (range 72.0 – 73.8). It was not possible to slaughter all animals exactly at 95kg. There was accordingly a range of slaughter liveweights (mean range 94 – 100kg) and cold carcass weights (mean range 68 – 72kg); the latter was therefore employed as a covariate in subsequent analyses of variance although the effect was not significant and accordingly omitted from presentations of results.

Table 1 presents means for hot P2 probe and carcass length. There was no effect of treatment; all mean P2 (probe) data were below the 12mm limit for top payment with only five individual pigs being higher (not related to treatment). There were no significant effects of treatment on a range of carcass lean and fat measurements (data not presented). Calculation of lean meat content was based on the industry-accepted prediction equation:

Table 1 Effect of treatment on Hot P2 probe, carcass length and lean meat %

	Diet					SED	P
	O	P	F	T	W		
P2 (probe) mm	11	10	10	10	11	1.5	0.285
Length cm	813	814	831	823	819	8.0	0.186
LM %	60.7	61.1	62.1	61.8	60.7	0.72	0.208

66.5-(0.95*Hot P2)+(0.068*cold carcass weight.).

There was no significant effect of treatment on lean meat % (Table 1). There have been long-standing concerns over the possible effects of legumes on indole and skatole concentrations in pig meat. Samples of shoulder fat were analysed for these two metabolites. Back transformed mean data were 0.023 and 0.055 µg/g respectively and 95% confidence intervals of 0.044, 0.067; 0.019, 0.027 respectively. Data are substantially below those thresh-holds where possible problems of taint may occur.

Conclusions Home grown peas and beans can be safely included into diets for growing finishing pigs at a rate of 300g/kg with no detrimental effects on carcass and meat quality, including lean meat content that is the basis for payments to producers. Accordingly home-grown legumes, at the levels employed in the current paper, may be used confidently in pig production systems.

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Effect of packaging and ageing times on shelf life and quality of steaks from commercial beef striploin

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Introduction Modified atmosphere (MA) packs, used for maintaining the desirable bright red colour of beef cuts during retail display are bulky since they require sufficient headspace to prevent meat from touching the impermeable top web. Whilst the high oxygen concentration (80%) maintains the bright red colour for longer than meat packed in air, it has also been shown to increase lipid and protein oxidation (Jakobsen & Bertelsen, 2000, Lund *et al*., 2008). Vacuum skin packaging (VSP) uses a barrier film which produces smaller pack sizes but no bright red colour. Using a semi-permeable VSP film over-packed in MA reduces pack size and allows the meat to bloom bright red (VSP-B). Ageing meat reduces the subsequent retail display life. This study explored the relationship between ageing and pack type on shelf life of beef sirloin steaks.

Material and methods 19 beef striploins were aged in vacuum at 1°C for 10d, cut in half and alternate ends aged for a further 11d (21d in total). After each ageing time, 20mm thick steaks were cut and packed in MA, VSP or VSP_B and displayed under simulated retail conditions at 3°C for 10d or, for VSP, 10d and 21d. Colour was measured daily for MA packs using a Minolta chromameter ("Chroma") and on day 10 for the opened VSP_B packs and days 10 and 21 for VSP packs opened and allowed to bloom for 1hr. After 10 or 21d display, steaks were analysed for lipid oxidation products (TBARS, Tarladgis *et al*., 1960). Instrumental texture of cooked steaks was measured using a Stevens CR analyser with a punch and die head. Trained taste panel assessment was carried out on meat aged for 10 or 21d and displayed for 7 d. Two-way analysis of variance was performed on the shelf life and texture data using GLM with pack type and ageing as factors (TBARS values were log transformed to normalise the variation). Sensory data was analysed using packaging and assessor as factors, with 8 replicates (Fizz, version 2.20,h Biosystemes, Coutomon, France).

Results There was a significant effect of pack type on chroma value in 21d aged but not 10d aged meat ($p<0.05$ Table 1). There was no effect of ageing on chroma of meat packed in VSP. Meat aged 21d and packed in MAP or VSP-B had a lower chroma at 10d display than that from 10d aged loins. TBARS values were significantly higher in MAP and VSP-B compared to day of pack meat or VSP meat at 10d or 21d display. These values were two-fold higher after the meat had been aged for 21 d. Sensory analysis showed that VSP steaks were more tender ($P<0.05$) and juicy ($P<0.001$) than MAP and VSP-B steaks (data not shown). There was no difference in beef flavour but abnormal flavour scores were significantly higher after MA displayed ($P<0.05$) than both VSP-types of packaging. Flavour liking and overall liking scores were also significantly higher in VSP-type packaging compared to MA ($P<0.001$). For meat aged 21 days, the trend was very similar although overall liking was less significant ($P<0.05$). Instrumental texture analysis confirmed that meat displayed in MAP was less tender than VSP ($P<0.001$).

Table 1 Effect of packaging type on shelf life of steaks from loins aged for 10 or 21 days (mean values, n=19)

Parameter	Ageing	day of pack	MAP10d	VSP-B 10d	VSP10d	VSP21d	sed P x A	Pack	Age	P x A
Chroma	10d	-	24.5^{b}	24.9^{b}	24.2^{b}	23.6^{b}	0.80	***	***	***
	21d	-	20.6^{a}	19.0^{a}	24.9^{b}	23.6^{b}				
TBARS¥	10d	0.2^{a}	0.9^{b}	0.8^{b}	-	0.2^{a}	0.06	***	***	***
(mg/kg meat)	21d	0.2^{a}	1.9^{c}	1.7^{c}	-	0.1^{a}				
Texture,	10d	21.4^{ab}	25.0^{c}	23.2^{bc}	19.4^{ab}	18.3^{a}	1.78	***	*	ns
(kg force)	21d	18.9^{a}	23.1^{bc}	20.7^{ab}	19.0^{a}	17.7^{a}				

Values within parameter with different superscripts vary significantly, *P<0.05, ***P<0.001, ¥back transformed means.

Conclusions Meat in smaller VSP-bloom packs has similar colour, lipid oxidation and texture to those in conventional MAP. Ageing significantly shortens shelf life and yields only a small improvement in tenderness. Sensory analysis showed that meat in MAP was poorer for texture and flavour than that from either VSP pack type. Using VSP would reduce space taken up in transport, storage and retail shelf and give a longer retail life than MAP though consumers may not appreciate the lack of bright red colour.

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026

Comparison of three winter finishing systems on vitamin E content and shelf life in lamb leg meat

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Introduction The colour stability of meat from late-finished lambs fed over the winter period is not as good as that from lambs finished on grass and slaughtered in autumn (SEERAD, 2004) possibly due to a low vitamin E content of the diet. This study compared the shelf life characteristics of leg meat steaks from lambs fed concentrates, or grazed on root crops (turnips) or grass over the winter period.

Material and methods 36 lambs finished on one of three diets; grass, root crops or concentrates (12 lambs/ group, 6 male, 6 female) were slaughtered commercially in the first week of March 2011. Hind legs were aged in vacuum at 1°C for 10d. After ageing, duplicate 20mm thick, leg steaks, containing the *semimembranosus* (SM) and *gluteobiceps* (GB) muscles, were cut and packed in a modified atmosphere (MA) (80% O_2, 20% CO_2) and displayed under simulated retail conditions at 3°C. The colour was measured daily using a Minolta chromameter ("Chroma"). Individual muscles were analysed for lipid oxidation products (TBARS, Tarladgis *et al*., 1960) after 7d display. Fatty acid composition (Teye *et al*., 2006) and vitamin E content (Liu *et al*., 1996) were measured in subsamples stored in vacuum at -20°C. Data was analysed by GLM anovar, TBARS values were log transformed to normalise the distribution.

Results Male and female results were not statistically significantly different and so data was pooled. Taking a chroma value of 18 as the point at which meat is considered to be unacceptably brown (MacDougall, 1982), SM from grass-fed lambs had a 2 day longer colour shelf-life compared to SM from lambs fed roots or concentrates (Figure 1). Results were similar for GB (not shown) except that all groups had one extra day of shelf life compared to SM. TBARS values for lipid oxidation were significantly higher in SM from root and concentrate-fed groups compared to the grass-fed group, and followed a similar trend for GB but were 20-26% lower than those found in SM.. Vitamin E concentration in muscles from grass-fed animals was two-fold higher than those fed concentrates, while those fed roots were intermediate. The % PUFA, P:S and n-6:n-3 ratios in the concentrate-fed animals were higher ($p<0.001$) than the root- or grass-fed animals (data not shown).

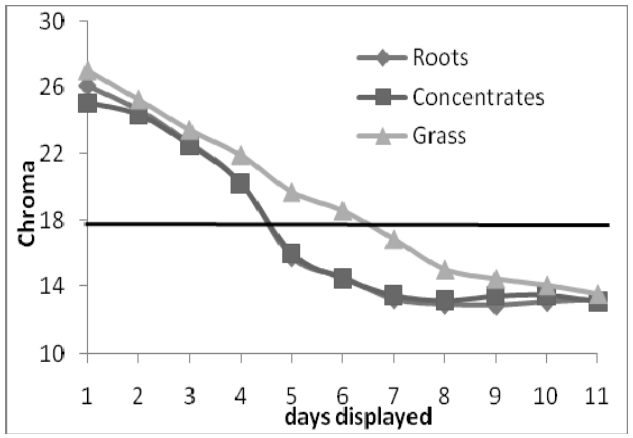

Figure 1
Effect of diet on chroma in SM packed in MA (n=12/group)

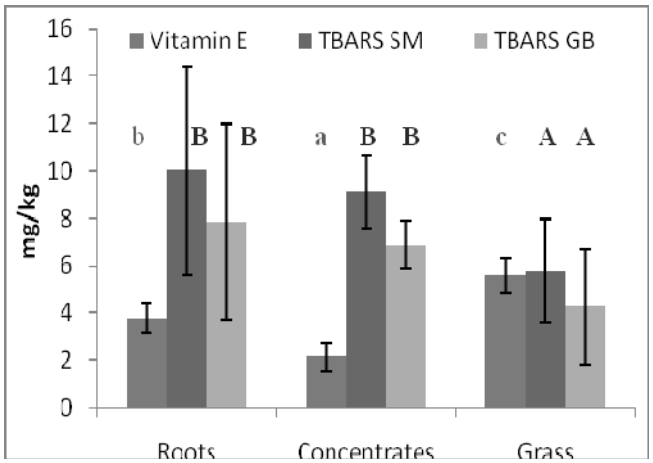

Figure 2
Vitamin E (mg/kg) and TBARS (mg/kg) in lamb leg (means ± sed)

Conclusions The higher levels of vitamin E found in muscle from grass-fed animals would explain the better colour shelf-life and lower TBARS seen in meat from this group. Although previous work has shown that vitamin E concentrations in loin muscle between 3and 4 mg/kg produce optimum colour shelf life, the SM value of 3.8 in root-fed lamb muscle was insufficient when displayed in MA as evidenced by the lack of difference in chroma value and TBARS between root- and concentrate-fed groups. These results suggest that finishing on grass, where available, over the winter period or supplementing with high doses of Vitamin E would ensure longer shelf life for meat from late-finished lambs.

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027

Incorporation of chlorophyll based markers into lamb finishing rations to aid visualization of faecal contamination in the abattoir – a spectrometric approach

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Introduction Contamination of carcasses with faecal matter commonly occurs during skinning and evisceration of the animal (Natasijevic *et al.,* 2009). Faeces of ruminants can harbour pathogens like *E.coli* which may cause illness in humans (LeJeune *et al.,* 2006). Small areas of faecal contamination may be undetectable by the naked eye and may harbour potential pathogens. Previous studies at IBERS have shown that fluorescent markers can be used to improve detection of contamination (Lee *et al.,* 2010). In this study the potential of concentrate feeds incorporating the markers against a control (marker free concentrate) to increase fluorescent intensity of the faeces to aid spectral imaging detection were determined.

Material and methods Eight male Cheviot sheep which were maintained on pasture till July 2010 were housed individually and fed control concentrate and barley straw (marker free diet) for 7 days to act as a wash-out period. The animals were then allocated at random to one of the four treatment concentrates containing either: Magnesium chlorophyllin (MgC, 1 g/kg dry matter (DM); Chlorophyll extract (CE, 10 g/kg DM); Chlorophyll extract+ dry grass fibre (CE+F, 100 g/kg DM) and Control (C, 0 g chlorophyll). The experiment was a replicated 4x4 Latin square design. Each period contained two weeks – a dosing week and a wash-out week. During the dosing week, animals were offered *ad libitum* barley straw and concentrate at 30% above maintenance. Faeces were collected on the last day of each washout period and on every day during each dosing period. Animals were moved temporarily from pens to a clean concrete floored collection area and left for up to 2 hours, after which time clean faeces (no food or bedding or urine contamination) were collected. The frozen faecal samples were thawed and spread uniformly in a black weighing boat and fluorescence excited by illumination at 395nm was measured using a spectrometer (Ocean Optics, Reading, UK, model SD2000). Data were analysed using general ANOVA with treatment*time as the fixed effect and blocking according to period.

Results Faeces from animals fed CE, CE+F and MgC had significantly ($P<0.001$) higher fluorescent intensity than animals fed the control diet (Figure 1). The faecal fluorescence reached maximum levels after 2-3 days of feeding the markers. The marker MgC has a unique peak position (671nm) compared to the other chlorophyll compounds.

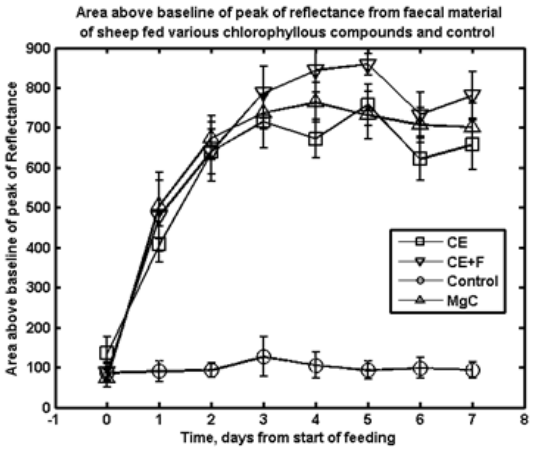

Figure 1 Faecal fluorescence over a period of 7 days

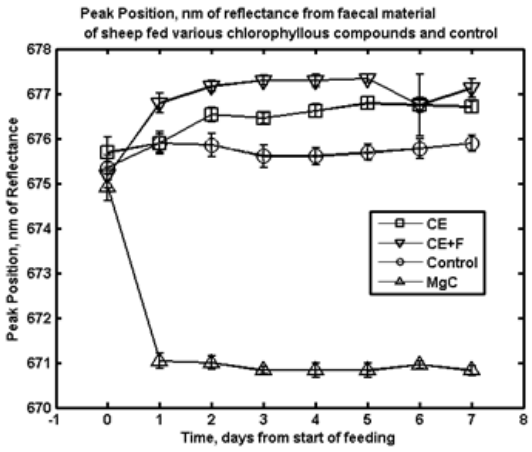

Figure 2 Fluorescence wavelengths of the markers and control

Conclusions Inclusion of chlorophyll based markers in concentrate diets significantly increased the fluorescence intensity of the faeces. This has the potential to improve faecal detection in the abattoir through spectral imaging and thereby provide safer food to the consumer. The project also aims to develop a visualisation system to detect the fluorescence emitted by MgC. It has now become compulsory throughout EU that all the meat industries should follow hygiene principles based on HACCP (FSA, 2010 -EU regulation 852/2004, Article 5). The increased sensitivity of faecal detection with the use of fluorescent markers along with appropriate on-line detection systems may become an important step in HACCP based systems in the abattoir.

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Microbial load of cheese produced from differently processed White Fulani cow milk

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Introduction Milk and its products are the most suitable media for the growth of micro-organisms. The problem of proliferation of different types of micro-organisms ranging from spoilage to pathogenic micro-organisms in milk and milk products has been a source of concern over the years. Hence, in most countries of the world, milk is pasteurised before consumption or made into products like cheese. However, some people believe that in processing milk into cheese, the former is heated to pasteurisation temperature and hence, boiled so; most pathogenic micro-organisms would have been killed by the time cheese is formed. The aim of this study therefore is to assess the effects of different processing methods on the microbial load of cheese.

Material and methods Fresh milk was collected by hand milking from 30 White Fulani Cows in mid-lactation and processed by: blending using a high speed mixer for 5 minutes (BM), fortification with skimmed milk at 6% w/v (MFSM), pasteurisation (at 62-66^{0}C for 30 minutes and rapidly cooling to 4^{0}C; PM) Blending followed by pasteurisation (BPM) and Skimmed Milk Fortification followed by Pasteurisation (SMFPM). Fresh Milk (FM) served as the control. West African soft unripened White Cheese was then made from these differently processed milks using extract from medium sized (8 x 15cm) *Calotropis procera* leaf juice as milk coagulant. Cheese was coagulated from 1000ml fresh milk using 7.50ml leaf extract i.e. 0.75% v/v (Ogunleke *et al*., 2009). The procedure for soft cheese manufacture was as outlined by Ogundiwin and Oke (1983).The process of cheese manufacture took 40minutes. Each treatment was replicated four (4) times. Using serial dilution method, cheese samples were plated on Nutrient Agar, MacConkey Agar and Potato Dextrose Agar to obtain the Total Viable count (TVC), coliform count and fungal counts respectively (Olutiola *et al*., 1991) 2 hours post cheese preparation. Data obtained were subjected to statistical analysis in a completely randomized design using the ANOVA procedures of SAS (1999). Treatment means of significant dependent variables were compared by Duncan's option of SAS (1999) ANOVA procedures.

Results Cheese produced from FM had the highest ($P < 0.01$) TVC of 2.77 x 10^5 cfu/g and coliform count of 1.20 X 10^5 cfu/g. This was followed by cheese produced from BM having TVC of 0.93 x 10^5 cfu/g. MFSM, PM, BPM, and SMFPM had nil TVC and coliform counts. The growth of fungi was not detected in any of the cheese samples.

Table 1 Microbial load of fresh cheese produced from differently processed milk

Milk type	TVC x 10^5 cfu/g	Coliform count x 10^5 cfu/g	Fungal count x 10^4 cfu/g
FM	2.77^a	1.20^a	nd
BM	0.93^b	0.17^b	nd
MFSM	nd^c	nd^c	nd
PM	nd^c	nd^c	nd
BPM	nd^c	nd^c	nd
SMFPM	nd^c	nd^c	nd
sem	0.02	0.04	nd

^{abc}: means with different superscripts within a column are significantly different ($p<0.01$): nd: below the detection limit
sem: Standard Error of mean

Conclusion The results show that milk fortification with skimmed milk, milk pasteurisation, milk blending followed by pasteurisation, and skimmed milk fortification followed by pasteurisation effectively suppressed microbial growth in cheese. Although milk blending significantly ($p<0.01$) reduced Total viable and coliform counts in cheese, it was however not as effective as the former methods.

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029

The effects of genotype and dietary lysine concentration on growth performance of weaner pigs

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Introduction Crossbreeding is common practice in commercial pig production and is used to improve heterosis; increasing litter size, robustness and productivity. Traditionally, the most commonly used commercial cross in the UK was a Large White terminal sire over a Large White X Landrace dam. More recently there has been an interest in other terminal sire breeds such as the Hampshire and Pietrain due to improved production and increased resistance to disease (BPEX, 2009). The aim of this study was to look at the effects of genotype and dietary lysine content on growth performance during the immediate post weaning stage and to identify whether different pig genotypes respond differently to varying lysine concentrations.

Material and methods A total of 708 piglets were used. All piglets had a Large White x Landrace dam but were sired by a Hampshire (234), Pietrain (234) or Large White (240) boar. Piglets were weaned at 26.8±1.45 days of age at a mean weight of 8.4±0.83 kg and placed on trial for 20 days. Piglets were weaned into fully slatted weaner accommodation and divided into six or eight piglets/pen, giving 10 replicates for each treatment. Pigs were given *ad-libitum* access to one of three diets differing in lysine content; high (H), medium (M) and low (L) (18.2, 16.2 and 14.2 g lysine /kg respectively). All pigs were individually weighed at day 0 (weaning), 7, 14 and 20. Average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were recorded weekly. General pig health was recorded daily and a daily total pen health and faecal score was given on a scale of 1-4. Data was analysed as a 3 x 3 factorial design.

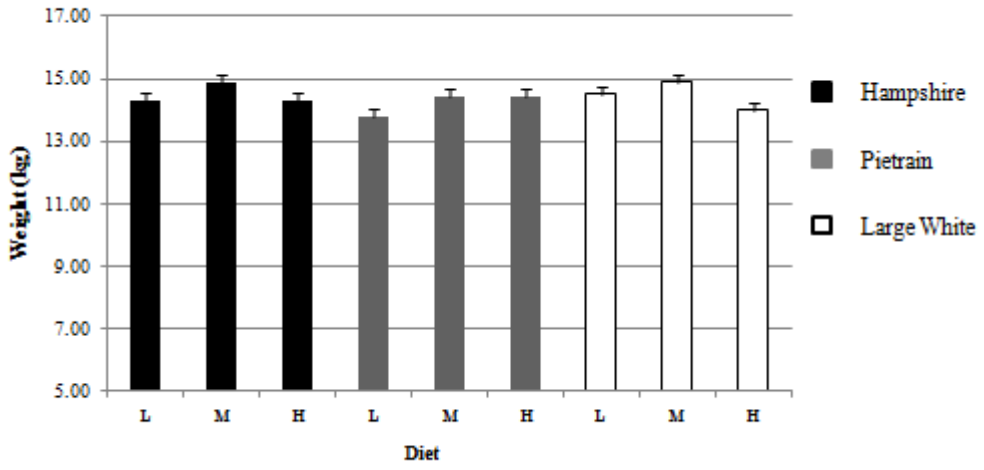

Figure 1 End weight (kg)

Results No difference in feed intake was observed between genotypes. Genotype did not affect piglet growth rate in the first two weeks of the trial. However in the third week, Hampshire and Large White piglets grew more rapidly than Pietrain piglets ($P<0.001$). Pietrain piglets also had a poor FCR in week three compared to Hampshire and Large White piglets ($P=0.001$). This difference did not affect overall performance as Genotype did not affect ADG over the whole 20 day trial period. Pigs fed the M diet completed the trial an average of 0.6 kg heavier (Figure 1; $P<0.05$) than those receiving either the H diet or the L diet. Piglets ate less of the H diet in weeks two and three of the experiment ($P<0.005$; $P<0.005$). FCR was highest for piglets fed the L diet compared to piglets fed the M and H diets ($P<0.001$). Pietrain piglets performed equally as well on both the H and M diets compared to the other genotypes for which the M diet produced the heaviest pigs ($P<0.1$). There was no difference in performance between male and female piglets.

Conclusions The results of this study suggest that in the first three weeks after weaning, lysine requirement is not substantially different between genotypes within the same environment, with 16.2 g/kg being optimal across all three genotypes.

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Prediction of digestible phosphorus and calcium retention and requirements for different pig genotypes

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Introduction Accurate knowledge of digestible phosphorus requirements ($digPh_{req}$) would allow the supply of digestible phosphorus (digPh) in a manner that more closely matches pig requirements. An accurate knowledge of digestible calcium requirements ($digCa_{req}$) is also important, because Ca binds with Ph to form an indigestible Ca-Ph complex. The accurate knowledge of $digPh_{req}$ and $digCa_{req}$ would therefore minimize Ph excretion and prevent the addition of unnecessary expensive inorganic Ph, so decreasing feed costs. The objective of this work was to develop a model to predict the $digPh_{req}$ and $digCa_{req}$ for different pig genotypes and compare these with the BSAS (2003) standards for the same genotypes. In addition, predictions were made for Ph and Ca retention, when diets were first limiting in protein.

Material and methods Maintenance requirements for both Ph (MPh_{req}) and Ca (MCa_{req}) were modelled as functions of the current protein (Pr) and mature protein mass (Pr_m). This was expected to be an advance over the expression of M_{req} as a function of liveweight (LW). The parameters of the MPh_{req} and MCa_{req} functions were estimated from Jongbloed (1987) and Letourneau *et al*. (2011), respectively. The efficiencies of Ph and Ca utilization (e_{Ph} & e_{Ca}) for maintenance were assumed to be close to unity, whereas the e_{Ph} above maintenance was estimated from Pettey *et al*. (2006) and Rodehutscord *et al*. (1999) and e_{Ca} was assumed to be the same as e_{Ph}. Body Pr content data were used to estimate the maximum Ph and Ca retention in the body (PhR_{max} & CaR_{max}) when pigs were fed a balanced diet, using isometric equations. Predictions for $digPh_{req}$ and $digCa_{req}$ for different genotypes were made and compared with BSAS (2003) standards for the same genotypes. Retention of Ph and Ca when pigs were fed insufficient lysine was also estimated and the composition of ash in Ph and Ca was assumed to be constant.

Results MPh_{req} and MCa_{req} were estimated to be $0.1293*Pr*Pr_m^{-0.27}$ and $0.2772*Pr*Pr_m^{-0.27}$ (g/day), respectively. The e_{Ph} and e_{Ca} above maintenance were estimated at 0.9; they were assumed to be constant across LW and genotypes. The PhR_{max} and CaR_{max} were found to be isometrically related to Pr: $PhR_{max}=0.0337*dPr/dt$ and $CaR_{max}=0.0553*dPr/dt$ (g/day), respectively. The predicted dPh_{req} for the three BSAS (2003) genotypes is shown in Figure 1 and their comparison with BSAS (2003) is in Table 1. When the ratio tlysine : Ash was below 0.45 g/g in the diet, the Ph : Pr ratio of empty body weight (EBW) was estimated: 0.06 - (0.04 x tLysine:Ash diet). Above the 0.45 threshold of tLysine:Ash in the diet, the Ph : Pr ratio in the body was constant at 0.04.

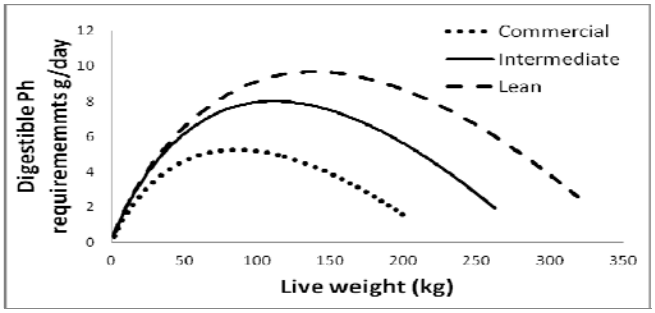

Figure 1 The $digPh_{req}$ for the three BSAS (2003) pig genotypes. The lean, intermediate and commercial pigs have: 50,40,30 kg Pr_m, and 55,48,39kg lipid respectively.

Table 1 New standards for $digPh_{req}$ and $digCa_{req}$(g/kg dry feed) compared with the intermediate pig genotype $digPh_{req}$ standards of BSAS (2003)

	Growers : **30-60 kg** LW	Growers: **60-90 kg** LW	Finishers: **90-120 kg** LW
$digPh_{req}$ BSAS (2003) intermediate pig genotype	2.5	2.4	2.2
New $digPh_{req}$ intermediate pig genotype	3.0	3.0	2.9
New $digPh_{req}$ commercial pig genotype	2.4	2.2	1.9
New $digCa_{req}$ intermediate pig genotype	4.8	4.8	4.5
New $digCa_{req}$ commercial pig genotype	4.0	3.7	3.2

Conclusion Compared to our findings, the BSAS (2003) $digPh_{req}$ are moderately underestimated, for pigs of an intermediate genotype, while, for the commercial BSAS (2003) pig genotype the same standards overestimate $digPh_{req}$. This is the first time $digCa_{req}$ has been estimated. When a diet is insufficient in protein the Ph:Pr ratio in the body increases, indicating continuing bone development. The current challenge is to predict the amounts of dPh and dCa intake from total dietary Ph and Ca. This will lead to robust predictions of retention and excretion of both these minerals.

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The effect of particle size and feed form on finishing pig performance

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Introduction Pelleting has been found to promote an improved feed efficiency compared with feed in meal form (Wondra *et al*., 1995). Particle size has also been found to affect the feed conversion efficiency of pigs (Wondra *et al*., 1995). However, few studies have investigated the combined effects of particle size and feed form. This study aimed to investigate the effects of feed offered in meal or pellet form with a fine or coarse particle size profile.

Material and methods Two trials were conducted, one on a research farm and a second on a commercial farm. In trial 1 (research farm) a total of 640 pigs were used in a 2 x 2 factorial design, over 8 replicates (8 time periods). At each time point pigs were weighed at 12 weeks of age and balanced onto treatment according to weight and sex (boars and gilts) and penned in groups of 20 (32 groups formed in total). Pigs were then offered one of the four dietary treatments. A finisher diet was formulated to contain digestible energy 13.6MJ/kg, crude protein 167g/kg and total lysine 9.6g/kg with the main ingredients being barley (41%), wheat (36%) and soya (19%). Dietary treatments involved how the finisher diet was processed. The coarse particle size diet was manufactured using a combination of two 14mm screens plus four 10mm screens. The fine particle size diet was manufactured using six 4 mm screens. No further processing was involved-for the meal diet and the pelleted diets were made using a Die size of 3.5mm, pressure at 2 Bar and a temperature of 70-75°C (Model of the press was CPM7932/11). Pigs were weighed and pen feed intakes were recorded at 15, 18, 20 weeks of age and finish (target 105kg). In trial 2 (commercial farm) only the effect of feed form was tested (i.e. meal vs pellets) using diets with a 'fine particle size'. At approximately 12 weeks of age pigs were weighed and transferred to finishing accommodation. At this stage they were balanced onto treatment according to weight and sex and penned in groups of 15 (a total of 120 pigs over 8 groups were used). Pig weight was taken at finish (target 105kg) and pen feed intake was recorded during the finishing period. In both trials pigs were offered feed through a wet and dry single space feeder (Verba). Two feeders were placed back to back in the trial on the research farm with 20 pigs per pen and 1 per pen in the commercial herd with 15 pigs per pen. In both trials the stomachs of a proportion of pigs (balanced for weight and sex) were examined for ulceration. The cold weight and back fat depth at P_2 (65 mm from the midline at the level of the last rib) was taken using the Ulster Probe 45 minutes after slaughter. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated on a per pen basis in both trials and data was statistically analysed using analysis of variance using a randomised block design (blocked for replicate) and the treatments in a 2 x 2 factorial arrangement.

Results The 'fine particle size' diet contained 0.5% particles >2mm; 12.7% 1.4 – 2mm; 59% 0.5 – 1.4mm and 27.7% under 0.5mm. The 'coarse particle size' diet contained 6.7% particles >2mm; 34.0% 1.4 – 2mm; 41.2% 0.5 – 1.4mm and 18% <0.5mm. In trial 1 (research farm) there was no significant interaction between particle size and feed form on pig or carcass performance. On the research farm, pigs that were offer a pelleted diet had a significantly improved FCR compared with pigs offered a meal diet. Pigs offered a diet with a 'fine particle size' also had an improved FCR compared with pigs offered a 'coarse particle size' diet (Table 1). No significant effect of feed form was found on the commercial farm but variation on the farm within the treatment groups was high as indicated by the SEM values (Table 1). Stomach ulceration was not apparent on either farm.

Table 1 Effect of feed form and particle size on pig performance

	Research Farm								Commercial farm			
	Meal	Pellets	SEM	P Value	Coarse	Fine	SEM	P Value	Meal	Pellets	SEM	P Value
Start weight (kg)	41.5	41.4	0.25	NS	41.3	41.6	0.25	NS	54.7	54.6	3.14	NS
End Weight (kg)	104.6	105.0	0.54	NS	104.1	105.5	0.54	NS	110	108	2.68	NS
ADG (g/day)	913	921	7.8	NS	910	925	7.8	NS	891	871	30.0	NS
ADFI (g/day)	2453	2347	25.1	<0.01	2412	2388	25.1	NS	2461	2311	65.4	NS
FCR	2.69	2.55	0.022	<0.001	2.66	2.58	0.022	<0.05	2.76	2.69	0.078	NS
KO%	75.9	75.9	0.26	NS	75.9	75.8	0.26	NS	75.8	76.7	0.409	NS
Back fat depth (mm)	12.7	12.9	0.20	NS	12.6	13.0	0.20	NS	14.2	13.8	0.373	NS

Conclusions No effect of feed form was found on the commercial farm, although it was noted that a numerical 3% improvement in FCR was present when feed was offered in pellet form compared with meal form. The variation within treatment on the commercial farm was two to three times greater than that observed on the research farm. It is likely that this contributed to the lack of significance observed on the commercial farm. Overall, the effects of pelleting and fine grinding individually improved feed conversion efficiency by 5.2 and 3 % respectively on the research farm. Furthermore, since no interactive effects were observed it could be concluded that the effects were cumulative.

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Lifetime performance of gilts bred on first to fifth observed oestrus

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Introduction Current recommendations are to inseminate gilts on their second or third observed oestrus to ensure that gilts have reached sufficient maturity and have adequate body reserves in order to sustain reproductive performance over subsequent parities (Close and Cole, 2000; Whittemore, 2006). Delaying breeding has the financial implications of increased non-productive days and extra feed costs. However, these could be offset by increased fertility and/or increased lifetime productivity. The aim of this study was to compare the lifetime performance of sows when they were inseminated on their 1st to 5th observed oestrus.

Material and methods In total, 157 gilts born between 2002 and 2006 were inseminated on either their 1st (n=19), 2nd (n=35), 3rd (n=51), 4th (n=30) or 5th (n=22) observed oestrus. Gilts were home bred and reared on the research herd at AFBI Hillsborough. The breed of gilts were F1 Landrace × Large White (n = 124) and F2 (Landrace x Large White) x Landrace (n = 33), these were evenly balanced across treatments. Gilts were offered a gilt developer diet (13.16 MJ/kg DE, 129.8 g/kg CP, 5.81 g/kg lysine) *ad libitum* from 65 kg until insemination. After which, gilts were offered the gilt diet at a rate of 2.2 kg/d until d 110 of gestation. From parity 2, all sows were offered a dry sow diet (13.21 MJ/kg DE, 140.7 g/kg CP, 6.04 g/kg lysine) at an approximate rate of 2.5 kg/d ± 0.5 kg (depending on condition score) during gestation. During lactation, both sows and gilts were offered a commercial lactation diet (13.5 MJ DE, 10.0 g/kg lysine, 180 g/kg CP). Litter and reproductive data were collected on a continual basis until all animals had completed their reproductive life. Piglets were recorded as being born alive or dead at birth and all piglets were individually weighed and recorded within 12 hr of birth and again at weaning (d 28 ± 2). Sow age at culling and the number of parities completed was recorded. Data were analyzed using regression analysis in Genstat version 12.1 with breed of sow and year of birth as covariates. Fisher's Least Significance Difference Test was used to assess pairwise differences between treatment means.

Results The average age of gilts served on their first observed oestrus was 210 days and as expected, insemination age increased significantly (P<0.05) as the number of observed oestrous cycles increased However, there were no significant differences (P> 0.05) between the weight of gilts at day 110 of gestation (average 196 kg). The observed oestrus that gilts were served on had no effect (P> 0.05) on numbers of piglets born alive (11.23 ± 0.638 piglets), dead (0.62 ± 0.159 piglets), total born (11.85 ± 0.657 piglets), total weight born (16.63 ± 0.754 kg) or average birth weight (1.43 ± 0.0439 kg) in the first litter. Furthermore, there was no significant effect of treatment (P> 0.05) on pre weaning mortality (0.12 ± 0.024 %), the number of piglets weaned (10.22 ± 0.607), average wean weight (8.89 ± 0.252 kg) or total weight of piglets weaned (90.46 ± 2.94 kg) in the first litter. Observed oestrus number at insemination had a significant effect on the total number of completed parities (P= 0.024) but not on age at cull (P> 0.05) (Table 1). Gilts inseminated on their first, second and third observed oestrus completed a greater number of parities than gilts inseminated on their fifth observed oestrus (P< 0.001) while those inseminated on their fourth were intermediate (Table 1). Observed oestrous number had a highly significant effect on total number of piglets born alive and dead, and total number of piglets weaned over the lifetime of the sows (all P< 0.001). However, this was not reflected in the total weight of piglets born alive, born dead or weaned (P> 0.05) (Table 1).

Table 1 Lifetime performance of sows when inseminated at different observed oestrus cycle numbers when gilts

	Observed oestrus number						
	1st	2nd	3rd	4th	5th	s.e.m.	P
Number of completed parities	6.3^{b}	6.0^{b}	6.6^{b}	5.4^{ab}	4.6^{a}	0.46	0.024
Age at culling (d)	1183	1127	1246	1173	1029	93.6	0.53
Number of piglets born alive	71.8^{c}	72.7^{c}	78.7^{d}	64.8^{b}	53.3^{a}	1.56	<0.001
Number of piglets born dead	7.1^{c}	5.1^{b}	5.0^{b}	4.8^{b}	3.5^{a}	0.43	<0.001
Number of piglets weaned	59.1^{cd}	56.7^{c}	61.4^{d}	49.7^{b}	42.5^{a}	1.40	<0.001
Weight born alive (kg)	105.0	107.2	118.7	94.7	80.1	11.73	0.171
Weight born dead (kg)	7.4	6.0	6.0	5.6	4.1	1.25	0.648
Total weight weaned (kg)	505	488	526	446	377	53.2	0.329

^{a,b,c} numbers with common superscripts are not significantly different (P>0.05)

Conclusions Observed oestrus cycle number at first insemination had no effect on first litter performance. However, inseminating gilts on their third observed oestrus optimised lifetime performance since these gilts both completed the greatest number of parities and produced the greatest number of pigs born alive. Delaying breeding after the third oestrus had a detrimental effect on lifetime performance and would have negative economic implications.

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Changes in commercial stud boar semen abnormality types throughout the year

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Introduction Boar semen quality is known to change throughout the year, with the total percentage of abnormalities increasing during the late summer and autumn months (Murase *et al.*, 2007). However little data is available in the literature on changes in specific types of abnormality under commercial conditions during differing months of the year. Therefore the aim of this work was to describe proportional changes in boar semen abnormality types throughout the year for different breeds and ages of boars.

Methods Semen abnormality data from three UK boar studs was collated and analysed according to month of the year, boar breed and age of boar at the time of collection. Age of boars in days was rounded to the nearest year for analyses, producing six age groups (one to six). The overall dataset contained 50,493 ejaculates from 1,043 boars of eight different breeds, over five years from 2005 to 2009. The semen abnormalities considered were; detached and malformed heads; damaged acrosomes; bent and coiled tails; proximal and distal droplets; any other abnormalities were grouped together as unclassified. Stud management was similar across all sites and years, with boars housed in buildings with little environmental control and the boars were worked on average twice fortnightly. Statistical analyses were carried out in MATLAB 7.11.0 (R2010b). Averages were calculated for each abnormality type and analysis of variance was applied to determine possible effects of month of collection, breed of boar and age of boar.

Results Spring and summer months were found to have higher proportions of detached and malformed heads ($P < 0.001$; Table 1), whereas abnormalities of the tail such as bent, coiled and proximal or distal droplets occurred more frequently during late summer and autumn months ($P < 0.001$). Damaged acrosomes occurred infrequently. Hampshire boars were found to suffer from the highest proportion of four different types of abnormality, although no clear trend in abnormality type by breed was identified. Younger boars generally had fewer abnormalities ($P < 0.001$) with the exception of distal droplets although not all abnormality types were affected by age. Interactions between age, month of collection and breed were found for several abnormality types ($P < 0.001$). Doses which were used were found to contain a significantly higher proportion of all abnormality types than discarded doses.

Table 1 Changes in abnormality type by month of the year

Abnormality	Month											
(%)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Detached head**	0.41^{ae}	0.44^{ac}	0.42^{ad}	0.44^{acd}	0.47^{bcde}	0.47^{bcd}	0.46^{bcd}	0.52^{bc}	0.49^{bcd}	0.42^{ae}	0.43^{ae}	0.37^{a}
Malformed head**	1.08^{bc}	1.12^{acd}	1.00^{bd}	1.19^{ace}	1.24^{ac}	1.27^{a}	1.24^{ac}	1.24^{ac}	1.14^{acd}	1.12^{acd}	1.04^{bde}	1.04^{bde}
Dam. acrosome*	0.00^{b}	0.01^{ab}	0.01^{ab}	0.01^{ab}	0.01^{ab}	0.02^{a}	0.01^{ab}	0.01^{ab}	0.01^{ab}	0.02^{a}	0.02^{a}	0.01^{ab}
Bent tail**	2.39^{cd}	2.02^{bd}	1.96^{bf}	1.84^{b}	1.99^{bf}	2.19^{def}	2.55^{ch}	2.65^{ac}	2.75^{ah}	2.66^{ac}	2.89^{a}	2.42^{ceh}
Coiled tail**	0.25^{ad}	0.22^{d}	0.21^{d}	0.23^{cd}	0.25^{ad}	0.24^{bcd}	0.27^{ad}	0.29^{ab}	0.31^{a}	0.24^{bcd}	0.29^{ac}	0.27^{ad}
Prox. droplet**	4.25^{def}	4.12^{ef}	4.12^{f}	4.57^{de}	4.59^{cd}	4.71^{cd}	5.33^{ab}	5.73^{a}	5.21^{b}	5.05^{b}	4.98^{bc}	4.50^{def}
Dist. droplet**	5.62^{abd}	5.15^{bc}	5.12^{c}	5.20^{ac}	5.63^{ad}	5.96^{de}	6.53^{f}	7.03^{g}	7.17^{g}	6.90^{fg}	6.98^{g}	6.24^{ef}
Other^{NS}	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02	0.02

Monthly values within rows for each abnormality type superscripted with different letters are significantly from each other (^{NS} Non-significant, * $P < 0.01$, ** $P < 0.001$)

Conclusions The amount of spermatozoa abnormality types present in the ejaculates of boars kept on commercial studs not only differed with the breed and age of the boar but also with month of the year. Ejaculates collected in summer and autumn months had increases in some abnormality types, suggesting that decreasing photoperiod and elevated temperatures were disrupting spermatogenesis and causing spermatozoa to become damaged or not to develop properly. It would therefore be advisable to provide methods for cooling down working boars when temperatures are raised. The data suggest that some abnormalities are more prevalent than others and so during the assessment of semen it may not be necessary to focus on all abnormality types, with only some of them occurring frequently enough to cause fertility problems. In addition seasonal boar culling strategies should consider boar age, as two to four year old boars produced the least abnormalities in the summer and autumn. Further work relating this to specific weather conditions may help elucidate whether temperature or day length changes play a larger role.

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Effectiveness of starter diets pre and post weaning on lifetime pig performance

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Introduction Creep and starter diets are the most expensive diets within pig production but the effect of creep diets offered pre weaning on pig performance post weaning are not consistent. The objective of the current study was to investigate the effects of offering creep feed pre weaning followed by a higher starter 1 diet allowance post weaning on the lifetime performance of pigs.

Material and methods In total 320 pigs were used in a 2 x 2 factorial design over 10 replicates. Litters of pigs either received creep pre weaning (29 Litters) or not (22 Litters). After weaning pigs received 2 or 6kg per pig of starter 1 diet. Starter 1 diet was used as the 'creep diet' pre weaning and contained 225g/kg crude protein, 17g/kg total lysine and 16.5MJ/kg digestible energy. In descending order of inclusion, the main ingredients in the starter 1 diet were cooked extruded soya, cooked wheat, cooked oats, whey powder and lactose in starter 1 diet. All pigs were then offered 6kg/pig of a starter 2 diet (215g/kg crude protein, 15.5g/kg total lysine and 15.5MJ/kg digestible energy). The starter 1 and 2 diets also contained 3.1kg/tonne of Zincotec. When pigs had finished their starter diet allowances, they were offered a grower diet to 12 weeks of age and then a standard finisher diet to slaughter (target 100kg). Pigs were weighed at birth and 10 days before weaning at which stage litters were balanced onto treatment (creep or not) according to their total number, total weight and average piglet weight. Where applicable starter 1 diet (with chromic acid) was offered *ad libitium* as 'creep feed' for a period of 10 days before weaning. Creep feed intake was recorded for the 10 day period and when pigs were 23, 25 and 27 days old the colour of their faeces was examined on an individual basis after extraction using a fecal loop. Pigs with green faeces on 2 out of the 3 occasions were classified as 'eaters' and these pigs were subsequently used post weaning. At weaning (28 days of age (+/- 2d)) pigs were weighed and balanced onto 'starter 1 diet allowance' treatment according to weight, sex and litter origin. Pigs were penned in groups of eight and the feed intake of each pen of pigs was recorded on a daily basis for 12 days after weaning. Pigs were individually weighed and pen feed intakes were recorded at 5, 6, 7, 10, 15, 20 weeks of age and slaughter. Average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated on a per pen basis. Data was statistically analysed using analysis of variance.

Results Average litter size was 10.4 pigs weaned and 83% of pigs offered creep were found to have consumed creep. Litters that received creep ate an average of 6.2kg (SD 3.3 kg) over the 10 day period pre weaning. However, offering creep had no significant effect on the growth rate of pigs over the 10 days pre weaning (305g/day). Creep feeding pre weaning had no significant effect on pig weight, growth rate or feed conversion ratio at any stage of growth between weaning and slaughter. However, pig feed intake was significantly higher at several time points during the 12 days after weaning (Table 1). There was no significant effect (P>0.05) of starter 1 diet allowance on the weight of pigs at 10 or 20 weeks of age (average 30.3 and 87.7kg respectively). However, pens of pigs that were offered 6kg/pig of starter 1 diet had a better ADG and FCR between 5 and 7 weeks of age compared with pigs offered 2kg/pig (Table 2). Overall, between weaning and 20 weeks of age, pigs that received 6kg of starter 1 diet had an improved ADG and tended to have an improved FCR (Table 2).

Table 1 Effect of creep feeding pre weaning on feed intake (g/pig/day) for 12 days after weaning

	Day post weaning											
	1	2	3	4	5	6	7	8	9	10	11	12
Creep	2.45	56.0	140	197	240	303	342	376	394	465	493	530
No creep	2.43	39.6	113	167	193	257	314	365	373	434	462	495
SEM	0.875	8.24	8.2	10.4	11.6	9.6	11.7	14.8	13.2	11.3	9.5	10.7
P value	NS	NS	<0.05	0.053	<0.01	<0.01	NS	NS	NS	0.059	<0.05	<0.05

Table 2 Effect of starter 1 diet allowance on pig performance between wean (Wn) and 10 and 20 weeks of age

	ADG (g/day)			ADFI (g/day)			FCR		
	5-7 wks	Wn–10	Wn-20	5-7 wks	Wn–10	Wn-20	5-7 wks	Wn–10	Wn-20
2kg	448	494	702	519	722	1490	1.16	1.47	2.13
6kg	486	517	725	516	719	1505	1.06	1.39	2.08
SEM	9.3	8.2	7.4	10.0	11.9	20.3	0.020	0.019	0.017
P value	<0.01	0.052	<0.05	NS	NS	NS	<0.01	<0.05	0.054

Conclusions While creep feeding pre weaning increased feed intake post weaning it did not equate to better growth performance post weaning. On the other hand, when a higher allowance of starter 1 diet was offered, lifetime benefits in growth rate and feed efficiency were found. However, offering a higher allowance of starter 1 diet represents a higher feed cost so the production performance benefits should be weighted against this extra feed cost.

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The effect of *ad libitum* feed post mating on the reproductive performance of gilts

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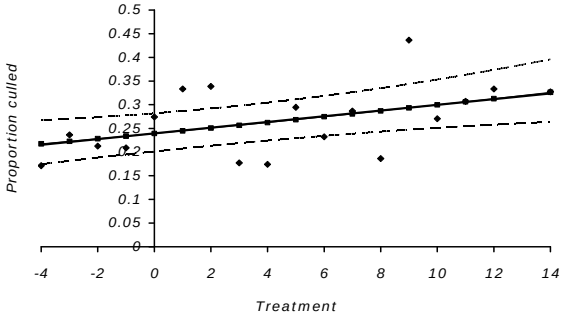
Introduction Offering gilts a high feed allowance pre-insemination has been shown to maximize ovulation rate (Aherne and Kirkwood, 1985). However, results from studies examining the effect of offering gilts a high plane of nutrition post insemination are inconsistent. For example, Jindal *et al*. (1996) found that continuing high plane feeding after mating increased embryo mortality, whereas Quesnel *et al.*, 2010 found no such effect. In the work by Jindal *et al.* (1996) and Quesnel *et al.* (2010), gilts were slaughtered on days 25 – 31 of gestation and this eliminated the capture of litter performance data. The aim of this study was to investigate the effect of *ad libitum* feeding post insemination on litter size at farrowing and on farrowing rates of gilts**.**

Material and methods In total, 1730 gilts on a large commercial herd were allocated to one of 19 treatments over 7 time replicates. During a 19-day period, all gilts were fed *ad libitum* for the first 14 days and for the remaining 5 days they were restrictively fed at 26 MJ DE/day. Gilts were served continuously over this 19 day period and treatment allocation was based on the duration of *ad libitum* feeding each gilt received. Due to the large number of animals involved, each day was considered a separate treatment. Therefore, there were 19 treatments which included *ad libitum* feeding for 1 to 14 days post insemination and restrictive feeding for 0-4 days pre insemination. For ease of interpretation, treatments will be called -4 – 14 days *ad libitum* feeding. Gilts were inseminated twice, once on the day of showing strong standing oestrus and a second time the subsequent day. All gilts were inseminated with AI (using the terminal Pietrain sire line PIC 408, Pig Improvement Company, Fermoy, Co Cork, Ireland). Gilts were kept in loose groups of 60 and liquid fed (1:5.5 mix ratio) *ad libitum* (12.70 MJ/kg DE, 163.1 g/kg CP, 8.78 g/kg digestible lysine diet, on a DM basis). Gilts were inseminated at approximately 31 weeks of age. Any gilts that were not served after the three week period were sent for slaughter and any gilts that repeated during gestation or were found to be not pregnant when pregnancy scanned at one month after insemination were recorded and sent for slaughter. Remaining gilts were then moved into gestation accommodation and kept in their same groups. From this stage until moving into the farrowing accommodation, gilts were restrictively fed 26 MJ DE/day of the same liquid diet. Gilts were moved into farrowing crates on day 108 of gestation (1 week prior to farrowing) and were liquid fed a lactation diet (14.46 MJ/kg DE, 203.8 g/kg CP, 11.6 g/kg digestible lysine, on a DM basis at a 1:3.8 mix ratio) at a rate of 38 MJ/day until farrowing. Data collected included numbers of piglets born alive, dead and total number born from each litter and the proportion of gilts not farrowing (1 - farrowing rate). Data was analysed using a generalized linear mixed model in Genstat 12.1 (using an appropriate distribution and link function).



◆ observed values, ▪ predicted values with upper and lower confidence limits

Figure 1 Effect of treatment on the proportion of sows culled before farrowing (P=0.006).

Results *Ad libitum* feeding had no effect (P< 0.05) on the numbers of piglets born alive, dead or total born. The average total litter size was 10.76 piglets, the average number born alive was 9.94 piglets and the average number born dead was 0.82 piglets. However, the percentage of gilts culled before farrowing was significantly affected by duration of *ad libitum* feeding (P= 0.006) and this was found to be a linear response (Figure 1). An odds ratio of 1.031 (lower confidence limit = 1.009, upper confidence limit = 1.054) was calculated. Therefore there was a 3.1% increased risk of the gilts being culled due to infertility for every extra day of *ad-libitum* feeding. Using the predicted values in Figure 1, it is estimated that there was an 8.7% increase in the number of gilts that failed to farrow between gilts which received 0 days *ad libitum* feed compared with those that received 14 days *ad libitum* feeding.

Conclusions This study found that *ad libitum* feeding after insemination did not affect litter size at birth in gilts. However, continuing *ad libitum* feeding post service did increase the number of gilts that were not pregnant. This has significant economic consequences due to the costs associated with buying in or breeding and rearing replacement gilts. This study suggests that to increase farrowing rates of gilts, if a high plane of nutrition is offered pre insemination, it should be reduced prior to, or immediately after, insemination.

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Effect of creep diet pre weaning and starter diet allowance post weaning on pig gut structure

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Introduction Pig producers often offer creep diets pre weaning and expensive starter diets post weaning to aid gut recovery and development and ultimately optimise lifetime performance. Magowan and Ball (2012) found that creep diets offered pre weaning increased feed intake post weighing but they had no effect on lifetime growth rate. Magowan and Ball (2012) also found improved lifetime performance when a higher allowance of starter 1 diet was offered post weaning. The objective of this study was to investigate the effect of offering a creep diet pre weaning and a higher allowance of starter 1 diet after weaning on pig gut structure.

Material and methods In total 96 pigs were used in a 2 x 2 factorial design over 8 replicates. Treatments included pigs consuming creep feed pre weaning or not and after weaning pigs being offered 2 or 6kg per pig of a starter 1 diet. The diets and dietary regime used are described by Magowan and Ball (2012). Pigs were weighed at 18, 28, 35 and 42 days of age. They received creep feed from 18 days of age and were weaned at 28 days of age. Pigs which ate creep feed pre weaning (eaters) were identified from those that did not (non-eaters), as described by Magowan and Ball (2012). Pigs that were classified as 'eaters' were subsequently used. The gut structure of pigs was examined on the day of weaning, just before the weaning process commenced, and at 35 and 42 days old. Across the 8 replicates and within each time point, pigs were balanced for weight and sex. Pigs were penned in groups of ten alongside those described by Magowan and Ball (2012) until removal for analysis. Pigs were not fasted and they were stunned by captive bolt, pithed and exsanguinated by incision of the jugular veins and carotid arteries. The entire gastrointestinal tract was immediately removed and three transverse segments were taken from the proximal duodenum, (10-15 cm from the pylorus), mid jejunum, and distal end of the ileum (approximately 10-15 cm from the ileo-caecal junction). Samples were placed into 10% neutral buffered formaldehyde (pH 7.4), and fixed for 48 hours. Transverse sections were prepared using standard paraffin embedding techniques. Samples were sectioned at 5 µm thickness and stained with haematoxylin and eosin (H&E), and examined with a light microscope (x10 eyepiece and x10 objective). On each stained section villous height and crypt depth were measured using a calibrated eyepiece graticule. A minimum of 15 straight intact villi and crypt in each intestinal section were measured. Villous height was measured from the tip to the crypt-villous junction, and the depth of the crypt was measured from the crypt-villous junction to the base. Data was statistically analysed using analysis of variance.

Results There were no significant interactions between creep feeding and starter diet allowance on villous height or crypt depth at any site at any time point. There was no significant effect ($P>0.05$) of creep feeding pre weaning on the villous height or crypt depth in the duodenum (485 and 222µm respectively), ileum (319 and 150µm respectively) or jejunum (374 or 185µm respectively) just before pigs underwent the weaning process. Creep feeding or starter diet allowance did not significantly affect ($P>0.05$) the villous height or crypt depth in the duodenum (446 and 196µm respectively) or ileum (318 and 158µm respectively) when pigs were 35 days old. However, villous height in the jejunum was greater ($P<0.05$, SEM 10.3) in pigs which received no creep pre weaning (364µm) compared with pigs that consumed creep pre weaning (329µm). There was no effect ($P>0.05$) of creep feeding on crypt depth in the jejunum (189µm) or of starter diet allowance on villous height (346µm) or crypt depth (189µm) in the jejunum when pigs were 5 weeks old. When pigs were 7 weeks old villous height and crypt depth in the duodenum were greater ($P<0.01$, SEM 16.2 and $P<0.05$, SEM 5.59 respectively) when pigs were offered 6kg/pig of starter 1 diet (536 and 216µm respectively) compared with when they were offered 2kg/pig (461 and 200µm respectively). Creep feeding had no effect ($P>0.05$) on villous height (499µm) or crypt depth (209µm) in the duodenum when pigs were 7 weeks old. Creep feeding or starter diet allowance had no effect ($P>0.05$) on the villi height or crypt depth in the ileum (353 and 171µm respectively) or in the jejunum (409 and 207µm respectively) when pigs were 7 weeks old. There were no significant interactions or effect of treatment on the ratio of villi height to crypt depth at any site along the small intestine when pigs were 42 days old. However, when pigs were 35 days old the ratio of villi height to crypt depth was higher in the ileum for pigs that consumed creep and were then offered 6kg/pig compared with when pigs received no creep pre weaning (Table 1). On the other hand the ratio was higher in the jejunum for the pigs that were offered 6kg/pig after weaning but received no creep pre weaning (Table 1).

Table 1 Effect of creep feeding and starter diet allowance on the ratio of villous height to crypt depth when pigs were 35 days old

	Creep		No creep			
	2kgs	6kgs	2kgs	6kgs	SEM	P Value
Duodenum	2.14	2.36	2.30	2.36	0.093	NS
Ileum	1.93^{a}	2.10^{b}	2.03^{ab}	1.95^{a}	0.050	<0.05
Jejunum	1.84^{ab}	1.70^{ab}	1.83^{a}	1.99^{b}	0.058	<0.05

Conclusions While a few significant differences in gut structure were observed as a result of creep feeding pre weaning or starter diet allowance post weaning, it is unlikely they had a significant contribution to the lifetime improvements observed by Magowan and Ball (2012). Overall, creep feeding pre weaning and starter diet allowance post weaning had little effect on gut structure.

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A genome-wide association study of bovine tuberculosis resistance in the Northern Ireland Holstein-Friesian dairy cattle population

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Introduction Bovine tuberculosis (bTB) caused by *Mycobacterium bovis*, is a zoonotic disease which affects cattle worldwide. Diagnosis and control is based on the single intradermal comparative tuberculin test (SICTT), supported by active abattoir surveillance. Despite many years of active testing, and slaughter of test positive cattle, bTB remains endemic in both the UK and Ireland indicating a need to investigate alternative strategies. One approach, which could prove beneficial synergistically with current eradication measures, would be genetic selection for increased resistance to bTB in cattle. Exploitable genetic variation exists for resistance to bTB; the heritability on the liability scale has been estimated 0.18 in Republic of Ireland and Great Britain Holstein-Friesian (HF) dairy cows (Allen et al., 2010; Bermingham et al., 2011). However, genetic markers associated with resistance would greatly facilitate selection. The aim of this study was therefore to undertake a genome-wide association study to identify loci that are associated with resistance to bTB in HF dairy cattle.

Material and methods Blood samples from cases were sampled at slaughter. Cases were defined as cattle with a positive reaction to the tuberculin skin test and a confirmed bTB lesion. Blood samples were collected from age matched controls traced to a subset of high prevalence case herds. Controls were defined as animals which were contemporaneous to cases from high prevalence herds and exhibited multiple negative tuberculin skin tests. In total, 3,715 blood samples were collected from 464 NI dairy herds between 2008 and 2009. DNA was extracted and quantified, and 1,324 female cattle (679 cases, 677 controls) were genotyped at 777,962 SNPs. Only SNPs with a minor allele frequency >1% were retained. Following quality control, 1156 cattle (594 cases, 562 controls), and 617,610 SNPs remained for inclusion in the analysis. Mixed model association analyses, using the genomic relationship matrix to remove population sub-structure, were fitted using GenABEL. Genome and chromosome-wide significance thresholds were estimated empirically. The heritability of resistance to bTB was estimated using ASReml. The odds ratios (OR) of significant bTB-associated risk alleles were estimated in the GLM package in R.

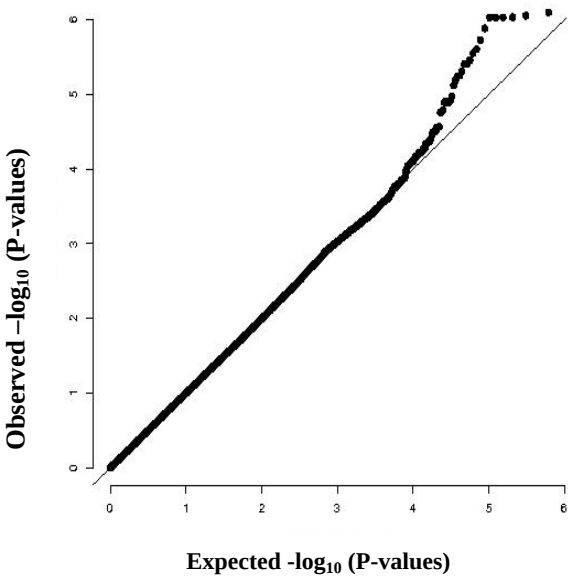

Figure 1 The Q-Q plot showing observed p-value for associations between genotyped SNPs and resistance to bovine tuberculosis (dots), compared to p-values expected under the null hypothesis of no association (diagonal line).

Results The heritability of bTB resistance was estimated at 0.21 (standard error 0.06). The number and magnitude of associations between SNPs and resistance to bTB exceeded expectation under the null hypothesis of no association (Figure 1 The signal of association across the 29 bovine autosomal chromosomes reached chromosome wise significance at the 5% level for three SNPs, and 10% level for two SNPs. The minor alleles at three of these SNPs reduced risk (OR <0.65 [95% confidence interval {CI} 0.39-0.81]) and two increased risk (OF >1.50 [95% CI 01.15-2.42]) of bTB, and together these SNPs explained 4.3% of the additive genetic variance in resistance to bTB in the NI HF dairy cattle population sample investigated in this study.

Conclusion Resistance to bTB is a moderately polygenic trait, with the five most significant SNPs associated with bTB in this study explaining 4.3% of genetic variance. These results need to be replicated across different cattle populations, phenotype definitions and *M. bovis* strain environments to provide robust evidence for association with resistance to bTB.

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Relationship between immune profile traits and functional traits in dairy cattle

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Introduction Long-term selection emphasis on milk production in the past has compromised the cows' health, fertility and welfare. Modern breeding programmes include traits linked to these important functions. However, the difficulty of on-farm recording of such traits calls for the development of alternative measurements. An approach might be to select for improved immunological performance, which requires an assessment of the association between immunological indicators and various economically important traits. The objective of this study was to estimate correlations of immune profile traits from blood sample analysis with yield, health, and reproduction traits.

Material and methods Data were from 248 Holstein cows raised on the Crichton experimental farm in Scotland. Blood of these cows was analysed for a number of traits related to the animals' immune system. Five such analyses took place at consecutive two-month intervals between July 2010 and March 2011. Blood serum traits considered were the concentrations of natural antibodies, tumour necrosis factor-alpha and haptoglobin. Circulating leukocyte traits included the percentage of peripheral blood mononuclear cells (PBMC) that were CD3+, CD4+, CD8+, γδTCR+, CD21+ or CD14+, the ratio of CD4+ to CD8+ cells within the PBMC population, the percentage of lymphocytes that were CD335+, and the percentage of total leukocytes that were lymphocytes, monocytes, neutrophils or eosinophils. The immune profile dataset was matched to individual cow records collected on the farm. These included weekly milk, fat and protein yield, milk somatic cell count (SCC), feed and dry matter intake, liveweight, and body condition score. Additional traits considered were the number of clinical mastitis and lameness episodes per lactation, and several reproductive traits (calving interval, days to 1st heat and 1st service, days between 1st and last service, number of services per conception, dystocia, and stillbirth). Correlations between immune profile and the other traits were calculated with a series of bivariate analyses based on animal repeatability models except for lactation traits with weekly repeated records per animal where a random regression model was used; the latter included third order polynomials for both the fixed curve and the random individual animal deviation. A Bonferroni correction (Holm, 1974) was implemented to statistically significant ($P<0.05$) estimates to account for multiple testing. All analyses were conducted using the ASREML software package (Gilmour et al, 2006).

Results After the Bonferroni correction, the only animal correlations that remained significantly different from zero were between the PBMC CD4+ to CD8+ ratio and SCC (-0.56±0.16 on the week of the immunological analysis and -0.44±0.13 for the average value across all weeks of lactation). This suggests that animals with inherently higher concentrations of PBMC CD4+ compared to CD8+ were associated with lower SCC and, consequently, fewer (sub)clinical mastitis cases. Phenotypic correlations that remained significant post Bonferroni correction are summarised in Table 1. Correlations with feed intake pertain to the week of the immunological analysis and are indicative of the potential impact of feed consumption on the immune profile. High levels of serum haptoglobin, an acute phase protein, were associated with response to clinical mastitis. Also increased concentrations of CD335+ lymphocytes were linked to foot infection leading to lameness. Elevated PMBC that were CD8+ were correlated with conception issues resulting in prolonged calving intervals. Finally, of interest is the association of low lymphocyte concentration with difficult calvings.

Table 1 Significant phenotypic correlations after the Bonferroni correction (standard errors in parentheses)

Immune profile trait concentration	Performance trait	Phenotypic correlation
Natural antibodies	Feed intake	-0.22 (0.04)
Lymphocytes	Feed intake	0.40 (0.08)
Haptoglobin	Clinical mastitis	0.32 (0.03)
Lymphocytes CD335+	Lameness episodes	0.47 (0.11)
PBMC CD8+	Calving interval	0.48 (0.14)
Lymphocytes	Dystocia	-0.42 (0.13)

Conclusions Immune profile traits were found to be associated with various lactation, health and reproduction traits in the cow sample population studied here. Such associations need to be verified with larger, independent datasets. Nevertheless, they provide a useful first insight into the utility of immunological measurements for the improvement of health and other important functions in dairy cattle.

Acknowledgments Crichton farm personnel for data collection; Ian Archibald (SAC) for data extraction; the Scottish Government for funding.

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Characterising heifer survival in UK dairy herds

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Introduction Premature mortality and culling causes great wastage to the dairy industry as a large number of heifers born never become productive or are culled before reaching their full lactation potential. The period prior to a cow entering the milking herd is largely unconsidered in genetic evaluations, with the exception of stillbirth and calving ease that are recorded at birth. There is a lack of knowledge on the genetic component of heifer survival from 48 hours of birth to first calving. The British Cattle Movement Service (BCMS) database traces birth, deaths and movements of all cattle in the UK and can provide useful information on longevity, such as heifer survival. The objective was to characterise data available from BCMS to provide information on heifer survival.

Material and methods Data from the BCMS database was validated, matched and combined to the databases of Milk Recording Organisations (MROs). The dataset obtained from BCMS contained only dead animals; thus live contemporaries were extracted from MROs. For this study, animals born in years 2002 to 2006 were extracted for data description and analysis. Survival of heifers at different age-points was investigated (2 to 180d, 2 to 450d, and 2 to 750d) (Table 1). Survival was treated as a binary trait (i.e. '1' if dead and '2' if live at specified age-point). Genetic parameters were estimated by univariate analyses in ASReml (Gilmour *et al,* 2006) using a sire model. The fixed effects and covariates for each trait were parity of dam (five classes: 1, 2, 3, 4, 5), age of dam at calving (in months), month of birth (12 classes), birth type (single, twin), heterosis and recombination, and herd-year.

Results In herds that recorded stillbirth the frequency of mortality was 7.4% which is similar to other UK studies (Brickell *et al*, 2007). However, on the whole stillbirth was under recorded in the dataset as it is not necessary for farmers to register dairy calves that die before tagging (i.e. within 36 hours of birth). Mortality of heifers was highest in the first month of life and tended to be highest in calves born during winter months (Figure 1). Heifer losses were 13.7% in the first 25 months of life and within the periods 2 to 28 days, 29 to 180 days, 181 to 450 days, and 451 to 750 days heifer losses were 3.1%, 5.4%, 2.5%, and 2.7% respectively. From 15 months there was a rise in numbers of heifers slaughtered at the abattoir, some of which would be due to conception failure, as indicated by insemination data which showed a proportion of these animals had been inseminated. Heritability estimates were very low (~ 1%) and similar for all survival traits but were significant ($p<0.05$) (Table 2).

Table 1 Summary of datasets for the analysis of survival

	2 to 180d	2 to 450d	2 to 750d
No. of animals	98,072	107,942	117,515
No. of sires	1,459	1,581	1,691
% dead	10.5	12.1	13.7
% dead on farm	95.6	94.8	88.9
% sent to abattoir	4.1	4.8	10.7

Table 2 Estimates of variance components of heifer survival

Trait	σ_s^2	σ_e^2	σ_p^2	h^2
2-180d	0.0002	0.0921	0.092± 0.0004	0.011± 0.003
2-450d	0.0003	0.1040	0.104± 0.0004	0.011± 0.003
2-750d	0.0004	0.1156	0.116± 0.0005	0.013± 0.003

σ_s^2 sire variance; σ_e^2 residual variance; σ_p^2 phenotypic variance; h^2 heritability

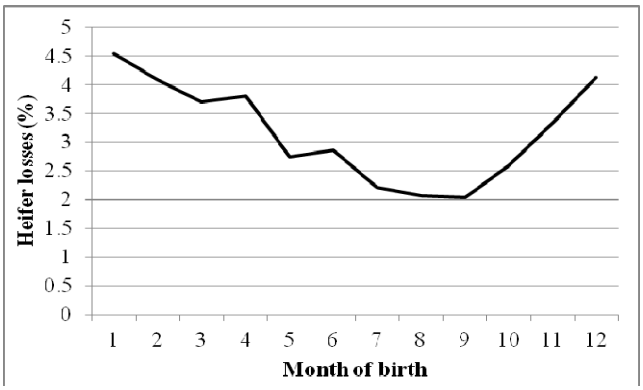

Figure 1 Calf losses by month of birth in the first month of life (2 to 28 days)

Conclusion Calf survival is influenced by many different environmental factors, but it appears that there is a genetic component contributing to survival, albeit small. Results emphasise that extra attention is required during winter months to keep calves alive born during winter. The study has found that BCMS data is a very valuable resource that could have other potential uses in addition to the purpose for which it was established.

Acknowledgements
Many thanks to RPA for permission to use BCMS data and funding from Defra under the Sustainable Livestock Production LINK Programme, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR.

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040

How direct and maternal gestation length genetically relate to fertility, milk production, type and lifespan in UK Holstein-Friesian heifers

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Introduction Worldwide, awareness is growing that an emphasis on the genetic merit for functional traits is needed when selection decisions are made (De Maturana *et al.*, 2007). Gestation length (GL) has been suggested as an interesting new selection trait and its potential as such has been evaluated (Norman *et al.*, 2010). Knowledge on genetic relationships with established traits of importance is however lacking which leads to a paucity of reliable information on what may be expected when GL is given an emphasis in selection indices. This study therefore has the simple objective of estimating the genetic correlations between GL, fertility, milk production, type and lifespan traits, using models that allow the full separation of direct and maternal genetic effects.

Material and methods Data from first-parity Holstein-Friesian heifers were provided by two milk recording organisations in the UK from 1995 to 2009 where GL was calculated from the last recorded insemination and calving date and restricted to 265-295 days. The dataset was matched to the data containing phenotypes of all other traits, which were recorded in the lactation following the recorded calving and extracted from national databases. Chosen traits of interest include fertility, (calving interval, days to first service, non-return rate at 56 days after service, number of inseminations per conception) milk production, (milk yield at day 110 in milk, accumulated 305-day milk, fat and protein yield), type (udder depth, chest width, rump width, rump angle, mammary composition, stature, body depth) and lifespan traits (days of productive life). Validity checks were performed on the matched dataset and calving interval was restricted to 300-600 days. The type traits were objectively scored on a categorical scale by a classifier at inspection and then adjusted by classifier as explained by Brotherstone *et al*. (1990). Time between calving and inspection was restricted to 0-8 months. The final dataset contained 27,845 heifer performance records, originating from 1,751 herds. Data were analysed using trivariate linear mixed models in ASREML v.3.0. GL is affected by both offspring and dam: the direct effect (GLd, gestation length prior to being born) and maternal effect (GLm, gestation length prior to giving birth) respectively. To allow estimation of both direct and maternal (co)variances, extended sire models were fitted. This means that in addition to the random genetic effect of sire of the cow to account for the additive direct effect of all traits but GL, sire of the calf was fitted to account for the additive direct effect of GL and sire of the cow to account for the maternal genetic effect of GL. In all models, trait 1 was consistently GL, trait 2 a trait of interest and trait 3 the accumulated 305-day milk yield (of the dam). Non-genetic fixed effects fitted were sex of the calf, herd, year*month of calving, age of the dam (months, covariate), and random an effect of herd-year. Additional fixed effects fitted for the type traits included year*month of inspection, stage of lactation at inspection, age at inspection (months, covariate) and a quadratic effect of age at inspection. For lifespan traits an additional quadratic effect of age at calving (months) was included. Obtained sire (co)variances were algebraically converted into direct and maternal (co)variances.

Results Estimated genetic correlations show that GLd is genetically related to milk production whereas GLm shows a genetic correlation with type rather than milk production. Lifespan and most fertility traits are not genetically related to GL. All significant genetic correlations are diagrammatically presented in Figure 1. In summary, high yielding individuals are genetically prone to be born relatively early; and individuals carrying their young relatively long before giving birth are likely to be wide and large animals.

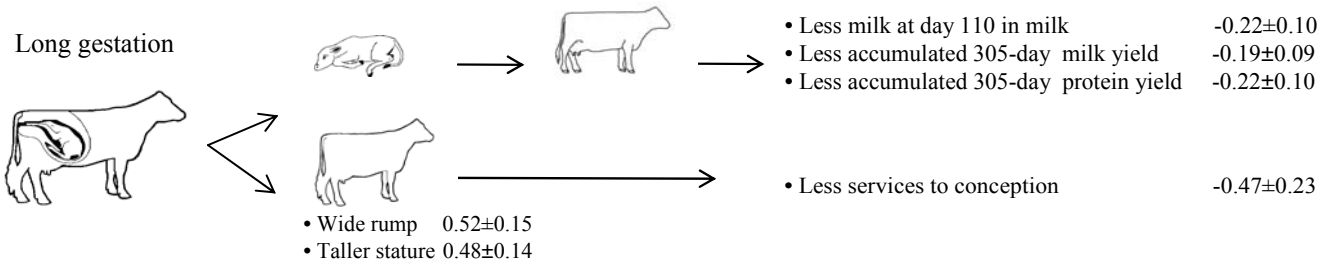

Figure 1 Diagram presenting the genetic correlations between gestation length, milk production, fertility and type

Conclusions This study shows that GL is genetically related to other important selection traits. This needs to be taken into account if GL is implemented into breeding indices. The economic importance of GL lies mainly in its association with calving interval, and its potential relationship to milk production. The first however appears phenotypic while the latter shows to be more complex than previously thought. Therefore, as yet, results suggest that GL is best being used as an indicator trait as opposed to being introduced as a new selection trait.

Acknowledgements The authors gratefully acknowledge funding from Defra under the Sustainable Livestock Production LINK Programme, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR. The authors further gratefully acknowledge Tracey Prichard and Raphael Mrode for their valuable input

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041

Association of herd size and seasonality, with somatic cell count in Irish and UK dairy cows

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Introduction Economic pressures, coupled with the phasing out of European Union milk quotas by 2015, and an increased demand for milk, require dairy farmers to adopt strategies to increase milk production efficiency. These include; milking more cows per herd, to gain economies of scale, reducing concentrate feed costs through optimal utilisation of grazed grass, and increasing the value of milk sold through reducing its somatic cell count (SCC). The first aim was to compare changes in cow level test day SCC associated with increasing herd size, and replacement rate between Irish and UK herds. The second aim was to investigate the impact of season on test day SCC for cows in these herds, while accounting separately for stage of lactation, and milk yield.

Material and methods Milk recording databases were provided by Irish Cattle Breeders Federation, and National Milk Records, UK. The study populations were 7,608 Irish dairy herds, with 10,256,636 records from 867,002 cows taken between 2005 and 2009, and 2,128 UK dairy herds, with 6,772,182 records from 474,669 cows taken between 2004 and 2006. Two samples of 500 Irish, and 200 UK herds were taken at random. Four-level linear models for test day ln SCC were developed, using data from herds in the first samples; random effects structure accounted for clustering of cows within herds, parities within cow, and recordings within parity. The models corrected for stage of lactation, milk yield, composition, year, and parity. The models were built by backward stepwise elimination of terms from a saturated model. Biologically plausible interactions, and herd level random effects were assessed. Factors remained in the model if $p \leq 0.05$. Data from the second sample datasets were used for cross validation of the models, with random effects set to 0.

Results In the Irish herds, cow level test day SCC increased with increasing herd size up to 290 cows, and then decreased. In UK herds, SCC remained constant with increasing herd size up to 180 cows, and then increased (Figure 1). Uncertainty in the estimates increased with herd size, due to a decline in the denominator. For the Irish herds, associations between SCC and calendar month were larger and more variable (95% confidence intervals shown) from February to August, and in December, compared to the UK herds (Figure 2). No association was found between replacement rate and cow test day SCC ($p>0.05$). Fixed effects from the final models were as good at predicting separate data, as they were at predicting that used for parameter estimation, indicating good model fit.

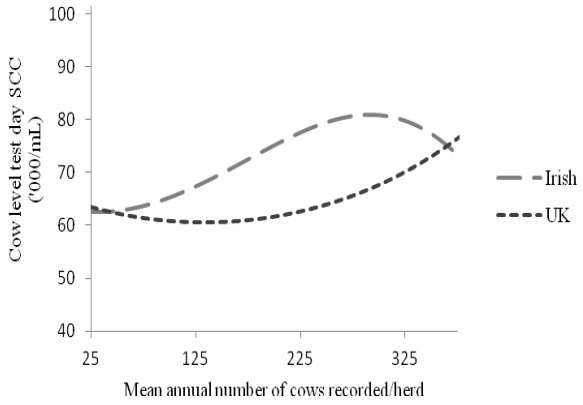

Figure 1

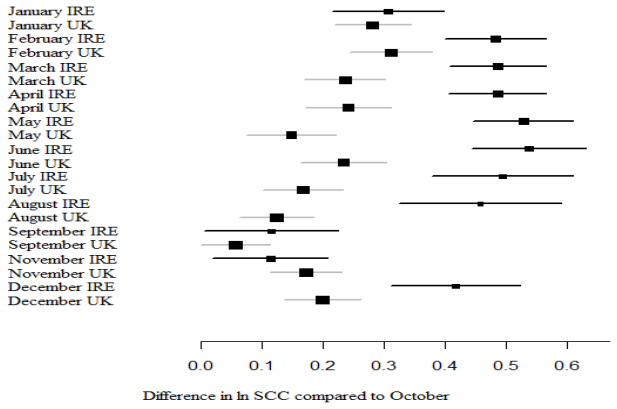

Figure 2

Conclusions Many herds in these countries failed to benefit from economies of scale for mastitis control (Figure 1), an important area for the cost effective expansion of dairy herds. Careful consideration must be given to mastitis control as herds expand. Figure 2 highlights the importance of mastitis control, during the grazing season for spring-calving herds, when bulk milk SCC, and farmers' interest in mastitis control may be low. The opposite trend was seen in UK herds due to non-seasonal calving patterns, and bulk milk SCC was highest when cows were at pasture. Monitoring strategies that do not rely on bulk milk SCC alone are recommended; these are particularly important to implement for spring calving herds.

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042

Effects of dietary incorporation of roughages and barley produced with selenium enriched fertilizers on the selenium content in milk and milk products

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Introduction Selenium (Se) is a trace element of importance both in human and animal nutrition owing to its involvement in many processes such as defence mechanism, antioxidant status or metabolism pathways. The Se content in forages and in feedstuffs produced in Belgium is rather low so that the Se intake in cattle could be below the requirements which results in low Se content in animal products such as milk and meat. The aim of the present work was to assess, on milk and milk products the effects of dietary incorporation of roughages and cereals produced with Se enriched fertilizer.

Material and methods The 46 Holstein dairy cows of the University of Liege herd, were divided in 2 groups balanced for parity number, date of calving and yield. The animals were housed in a free stanchion barn with straw as bedding. They were offered a diet based on maize silage (0.30 on a DM basis), grass silage (0.36), barley (0.10), dehydrated lucerne (0.04), sugar beet pulp (0.10) and soya bean meal (0.10). In the Se group, maize, grass and barley were grown with nitrogen fertilizer enriched with Se spread at a rate corresponding to 8, 4 and 4g of Se as selenate per ha. In the control group, the fertilizer was without Se. The 2 groups of cows were milked separately, the milk being stored in 2 different tanks. Samples of the tanked milk were taken at regular intervals for chemical analysis. Volumes of 100 L milk were taken to produce butter and cheese in a pilot dairy unit. The experiment was conducted over a one month period, the cows being on their specific diets since 2 weeks. The data were analyzed according to an Anova II model, including the effects of milk products, Se treatment and the interaction. The level of significance was set at 5% (SAS 1999). It was not possible to measure Se in butter owing to technical problems associated to fat.

Results The dietary Se content was 56 and 140 µg/kg DM in the control and the Se groups respectively. The diet high in Se affected the fat content (42 vs 40 g/l) but had no effect on protein and urea contents. The Se content in milk was also significantly increased (16,2 vs 13.3 ng/ml - P<0.000). Figure 1 shows the changes in Se content in milk during the experimental period. The Se content in the milk varied according to the time in both groups but was higher in the Se group as expected. Figure 2 describes the Se content in the milk products. It was in the hard cheese and in the cottage cheese that the Se content was the highest and in the whey the lowest. These differences have to be ascribed to differences in DM content of the product. In all the milk products the Se content was higher when the cows received the diet enriched in Se. It could be speculated that the Se spread as selenate from the fertilizer was absorbed by the forages and cereals. It was then transformed in an organic form, the plant acting as bioreactor. It is expected that the organic Se from the plant was available in the milk as organic selenium to be used by the consumer.

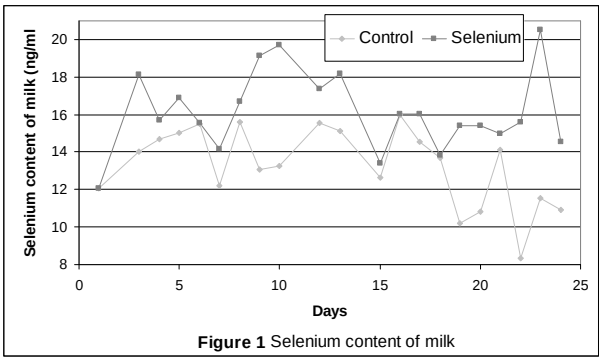

Figure 1 Selenium content of milk

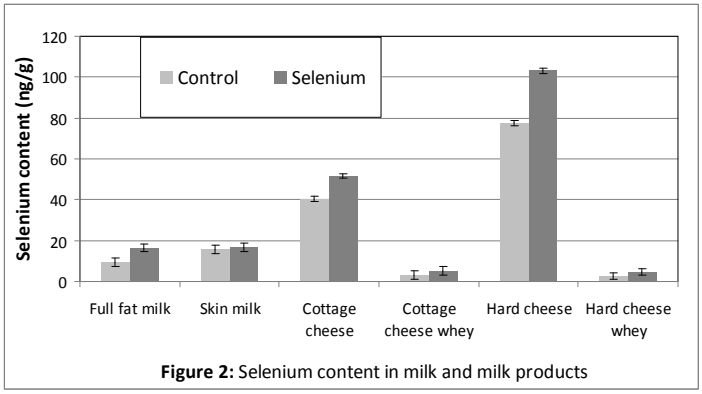

Figure 2: Selenium content in milk and milk products

Table 1 Average chemical composition and selenium content in milk samples taken on 21 occasions

	Control	Selenium	SEM	P < F
Fat (g/l)	40	42	0.35	0.002
Proteins (g/l)	35	35	0.14	0.710
Urea (mg/l)	229	241	7.36	0.271
Selenium (ng/ml)	13.3	16.2	0.46	0.000

Conclusion Selenium enriched roughages and cereals by use of fertilizers with Se improved largely the Se content of milk and milk products. This could be of interest to increase the Se intake of the consumers and therefore could be a method to improve human health.

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043

Use of pre-treatment with parenteral tetrathiomolybdate and fractional changes in liver copper concentration to improve the assessment of copper supplements by hepatic copper repletion in cattle

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Introduction Copper (Cu) supplements are commonly evaluated for their ability to arrest depletion of liver Cu in animals given Cu-deficient diets but assessments must allow for the fact that rate of decline in liver Cu is proportional to initial concentration (ILCu). Balemi *et al* (2010) reported a dominant effect of ILCu in a comparison of six supplements in dry cows, despite selecting individuals with relatively low ILCu from the herd. Tetrathiomolybdate (TTM) is a Cu-chelator, produced by rumen synthesis when the diet is rich in molybdenum (Mo) and sulphur (S). In sheep, Cu toxicity can be prevented by using parenteral TTM to deplete liver Cu but there have been no comparable studies in cattle. This paper reports the use of TTM to minimise variation in ILCu in calves.

Material and methods Eighteen weaned Friesian calves, weighing 200-250 kg, were group-fed a barley-based diet, containing 4mg Cu kg^{-1} DM, made Cu-deficient by addition of molybdate (3 mg Mo kg^{-1}) and sulphate (3g S kg^{-1}) to a complete micronutrient pre-mix. The daily feed allowance was 7 kg/day and oat straw was available *ad libitum*. The intention was to compare the ability of two Cu supplements to attenuate liver Cu depletion in one experiment but initial liver biopsy Cu ranged so widely (3.15-14.17mmol/kg DM) that two were arranged, each with three treatments and three replicates. Expt L used nine calves with the lowest ILCu but Expt H use the other nine and they were given 235mg TTM in each of three subcutaneous injections after 42-48 days. In both experiments, calves were ranked again for liver Cu after 50 days depletion. Six days later (d 0), calves were allocated randomly within tiers of three to receive 100mg Cu subcutaneously as CuCaEDTA, either in an oily base ('Coprin', C) or a revised formulation in an aqueous base (RC), or no injection (O). Thereafter, liver biopsies were obtained every 28 days and plasma samples every 14 days for 16 weeks for determination of liver and plasma Cu by atomic absorption spectrophotometry. Differences between Expts H and L prior to injecting Cu were ascertained by students 't' test, with liver Cu analysed as a fraction of pre-depletion (d -56) values (FILCu). Subsequent effects of Cu treatment within each experiment were assessed by two-way, repeated measures (mixed model) ANOVA (GraphPad Software, La Jolla, CA, USA), with FILCu expressed as a fraction of pre- injection (d 0) values. In the absence of significant effects (*i.e.*, $P > 0.05$) of Cu injection on plasma Cu, the same model was used to ascertain time and pre-treatment effects in pooled data.

Results After 50 days depletion (*i.e.*, d -6), FILCu was marginally lower (0.64 ± 0.066 v 0.80 ± 0.090 SEM; $p = 0.09$) but plasma Cu significantly higher (16.6 ± 0.52 v 13.3 ± 0.49 SEM µmol/L; $p < 0.001$) in H than L calves. Despite the injection of Cu, mean plasma Cu had declined by d 42 to minimum values of 13.4 and 12.2 µmol/L in H and L calves , respectively, but partially recovered thereafter (time effect $P< 0.001$) and was always higher in H than in L calves, the average difference being 2.0 ± 0.36 umol l^{-1} ($P < 0.001$). The changes in liver Cu following the injections of Cu are summarised in Table 1.

Table 1 Fractional change in liver Cu following the injection of two supplements in two experiments

	Days post-injection of Cu						Effect (P)		
Expt	Injection	28	56	84	112	s.e.m.	Injection	Time	Interaction
L	O	0.47[a]	0.36[a]	0.29[a]	0.15				
	C	1.14[bc]	1.10[b]	0.78[b]	0.44	0.035	0.006	0.0001	0.026
	RC	0.91[bc]	0.67[ac]	0.54[ab]	0.20				
H	O	0.75	0.68	0.53[a]	0.18				
	C	0.94	0.87	0.88[b]	0.41	0.034	0.057	0.0001	0.41
	RC	0.96	0.73	0.68[ab]	0.20				

[abc] different superscripts within columns and experiments indicate significant differences between groups.

In Expt L, both supplements arrested the decline in liver Cu seen in Group O, with C more effective than RC and sustaining liver Cu for 56 days. In Expt H, the effects of both supplements were marginal. The difference between experiments can be attributed to the relatively slow rate of Cu depletion in Group O in Expt H, there being no indication that TTM reduced the transfer of injected Cu to the liver. Differences between experiments could be attributable to either the use of TTM or the difference in ILCu. If high ILCu reflected inherently superior retention of dietary Cu in H calves, this should have continued to raise liver Cu in all groups. Plasma Cu does not reflect variation in liver Cu when the latter is within normal limits (0.3-6.0 mmol/kg DM) but is increased by TTM.

Conclusions TTM only marginally increased depletion and probably had sustained residual effects on repletion by slowing turnover of Cu in liver and plasma, thus affecting evaluation of supplements. Use of FILCu detected treatment effects with small groups.

Reference

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044

Dairy cows milked by an automatic milking system located at pasture: practical aspects on technical uses of the paddocks

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Introduction The number of farms equipped with automatic milking systems (AMS) is increasing and will probably increase to a larger extent in the future owing to reduced labor costs. In most of those farms, the cows remain indoors without any grazing opportunity. The benefits from grazing practices such as reduced feed costs or improved animal welfare and health are thus lost. Grazing is positively perceived by the consumers. Grazing remains possible with cows milked with an AMS when pastures are close to the farm. A mobile AMS was developed at the University of Liège (Dufrasne *et al*. 2010) for pastures far away from the farm. The AMS is located in the barn during the winter period and moved to a specific location on pasture during the grazing season. Different practical aspects on the technical uses of the paddocks were studied.

Material and methods The Holstein dairy cows of the University of Liège were milked indoors with an AMS for the first time on 20/04/2010. The cows grazed from 22/06/2010 until 20/10/2010 on a permanent pasture with a rotational system with 11 paddocks. The flora was composed mainly of grasses (70%) and white clover (15%). The paddocks were cut for grass silage on 15/05/2010. The areas were comprised between 1.33 and 2 ha. The distance between the paddocks and the AMS was comprised between 100 m and 425 m. The AMS was located in a 0.4 ha paddock. The cows were fetched twice a day at 6.00 am and 16.00 pm to a waiting place in order to pass in the AMS twice per day. Furthermore, the cows had free access to the AMS during the whole day. The cows could see the AMS from 6 paddocks only. The AMS was lit during the night. A drinking bowl was present in each grazed paddock and a water through was available in the waiting place and at the exit of AMS. At the beginning of the grazing period, the days in milk were 172±61 days, the animals weighed 645±63 kg, the calving number was 2.3±1.15 and the animal produced 29.9±5.4 l milk daily. In the AMS, they received on average 2.15kg concentrate per day with a crude protein content of 170g/kg DM. Until 08/08/2010, the cows received also 6 kg DM maize silage owing to low grass availability and to hot and dry weather. The cows received the same amount of maize silage from 11/10/2010 in order to prepare them to the winter diet. The data were analysed with a GLM procedure including the effect of animal, days in paddock, distance, rotation cycle number and complementation.

Results Over the whole grazing season, the cows produced on average 19.6kg/day. The average milking number assessed as the total number of successful milkings during the AMS visits was 2.1 per day. 95% of the cows passed more than twice a day at least one time during the grazing season. Grass crude protein, neutral detergent fiber and water soluble carbohydrate contents were 232±50, 447±63 and 73±32g/kgDM. From these results, grass quality could be considered as high. The entry and exit grass heights recorded on 50 locations in the paddocks were 10.2±1.9 and 3.2±1.0 cm respectively. As shown in Figure 1, the milking number increased when grass heights decreased. Milking numbers less than twice were due to difficulties to fetch some cows to the waiting paddock when grass availability was high such as at entry in the new paddocks. The variations expressed as percentage of the total variation in the statistical model calculated on the data on milk yield and milking number are given in Table 1. The animal effects were the highest explaining 78 and 53% of the total variation respectively (P<0.001). As expected the days in milk effect was rather high in the variation on milk yield. The other parameters explained also significantly the variation but to a lesser extent.

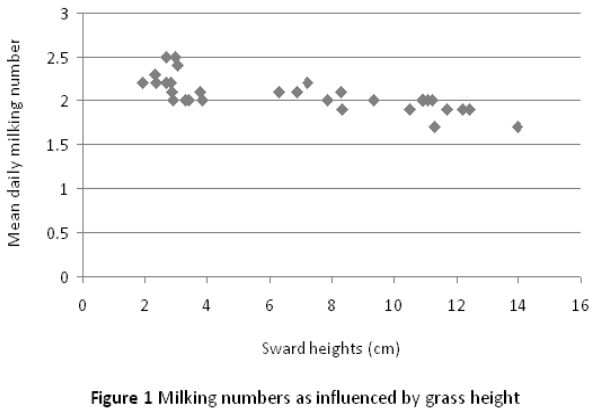

Figure 1 Milking numbers as influenced by grass height

Figure 1 Milking number as influenced by grass height

Table 1 Part of the variation in milk yield and milking number Explained by management parameters

Parameters	Milk yield		Milking number	
	Variation (%	Sign	Variation (%)	Sign
Animal	77.6	P<0.001	52.8	P<0.001
Days in milk	12.4	P<0.001	31.1	NS
Maize silage sup	nd	-	0.5	P<0.05
Rotation cycle	2.5	P<0.001	0.5	P<0.05
Days in paddock	2.7	P<0.001	2.7	P<0.001
Distance	2.3	P<0.001	3.2	P<0.001
Milking number	2.7	P<0.001	-	-
Milk yield	-	-	7.3	P<0.001

Conclusion An AMS could be satisfactory used on pasture. Animal effects largely explained variations in milk yield and the number of times cows were presented for milking.

Reference

Dufrasne, I., Robaye, V., Istasse, L., Hornick, J.L. 2010. Grassland Science in Europe. 15, 217-219.

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045

Alkagrain for finishing beef cattle

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Introduction There is growing interest in feeding Alkagrain to dairy and beef cattle in the UK as well as in Australia and Ireland. Alkagrain is alkaline preserved processed mature cereal grain. Alkagrain is normally produced by combine harvesting then processing (crimping, rolling, milling or kibbling) a dry cereal crop and mixing with Home n' Dry pellets prior to storage (Home n' Dry contains 146% CP and is formulated from urea and selected soya protein sources, FiveF Agri LLP). This rapidly releases ammonia into the cereals killing off moulds and bacteria, raising the pH into the alkaline range (pH 8.0-9.0). The typical inclusion rate for Home n' Dry is 30kg/t which increases the protein content of the cereals by approximately 4.3 percentage units. Alkagrain allows increased inclusion of own grown or locally grown cereals in beef rations reducing the need for imported feed ingredients. Alkagrain is stored in a clamp and therefore farmers do not need purpose built dry grain storage. The objective of this experiment was to compare the effect of feeding either Alkagrain or a conventional barley based ration on the performance of intensively finished dairy-bred bulls.

Material and methods Thirty Holstein and four Continental cross Holstein bulls weighing 300kg were allocated in a randomised block design according to breed and live weight and fed the following diets *ad libitum* through to slaughter; Barley Mix containing (kg/t) 775 rolled barley, 75 soya-bean meal, 75 rapeseed meal, 50 molasses, 25 minerals; Alkagrain containing (kg/t) 946 rolled barley, 30 Home n' Dry, 24 minerals. The Barley Mix and Alkagrain were analysed to contain 846 and 860g DM, 156 and 150g CP/kg DM, 473 and 502g starch/kg DM respectively. The cattle were housed in straw-bedded yards with 2 pens per treatment and were selected for slaughter by S Marsh (Senior Lecturer – Beef Cattle Specialist, Harper Adams University College) at EUROP fat class 3. The data were analysed using ANOVA. Liver scores were analysed using Kruskal-Wallis analysis of variance.

Results There were no significant differences ($P > 0.05$) in cattle performance or carcase classification. The Alkagrain fed bulls recorded a lower liver damage score however this was not significantly different.

Table 1 Animal Performance

	Barley Mix	Alkagrain	s.e.d	Sig
Start weight (kg)	300	300	15.5	NS
Slaughter weight (kg)	559	563	14.5	NS
Days to slaughter	196	201	10.7	NS
DLWG (kg)	1.32	1.31	0.074	NS
Carcase wt (kg)	284	285	8.9	NS
Kill out (g/kg)	507	506	60.0	NS
Liver score[1]	2.12	1.35		=0.103*

[1]Liver assessment: 1= Healthy liver, 3 = Slight abscesses, discolouration and/or swelling, 5 = Severe abscesses
* The chi-squared probability is presented for the liver scores and was non-significant. The arithmetic means are presented.

Table 2 Feed intakes, feed conversion ratio (FCR) and feed cost per kg gain

	Barley Mix	Alkagrain
Total feed intake (kg)	1,758	1,666
FCR (kg: kg LWG)	6.79	6.43
Feed cost (p/kg LWG)	1.16	1.07

Feed costs were reduced by £18 per bull and 9p (7.7%) per kg LWG with Alkagrain based on the costs prevailing at the time of the study.

Conclusions Overall performance of the bulls was very good, both achieving and exceeding recognised targets for intensive cereal beef production. Feeding Alkagrain had no significant effect ($P > 0.05$) on slaughter weight, DLWG, carcase weight and carcase grade. The FCR appears relatively high compared to the target of 5.4:1 for cereal beef production but it must be taken into consideration that the experiment did not include the period of growth from 110kg to 300kg. During this rearing phase dairy-bred bulls at Harper Adams typically record an FCR of 3.3:1 with a DLWG of 1.56kg. The Alkagrain bulls recorded lower liver damage scores however this was not significantly different. Liver abscesses are associated with mild acidosis from feeding high starch based diets (Plaizier *et al.,* 2009). Replacing Barley Mix with Alkagrain will reduced feed costs per bull. From the experiment it can be concluded that feeding Alkagrain to 300kg intensively finished beef cattle can help improve financial performance and be used as a 'complete concentrate feed' for intensively fed cattle apart from the addition of a mineral supplement.

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An assessment of alternative test length periods when measuring liveweight change in finishing cattle during feed efficiency studies

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Introduction Measuring residual feed intake (RFI) is an expensive test to undertake in either experimental or commercial practice due to the length of time required to undertake the test. Following an acclimatisation period, typically, animal livewight (LW) is measured on a fortnightly basis during a standard 70 day RFI test period with cattle. However, several authors have indicated that it should be possible to reduce the length of the standard 70 day test when studying feed efficiency in cattle (Archer, *et al*, 1999; Graham *et al*, 1999; Kearney *et al*, 2004 and Wang *et al*, 2006). More frequent weighings on a weekly basis is one method to achieve this reduction in test length which would in turn reduce the cost of RFI studies. The objective of this study was to assess the consequences of alternative test lengths on the precision of liveweight gain (LWG) measurements in RFI studies with finishing steers.

Material and methods Data on steer LW and LWG was obtained from a total of 36 finishing steers over an RFI measurement period of 84 days in the run up to slaughter. Steers were approximately 15 – 18 months old during the test period with breed type being either Aberdeen Angus x Limousin (AAxLIM) or Limousin x Aberdeen Angus (LIMxAA) and were offered one of two complete diets on an *ad libitum* basis. The diets consisted of either, concentrates plus straw (689, 200, 81, 20 and 10 kg/t of barley, maize dark grains, straw, molasses and minerals, respectively) or a mixture of the same concentrate ingredients and forage (154, 87, 410, 345 and 4 kg/t of barley, maize dark grains, grass silage, wholecrop barley and minerals, respectively). All steers were bedded on wood sawdust to ensure no consumption of bedding. Each animal was weighed on a weekly basis over the 84 days of the test period and LWG determined by linear regression of LW against days on trial for alternative test length periods of 84, 77, 70, 63 or 56 days from the start of the measurement period using Genstat 11. From this regression analysis the % of variance accounted for (R^2), the slope of the fitted regression equation (LWG), the standard error of the slope along with the error bound (s.e./slope x 2) expressed as a % were obtained and analysed for the effects of test length using the residual maximum likelihood (REML) procedure in Genstat 11.

Results While feed consumption was also measured, no discussion of feed intake data will be undertaken here as only an assessment of the LWG data is required to address this specific objective. Results on the precision of LWG estimates and the LWG observed over the different test durations are given in Table 1. Generally the precision (as determined by R^2, s.e. of LWG and error bound) of the LWG estimates declined significantly ($P<0.05$) as the test length duration was reduced from 84 to 56 days. The rate of LWG observed over the 56 day test length was significantly higher ($P<0.001$) than the rate of LWG for the longer test lengths in this finishing study as the steers approached the slaughter date subsequent to the end of the measurement period.

Table 1 Duration of test length when measuring LWG in finishing beef cattle and its effect on precision of LWG measurement and LWG estimates Test length (days)

	84	77	70	63	56	s.e.d.	Significance
R^2	95.6[a]	94.8[b]	93.8[c]	92.7[d]	92.4[d]	0.339	*
LWG (slope)	1.19[a]	1.18[a]	1.19[a]	1.20[a]	1.25[b]	0.013	***
s.e. of LWG	0.070[a]	0.079[b]	0.092[c]	0.105[d]	0.120[e]	0.0028	**
Error bound (%)	12.0[a]	13.7[b]	15.6[c]	17.9[d]	19.4[e]	0.499	**

Values not sharing common superscripts differ significantly ($P<0.05$).

Conclusions Along with other factors, the precision required from any estimates of LWG should be considered when deciding on the test length needed during RFI studies in finishing cattle. Where LWG estimates with an R^2 above 90% and error bound below 20% are acceptable then test lengths of 56 days are adequate. As cattle approach slaughter condition at the end of a finishing period of growth then LWG may decline as suggested from the data in Table 1. Selecting the optimum test period for RFI studies in relation to the slaughter date in finishing cattle might benefit from further study.

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047

Effect of different level of yeast probiotic and prebiotic and their symbiotic interaction on Holstein female suckling calves

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Introduction Probiotics and prebiotics have been shown to have different functions such as increasing feed efficiency and weight gain and improve immune system (Heinrichs *et al*., 2003: Timmerman *et al*., 2005). A probiotic is a culture of a single bacteria strain, or mixture of different strains, that can be fed to an animal to improve some aspect of its health. Probiotics are also referred to as direct fed microbials (DFM).The aim of the current experiment was to study the effects of feeding diet containing probiotic, prebiotic and probiotic + prebiotic (synbiotic) on performance and some plasma parameters.

Material and methods Nine groups of ten male calves, were fed for 12 weeks, iso-caloric, iso-nitrogenous diets. 90 Holstein calves were used to investigate the effects of probiotic and prebiotic on performance and some blood parameters. A completely randomized design with 3×3 factorial arrangement was used to assess the effect of probiotic (0, 2 and 4%) and prebiotic (0, 2 and 4%) on above parameters. Data were analyzed by General Liner Model procedure of SAS program (SAS1996) and means were compared by Dunkan test. Calves received whole milk twice daily. Water and starter were offered *ad libitum* throughout the trial. Body weight was measured at birth and thereafter weekly until 12 wks of age. Starter intakes were measured daily. Blood samples were collected at the end of experiment and analyzed for glucose (Glu), albumin (Alb), Globulin, total protein (Tp), triglyceride (Tg), Cholesterol, β-hydroxy butyric acid (BHBA), blood urea nitrogen (BUN), Ca, P.

Result Analysis of DMI, feed efficiency and calves' height revealed no significant difference during the trial between treatments. Effects of probiotic different levels was significantly different on ADG. Prebiotic different levels in diet had no significant effect on ADG, final weight, height and dry matter intake compare with control group while above parameters were affected by probiotic levels especially diet with 2g probiotic per calve per day showed the best performance. No treatment differences in Tg, BHBA and BUN concentration were detected during the trial (P>0.05).

Table 1 Effects of Probiotic and Prebiotic different levels and their interaction effects (probitic*prebiotic) on Holstein suckling calves performance and blood parameters

	Probiotic Levels			Prebiotic Levels			Significant		
	0	2	4	0	2	4	Pro	Pre	Pro*Pre
DMI (Kg/Day)	0.902	0.899	0.894	0.894	0.905	0.896	NS	NS	NS
ADG (Kg/Day)	0.643^{b}	0.702^{a}	0.688^{ab}	0.67	0.683	0.67	*	NS	NS
FE (%)	51.5	53.5	53.5	52.3	53	53.2	0.094	NS	NS
Height[1] (cm)	95.55	96.58	96.49	96.08	96.30	96.25	NS	NS	NS
Glu (mg/dl)	89.98^{a}	82.08^{b}	85.22^{ab}	83.03	86.28	87.98	0.047	0.268	0.749
Alb (g/dl)	3.79^{b}	4.13^{a}	4.04^{a}	3.97^{b}	4.09^{a}	3.09^{b}	0.001	0.058	0.19
Globulin (g/dl)	2.74	2.55	2.7	2.82^{a}	2.72^{ab}	2.45^{b}	0.21	0.015	0.009
Tp (g/dl)	6.47	6.72	6.73	6.74^{a}	6.84^{a}	6.34^{b}	0.087	0.001	0.002
Tg (mg/dl)	32.46	38.24	37.98	33.55	38.43	36.71	0.13	0.251	0.06
Cholestrol (mg/dl)	109.1	107.1	101.3	92.34^{b}	117.90^{a}	107.28^{a}	0.687	0.026	0.13
BHBA (mmol/l)	0.27	0.32	0.3	0.28	0.31	0.3	0.265	0.657	0.844
BUN (mg/dl)	13.33	14.54	15.37	14.64	14.87	13.73	0.751	0.341	0.19
Ca (mg/dl)	12.09^{b}	13.09^{a}	13.04^{a}	12.61	12.83	12.77	0.006	0.741	0.11
P (mg/dl)	7.34^{c}	7.95^{b}	8.90^{a}	7.50^{b}	8.53^{a}	8.17^{a}	0.001	0.008	0.15

1. Height at 12 weeks old
Means with different superscripts in the same row differ significantly (P<0.05).

Conclusions Calves that received 2 gr per day probiotic in their diet have the best situation of health (see blood parameters in Table 1) and performance. Prebiotic had no effects on measured parameters in this study but adding 4 gr prebiotic per day in calves diets showed positive effects on some blood parameters related to animal health.

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Changes in hepatic lipid metabolism during early postnatal life: an explanation for the reduction in neonatal mortality in Meishan piglets?

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Introduction

Chinese Meishan pigs are well recognized for their low rate of neonatal mortality and high prolificacy in comparison to European breeds, such as the Large White. A major reason behind newborn death in European porcine breeds is hypoglycemia due the limited capacity of the liver to process long-chain fatty acids during the first week of postnatal life (1). Utilizing a comprehensive lipidomics platform, we compared metabolic alterations in the livers of Meishan and Large White piglets during the first three weeks of postnatal life.

Material and methods The liver lipid profile and glycogen content of sixteen Meishan (M) and an equal number of Large White (C) piglets were used for this study. The piglets of each breed were randomly allocated into three groups: one group of M and C offspring were euthanized at birth and the remaining groups during lactation at 7 and 21 days, respectively. Fatty acids were analysed using gas-liquid chromatography to screen 20 species of lipid. The lipidomics data were analysed using empirical Bayes moderated t-statistics, unsupervised hierarchical clustering and principal component analysis (PCA), which were performed using ArrayMining version 1.0 (2). In addition, it was used a rule-based machine learning system (BioHEL) (3), which is able to identify and quantify interactions between lipids in the form of production rules.

Results

During the first 21 days of postnatal life, this systematic profiling led to the detection of six significant changes in fatty acids in Meishan offspring (q-value <0.05). By contrast, in the C group, no changes in the lipid population were observed. The PCA and the unsupervised hierarchical clustering indentified the lipid profile of the Meishan breed as an independent cluster at birth, in relation to the other two age groups. However, these analyses of the C group recognised the majority of the breed as a single cluster. BioHEL was able to generate two alternative rules predicting with perfect accuracy which samples belonged to newborn M group, one involving glycogen and C18:1n9C and the other one involving C18:1n9C and C22:0.

Conclusion

These results show that the Meishan breed may have an endocrine network that facilitates liver responses to hypoglycaemia, which may develop during gestation, thereby increasing the chances of survival of this breed during the neonatal period.

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049

Effects of spray-dried porcine plasma on weaner pig performance and resilience to sub-clinical post weaning colibacilossis

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Introduction Spray-dried porcine plasma (SDPP), a food grade slaughterhouse co-product with a high nutritional value for monogastric livestock such as newly weaned pigs, may at least partially protect weaned pigs from the consequences of sub-clinical post weaning colibacillosis (Torrallardona, 2010). Although the basis of this effect is not known, it may be related to the presence of immunoglobulins in SDPP and a positive effect on feed intake (Torrallardona, 2010). Here we test the hypothesis that SDPP inclusion in typical UK weaner pig diets increases resilience to sub-clinical post weaning colibacillosis.

Material and methods Sixty-four pigs, weaned at 26.7±0.3 day old, were divided in 16 pens with weaning weight of 8.97±0.11 kg, and 2 male and 2 female pigs per pen. Pigs were offered one of two iso-energetic weaner pig diets (16.9 MJ DE/kg), one commercial UK diet with dried skim milk powder (DSMP) at 50 g/kg and one with SDPP included w/w at expense of DSMP. Lactose levels were kept constant by including whey, mass balance was achieved by slightly reducing the main grain ingredient (micro-wheat), and pure amino acids were used to balance at 16.7 g lysine/kg. SDPP and DSMP diets were fed *ad libitum* for two weeks post weaning (P1, d_0-d_{14}), and pigs were either kept uninfected, or trickle infected with 10^9 cfu Nal-resistant enterotoxigenic *E. coli* (ETEC) per pig on 5 occasions between d_4 and d_{13} through offering inoculated food as previously described (Athanasiadou *et al*., 2011). Pen dividers were used to assure infective agent uptake by individual pigs. Pigs were fed commercial diets from d_{14} to d_{35} (P2) to assess carry-over effects. Feed refusals were taken daily during P1 and weekly thereafter to calculate average daily feed intake (ADFI). Pigs were weighed weekly from d0 onwards to calculate averaged daily gain (ADG). Feed conversion ratio (FCR) was calculated as ADFI/ADG. Faeces scores were taken daily on a scale from 1 (solid) to 4 (severe diarrhoea). Freshly voided pen faecal samples were collected twice weekly until d_{21} and weekly thereafter for assessment of lactobacilli (L), coliforms (C) and Nal-resistant ETEC through standard enumeration methods. Data was analysed using a 2 x 2 factorial ANOVA, with pen as experimental unit. Faeces scores and bacterial counts were averaged over time within P1 and P2 as there were no interactions between treatments and time.

Results Feeding and infection treatment interacted for ADFI and ADG during P1 (Table 1); the consistently higher ADFI and ADG of SDPP pigs over DSMP pigs were more pronounced during ETEC challenge. This interaction did not carry over, though ADFI and ADG of SDPP pigs remained higher than DSMP pigs during P2, whilst previous ETEC exposure resulted in higher ADG. Feeding and infection treatment did not affect FCR. SDPP pigs had slightly higher faeces scores during P1. Feeding and infection treatment did not affect faecal bacterial counts (P>0.20), averaging 9.35±0.09 and 8.65±0.09 cfu/g for lactobacilli, 7.12±0.14 and 5.35±0.12 cfu/g for coliforms, 1.34±0.03 and 1.65±0.04 for L:C ratio and 5.62±0.26 and 0.67±0.24 cfu for ETEC during P1 and P2, respectively.

Table 1 Effect of SDPP feeding and ETEC exposure on performance and faeces score of weaned pigs

	Days post	DSMP		SDPP			P-values		
Parameter	weaning	Sham	ETEC	Sham	ETEC	s.e.d.	Diet	Inf	D x I
ADFI	0-14	430	383	461	478	22	0.002	0.350	0.063
(g/d)	14-35	1073	1075	1156	1185	33	0.001	0.524	0.573
	0-35	816	798	878	902	24	0.001	0.854	0.239
ADG	0-14	354	311	373	380	19	0.008	0.213	0.099
(g/d)	14-35	763	773	798	850	20	0.002	0.046	0.155
	0-35	599	588	628	662	17	0.001	0.354	0.082
FCR	0-14	1.22	1.23	1.24	1.26	0.03	0.243	0.304	0.879
	14-35	1.41	1.39	1.45	1.39	0.04	0.412	0.215	0.454
	0-35	1.36	1.36	1.40	1.36	0.03	0.402	0.439	0.509
Faeces score	0-14	1.14	1.26	1.40	1.38	0.08	0.007	0.398	0.213
	14-35	1.07	1.06	1.08	1.05	0.06	0.942	0.662	0.770

Conclusions The data support the view that inclusion of SDPP at the expense of DSMP increases pig performance, especially in the face of sub-clinical post weaning colibacilossis. As per EU directive 1292/2005, food grade SDPP of non-ruminant origin is permitted as a feedstuff for monogastric farm animals. Thus, subject to authorisation, registration, permission and safety requirements under UK regulations for feedstuff use, SPDD may be an alternative protein source for newly weaned pigs.

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Dietary conjugated linoleic acid (CLA) and its effects on proliferation and production of interleukin 2 (IL-2) of peripheral blood mononuclear cells in pigs infected with porcine respiratory and reproductive syndrome virus (PRRSv)

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Introduction There is evidence that CLA modulates the immune system by increasing lymphocyte blastogenesis, lymphocyte cytotoxic activity and inflammatory response**.** Studies *in vitro* and *in vivo* with CLA using murine models or several cells lines are equivocal, particularly in relation to production of some cytokines e.g. interleukin (IL) IL-2 (Ramírez-Santana *et al.* 2009). In pigs, CLA increased lymphocyte proliferation and the circulating number of $CD8^+$ lymphocyte subsets in piglets early-weaned (14 days old) infected with porcine circovirus (Bassanganya-Riera *et al.* 2001). PRRS is a viral disease considered as a major cause of reproductive loss and respiratory disease in pigs with an economic impact worldwide. The aim of this study was to evaluate the effect of dietary CLA on the proliferation of peripheral blood mononuclear cells (PBMC) and IL-2 production in pigs infected with PRRSv.

Material and methods The experiment was carried out in the Animal Experimental Unit of Centro de Investigación en Alimentación y Desarrollo (CIAD, Sonora, México). Pigs of 4 weeks of age were allocated (6 per treatment) to CLA 0%, CLA 1% and CLA 2% diets. Fourteen days after supplementation with CLA (Lutalin™) the pigs were inoculated intramuscularly and intranasally with American NVSL-97-7895 strain for 28 days. Blood samples were collected on day 0 (baseline), day 14 (infection), and days 14 and 28 post infection (pi). PBMC ($5x10^6$) were dyed with CFSE, stimulated with phytohaemagglutinin PHA (5 µg/mL) and re-stimulated with PRRSv and incubated at 37°C in a 5% CO_2 atmosphere for 72 h. Effects on PBMC were determined by flow cytometry (BDFACS Canto II) using BDFACSDiva v 6 1.1 software. Production of IL-2 in PBMC was quantified using an ELISA kit (BioSource) and viral loads (particles/ml of blood) by real time PCR (Tetracore). Data were analysed by ANOVA using NCSS 2001. Differences among treatments were determined by Tukey test at 95%. The Ethics Committee of CIAD granted approval for the study.

Results PBMC proliferation stimulated by PHA was significantly higher on day 14 (previous infection) and day 14 pi in pigs fed 1% CLA than in those fed 0% CLa and 2% CLA. During the rest of the experiment, no difference was found among the groups (P>0.05). The proliferation of PBMC re-stimulated by PRRS was lower (p <0.05) in pigs supplemented with CLA on days 14 and 28 pi compared to the CLA 0% group. However, data showed that on day 14 before infection, IL-2 production stimulated by PHA was higher in the groups fed 1% CLA and 0% CLA than in the group fed 2% CLA. The IL-2 re-stimulated with PRRSv was lower for the 2% CLA diet during the course of the experiment compared with 1% CLA and 0% CLA. No change was found in viral load on days 14 and 28 pi irrespective of treatment.

	PBMC PROLIFERATION (%)											
Day	CLA 0%				CLA 1%				CLA 2%			
	PRRS	se	PHA	se	PRRS	se	PHA	se	PRRS	se	PHA	se
0	5.5^{A}	1.30	65.9^{a}	1.93	7.0^{A}	1.30	52.8^{ab}	1.93	2.7^{A}	1.30	38.0^{b}	1.93
14	4.7^{A}	1.03	27.2^{a}	2.90	3.9^{A}	1.03	49.1^{b}	2.90	6.1^{A}	1.03	37.5^{a}	2.90
14 pi	27.2^{A}	2.25	28.5^{a}	1.96	3.8^{B}	2.25	56.9^{b}	1.96	3.6^{B}	2.47	43.2^{a}	2.15
28 pi	29.6^{A}	2.26	39.4^{a}	2.10	3.1^{B}	2.26	41.0^{a}	2.10	5.3^{B}	2.77	38.9^{a}	2.58
	IL-2 (pg/mL)											
0	30.7^{A}	0.62	38.3^{a}	11.28	9.99^{B}	0.62	57.6^{a}	11.28	10.29^{B}	0.62	74.5^{b}	11.28
14	30.6^{A}	0.71	90.5^{a}	16.73	9.56^{B}	0.73	111.9^{a}	16.73	9.39^{B}	0.72	72.1^{b}	16.7
14 pi	28.4^{A}	0.72	120.7^{a}	8.20	9.49^{B}	0.79	44.0^{b}	8.20	8.68^{B}	0.72	22.8^{b}	8.98
28 pi	29.1^{A}	1.07	57.6^{a}	10.62	29.96^{A}	1.02	88.2^{a}	10.62	8.67^{B}	1.25	32.6^{b}	13.00

Mean values within a row with different superscripts are different $P<0.05$; capitals superscripts indicate ANOVA for PRRS and lower superscript ANOVA for PHA; se- standard error

Conclusions Our results suggested that CLA had no protective effect on the PRRSv response as measured by PBMC proliferation and IL-2 production.

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Identification of risk factors associated with poor growth performance in pigs

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Introduction Weight variation is a common problem in the pig industry and has financial, welfare and environmental implications. While this variability in weight can occur at different stages of the production cycle, birth weight is considered a predictor of future growth potential with early differences in body weight often being exacerbated throughout production. To address this problem, the factors that contribute to this weight variation need to be understood. The aim of this paper was to identify the risk factors associated with slow growth in pigs, paying particular attention to the effect birth weight and weaning weight have on subsequent performance.

Material and methods Two industry databases (D1 & D2), containing individual records for approximately 44,000 and 90,000 pigs respectively, were obtained from international breeding companies. Birth weight (BW), weaning weight (D2) or intermediate weight (at ~45kg, D1) and final weight were recorded in both datasets. For the majority of pigs the following potential determinants were also present; sow parity number, total number of piglets born per litter, date of birth, gender, gestation length (D2) and genetic line (D1). Absolute (g/day) and relative (g/day/kg body weight) growth rate were calculated for each pig for each growth stage recorded. Pigs were then categorised into 3 groups, using percentiles, denoting high, medium and low growth rates. Pigs were also assigned to birth weight and weaning/intermediate weight groups based on percentiles (8 categories). To identify potential risk factors for poor postnatal growth, an ordinal logistic regression model was constructed; highly correlated variables (0.7 or above) were discounted by retaining only the variable with the greatest effect in the model. Three separate multivariate models were run, one for each growth period. To examine the effect of birth weight and weaning weight on the lifetime growth rate of pigs, a continuous linear plateau model was fitted to the data for all pigs (Piegorsch and Bailer 2005) using the Levenberg-Marquardt fitting algorithm. This model allowed identification of a break point for birth and weaning weight, above which a plateau occurs at the maximum growth for the population of pigs. Once the breakpoint had been estimated, linear regression was applied to the data below the breakpoint to identify the variables which were influencing lifetime growth of this population of pigs.

Results The effect of BW and weaning (or intermediate) weight on lifetime growth rate was similar for both datasets; the outcomes of the analysis of D2 are given as an example in Table 1. Piglets in the lowest BW or weaning weight groups were more likely to be in a low growth rate group; the converse was the case for the higher BW groups. As the number of piglets born alive per litter was increased, piglets had increased odds of low growth rates. Females were more likely to exhibit lower growth rates than males post-weaning, while pigs born January-April were most likely to be in the lowest growth rate category. The linear plateau model (Figure 1) indicated that lifetime growth rate increased linearly with birth weight until a threshold value of 1.8kg was reached (D1 & 2), with a plateau growth rate of 604 g/day and 658g/day for D1 and D2 respectively. Linear regression applied to data prior to the breakpoint indicated that weaning/intermediate weight was the best predictive factor for lifetime growth rate of pig

Table 1 Significance of parameters for Logistic Regression model for each stage of pig growth (D2)

	Significance level		
Risk Factor	Birth to Weaning	Weaning to finishing	Birth to finishing
Litter size (total born)	<0.0001	0.7106	<0.1740
Sex	0.0183	<0.0001	<0.0001
Sow Parity	<0.0001	<0.001	<0.0001
Piglet Gestation	<0.0001	0.0323	0.0794
Month of Birth	<0.0001	<0.0001	<0.0001
Birth weight	<0.0001	<0.0001	<0.0001
Weaning weight	-	<0.0001	<0.0001

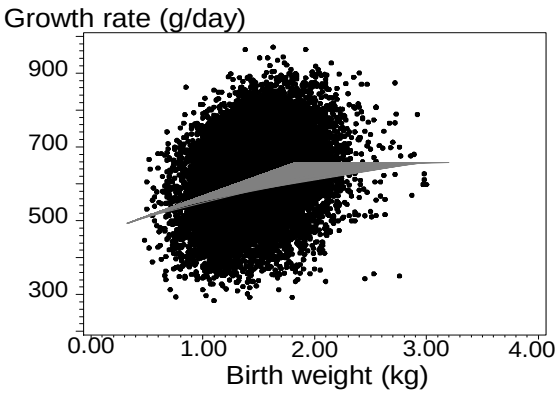

Figure 1 Breakpoint estimation for the effect of birth weight on lifetime growth rate for D2

Conclusions The results suggest that there are a number of variables affecting the growth of pigs, the influence of which will vary depending on their period of growth. As weaning weight is the best predictor of the lifetime growth rate of those lighter piglets born below 1.8kg, this suggests interventions to increase weaning/intermediate weights could maximise the lifetime growth of low birth weight piglets.

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Nutritional value of diets for growing / finishing pigs containing high levels of home grown legumes compared with one based on soyabean meal 1. Growth performance

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Introduction In order to remain competitive in the global market, comply with government policy that promotes sustainable pig farming and reduce environmental impact, the British Pig Industry must seek viable and sustainable solutions to the sourcing and level of dietary energy and nutrient inputs whilst maintaining a desirable level of output. Such sustainability may be enhanced by increasing utilisation of UK grown feed ingredients, as opposed to relying on imported feedstuffs. As home-grown legumes eliminate the need to rely upon imported N-containing raw materials, they may also reduce the energy demands associated with its transport and thus the global warming potential per kg of pig live weight produced (Crépon, 2006) but to an extent that is debateable. A crucial aspect of comparisons between individual raw materials is that compound diets are formulated to be balanced for energy and nutrients. The objective of the current study was to compare the effect of replacing soyabean meal with home grown peas or beans on growth performance.

Material and methods Diet formulations were conducted using the Premier Nutrition feed data-base. Five grower (one containing 140g hipro soyabean meal (GO) and four others containing 300g of White-Flowered Peas Prophet (GP), Spring Coloured-Flowered Faba Beans Fuego (GF), White-Flowered Faba Beans Tattoo (GT) and Winter Coloured-Flowered Faba Beans Wizard (GW) / kg) were formulated. Similarly, five finisher diets were formulated containing either 120g hipro soyabean meal or 300g of the four other legumes / kg (giving FO, FP, FF, FT and FW. Diets were iso-energetic (NE 9.3 and 9.0 MJ/kg respectively for grower and finisher) and balanced, by including pure amino acids, for standard ileal digestible lysine (8.1, 7.1 g/kg), and methionine (2.4, 2.1), threonine (5.3, 4.6), and tryptophan (1.5, 1.3) . Other dietary raw materials were wheat, barley, molassed sugar beet pulp, wheatfeed, rapeseed meal, soya acid oil, macro minerals and micro-mineral vitamin premix. Each grower diet was fed to eight replicates of a pen containing an individually housed commercial white hybrid entire male pig of initial weight 25kg located in the University of Nottingham's experimental pig growth facility. Transfer to the finisher diets was at 55kg and the experiment continued until animals weighed 95kg. Feed intake and animal weight was recorded weekly. Daily liveweight gain was calculated as the linear slope of the response of liveweight again against time. Solving the linear regression allowed an estimate of the actual day an animal was at 25kg and reached 55kg (for the grower phase) which allowed measurement of the precise amount of feed required to grow over this liveweight range and hence feed conversion ratio could be determined. Such procedures were conducted for Grower (25 – 55kg), Finisher (55 – 95kg) and Overall (25 – 95kg).

Results Performance (Daily liveweight gain, DLWG; Feed Intake, FI; Feed Conversion ratio, FCR) is presented in Table 1a (Grower), Table 1b (Finisher) and Table 1c (Overall)

	Table 1a Grower							Table 1b Finisher						
	Diet					SED	P	Diet					SED	P
	GO	GP	GF	GT	GW			FO	FP	FF	FT	FW		
DLWG	0.92	0.95	0.99	0.96	1.02	0.035	0.065	1.13	1.19	1.17	1.10	1.14	0.045	0.400
FI	47.9	47.9	46.4	46.9	46.9	1.8	0.838	122	119	118	116	122	4.2	0.588
FCR	1.92	1.91	1.86	1.85	1.87	0.074	0.834	3.05	2.97	2.95	2.90	3.04	0.104	0.588

There was no significant effects of treatment on FI or FCR, although there was a strong trend (P=0.065) for DLWG (Grower) suggesting higher gain upon home-grown legume inclusion. There were no significant effects of treatment (finisher); overall there was no significant effect of treatment on DLWG. There were strong trends of an effect of treatment on both FI and FCR (P=0.067).

	Table 1c Overall						
	Diet					SED	P
	OO	OP	OF	OT	OW		
DLWG	1.05	1.08	1.10	1.06	1.05	0.034	0.422
FI	177	168	172	160	174	5.9	0.067
FCR	2.72	2.58	2.65	2.46	2.67	0.091	0.067

Conclusions The results demonstrate that, when diets are accurately formulated and balanced for both energy and nutrients, performance of growing / finishing pigs on diets based on home-grown legumes is no different from when animals are fed a diet based on hipro soyabean meal.

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Short term feeding behaviour of newly weaned piglets

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Introduction Short Term Feeding Behaviour (STFB) describes food intake patterns of animals at the level of feeding events, such as visits to feeders, and meals (Howie *et al*., 2009). A Meal Criterion (MC) is the shortest interval between visits that can be considered an interval between meals. Log-normal models, based on the concepts of hunger and satiety, have been used to calculate MC (Tolkamp *et al.,* 1998) but have not been tested using data collected with newly weaned piglets. Data from a study into the short term feeding behaviour of such piglets, recorded continuously with Leeds University's Feeding Behaviour System (LUFBS), were used to examine the suitability of this methodology and calculate meal characteristics.

Material and methods The feeding behaviour of sixteen piglets equipped with transponder ear tags (four groups of four piglets per pen) was recorded for two weeks post weaning (24h per day) using the four-spaced LUFBS in each pen. Pens were balanced for weaning weight, litter origin and gender and piglets were offered *ad-libitum* access to feed (16.45 MJ DE, 1.6 g lysine/kg). Animals were weighed at weaning (d0), d7 and at the end of the experiment (d13). GENSTAT 12 was used for all statistical analysis and model fitting. Feeding intervals were $\log_e$ transformed and plotted as a frequency distribution. A three population model (GGW) was fitted to the log-transformed observed interval length as described by Yeates *et al.* (2001) and the probability of animals starting feeding in the next 5 minutes (Pstart) was calculated to analyse the pattern of feeding of the piglets post weaning. The nadir of each Pstart curve was recorded as an estimate of MC (Howie *et al.*, 2009). Pstart was calculated and plotted for day and night separately and each day post weaning. A line of best fit was plotted for the longer interval lengths after the nadir of the graph to investigate the plateau. The difference in Pstart between 240 and 30 minutes was calculated.

Results Growth of the piglets was acceptable at 159 (±26) g/ piglet/day. A frequency distribution was plotted of the feeding intervals which confirmed the classic pattern of intervals with a large initial peak, suggesting within meal intervals, and a smaller second peak of between meal intervals (Figure 1). The parameters of the GGW model suggested a MC of 29.1 minutes when night time data was fitted to the model (fitting unsuccessful for 24h data), which would result in 6.26 meals/day per piglet. However, the MC was longer than previously found in pigs of 16.9 minutes (Morgan *et al.*, 2000a). Pstart (Figure 2) was calculated and did not increase post weaning, but plateaued after the initial drop, unlike that observed in more mature pigs. This suggested entirely random behaviour and therefore the log-normal model was not suitable for this data. The difference in Pstart between 30 and 240 minutes was -0.014 confirming the lack of increase. Pstart was investigated for night and day (Morgan *et al.*, 2000b), and for each day post weaning. Table 1 shows a range of this data. The mean nadir for days 1 – 13 was 31.6 (±1.2), an estimation of the MC using Pstart.

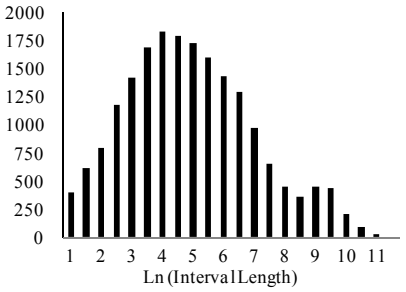

Figure 1 Frequency distribution of intervals

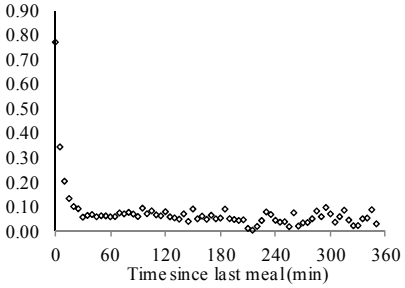

Figure 2 Probability of feeding in next 5 minutes

Table 1 Pstart parameters

	Nadir (min)	Line of best fit* (y =)	Difference (30-240 min)
d1	20	0.00187x - 0.1290	0.393
d2	35	0.00120x - 0.0392	0.252
d4	30	0.00029x - 0.0102	0.061
d7	30	0.00036x + 0.0352	0.075
d10	35	0.00011x + 0.0007	0.023
d13	25	0.00013x - 0.0047	0.027
night	30	0.00001x + 0.0833	0.004
day	35	0.00002x + 0.0569	-0.021

*mean r^2 = 0.22 (±0.059)

Conclusions The MC for these piglets was higher than previously found for older pigs, which may be a factor of the weaned piglets' stress and inexperience. Pstart concludes that the STFB of weaned piglets was not governed by hunger and satiety in this experiment and therefore log-normal methods are inappropriate for this data. At times before the nadir of the Pstart graph, Pstart is higher and indicates intervals within meals. The almost plateaued, constant and lower Pstart at longer intervals represents intervals between randomly occurring meals. The MC was estimated at the nadir of the Pstart graph at 31.6 minutes. Apparent random behaviour, where the Pstart did not increase with time, has been observed before when data were inappropriately pooled across day and night (Morgan *et al.*, 2000b). However, splitting the data in this experiment into day and night and individual days post weaning, did not conclude an increase in Pstart and therefore log-normal methodology remains inappropriate for analysis of STFB of weaner piglets.

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Effects of 1 week treatment with beta-adrenergic agonist or growth hormone on the growth and feed conversion efficiency of growing gilts

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Introduction Pork is the most highly consumed meat in the world making up approximately 43% of total global meat consumption according to the analyses of livestock and poultry markets produced by the Foreign Agricultural Services (FSA) and USDA in April 2011. Feed conversion efficiency (FCE) and carcass composition of the animals are major variable factors which impact on the productivity and profitability of the pig industry. This study aimed to determine the response of a UK White Duroc x (Landrance x Large White) to two types of growth promoters, a β-adrenergic agonist (Ractopamine^{TM}) and growth hormone (Reporcin^{TM}) for a 7day period.

Material and methods Forty five (45) White Duroc x (Landrance x Large White) gilts weighing about 85(±5)kg were sourced from PIC (Alpha Building, Nantwich, Cheshire), acclimatized to the feed and environment for 5days, before being allocated to one of three treatment groups (n=15 per group): Control group (n=15) fed a standard commercial diet (high energy (14MJ/kg), high protein (16.7% CP)) *ad-libitum*; β-adrenergic agonist group (BA, n=15) also fed *ad-libitum* the standard commercial diet containing Ractopamine (10mg/kg); and growth hormone group (GH, n=15) fed the commercial diet *ad-libitum* and administered Reporcin (10mg) by intramuscular injection on days 0, 2, 4, and 6. Feed intake was measured daily and body weights measured on days 0, 3 and 7. Within 15 minutes of slaughter, skeletal muscle tissues (*Semitendinosus* (ST), *Vastus Lateralis* (VLat) and *Vastus Intermedius* (VInt)) were dissected from the right-side half of the carcass and their weights recorded together with that of the liver and the whole carcass. A sample of *Longissimus Dorsi* (LD) was taken, snap frozen in liquid nitrogen and stored at -80^{o}C prior to glycogen determination according to Dreiling *et al*. (1987). Data were analysed by ANOVA (Genstat) and *Post Hoc* Dunnett's test.

Results GH significantly increased liver weight, including liver weight expressed as a % of body weight, and reduced feed intake (daily and total), but had no significant effect on body weight gain, feed efficiency, carcass weight or any of the whole muscle weights. BA significantly reduced LD muscle glycogen content and increased VLat muscle weight (p=0.062) and VLat muscle weight expressed as a % of body weight (p=0.055). Body weight gain, feed efficiency, carcass weight and ST muscle weight were highest in the BA-treated pigs, but none were statistically significant.

Table 1 Effects of treatment on gilts with BA and GH in a 7 day period

Measurement	Control	BA	GH	SED	P-value
Initial BWt on day 0 (kg)	87.07	86.90	86.03	2.11	0.871
Final BWt on day 7 (kg)	93.63	94.00	91.70	2.22	0.543
Change in BWt (kg over 7days)	6.57	7.10	5.67	0.97	0.340
Total Feed intake (kg over 7days)	20.223	20.827	17.869	1.207	0.047
Daily Feed intake (kg/d)	2.829	2.928	2.509	0.170	0.049
Feed conversion efficiency (kg gain/kg feed)	0.327	0.336	0.315	0.041	0.879
Carcass weight (kg)	74.96	75.62	73.59	1.93	0.567
Liver wt (kg)	1.573	1.551	1.859	0.067	<0.001
% Liver wt (g/100g BWt)	1.68	1.65	2.02	0.05	<0.001
LD muscle glycogen (mg/g)	6.568	4.779	6.685	0.741	0.015
Whole muscle weights:					
ST wt (g)	434.21	454.58	417.46	21.23	0.231
% ST wt (g/100g BWt)	0.465	0.484	0.454	0.018	0.267
VLat wt (g)	340.62	376.87	342.51	16.56	0.062
% VLat wt (g/100g BWt)	0.364	0.401	0.373	0.015	0.055
VInt wt (g)	121.08	119.03	123.21	5.99	0.786
% VInt wt (g/100g BWt)	0.1297	0.1267	0.1347	0.0060	0.415

Conclusion Over a 7 day treatment period GH decreased feed intake with no effect on muscle weights, whereas BA tended to increase *Vastus Lateralis* muscle weight and decrease LD muscle glycogen content. Overall BA was a more potent anabolic agent than GH (over this short period and dose), in terms of its effects on muscle growth.

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055

Estimation of residual energy intake and its genetic background during the growing period in commercial pigs

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Introduction The method of defining feed efficiency has substantial influence on effectiveness of selection on this trait. Residual feed intake (RFI), which is the difference between actual feed intake and that predicted on the basis of average requirements for production (e.g. ADG and body composition) and maintenance, has been discussed as an alternative measure of feed efficiency (Kennedy *et al*., 1993). The aims of this study were to develop the best model for estimation of residual energy intake (REI) and to estimate the heritabilities (h^2) and genetic correlations between REI and production traits at different stages of growth.

Material and methods Data from 315 pigs of a F_2 population were used which originated from a 3-generation full-sib design population obtained from crosses of Pietrain grand-sires with commercial dam line. Average daily protein (APD) and lipid deposition (ALD) rates were calculated as the difference between protein or lipid amounts measured using the deuterium dilution technique at the following target weights: 60 kg, 90 kg, 120 and 140 kg. Of the F_2 animals, 48 gilts and 46 barrows were single-housed and fed manually. The remaining 117 gilts and 104 barrows were group-housed and fed using electronic feeders which recorded individual feed consumption. Pigs were fed *ad libitum* with two different pelleted diets, according to the growth stages, containing 13.8 MJ/kg ME during growth from 60-90 kg body weight and 13.4 MJ/kg ME during growth from 90-120 kg and 120-140 kg body weight. Average daily gain (ADG) was calculated within each stage of growth and the entire growing period. The REI was estimated, as the difference between actual metabolisable energy intake (EI) and predicted using four different models that included besides other fixed effects (sex, housing type, halothane genotypes, herd and batch) 1) non-adjusted ADG and backfat thickness (BF) (REI1) 2) non-adjusted APD and ALD (REI2); 3) adjusted ADG and BF (REI3) and 4) adjusted APD and ALD, as a unique method (REI4). The genetic parameters and EBVs for REI traits at different stages of growth and during the entire growing period were obtained in a REML analysis using the above models and animal as a random effect using ASREML (Gilmour *et al*., 2009). Correlations between EBVs were estimated as an indication of genetic correlations because the multi-trait analysis did not converge. The BF (cm) was measured on slaughter pigs only so that the REI models 1 and 3 could only be carried out over the entire growing period.

Results The coefficient of determination (R^2) associated with the four REI models indicated that models 1 and 3 explained 2.5% lower variation of EI than models 2 and 4. The phenotypic variation of REI reduced by 8.6% when using model 2 and 4 compared to model 1 and 3. Using model 4, REI explained 29%, 35%, 35% and 21% of variance in EI for 60-90 kg, 90-120 kg, 120-140 kg and the entire growing period, respectively.

Table 1 Estimates of root mean square error (RMSE), coefficient of determination (R^2), phenotypic variance (σ_p^2), heritability (h^2) and its standard error (SE) of residual energy intake measurements during the entire growing period

Trait[1]	RMSE	R^2	σ_p^2	h^2	SE
REI1	1.99	0.77	4.43	0.44	0.20
REI2	1.89	0.79	4.05	0.44	0.21
REI3	1.99	0.77	4.43	0.44	0.20
REI4	1.89	0.79	4.05	0.44	0.21

The h^2 (SE) and σ_p^2 of REI4 for 60-90 kg were 0.41 (0.20) and 7.96; for 90-120 kg were 0.35 (0.20) and 8.99; and for 120-140 kg were 0.54 (0.20) and 12.26. For the entire growing period, the EBV correlations of REI4 with ADG, BF, EI, APD, ALD or total nitrogen excretion (TNE) were 0.22, 0.13, 0.66, 0.24, 0.29 and 0.67, respectively, and all significantly different from zero ($P < 0.05$).

Conclusions This study showed that estimation of REI by adjusting for ADG and BF, used as approximation for lean and fat growth, provided similar results to those adjusted for APD and ALD, which are biologically more related to energy requirements. BF was measured on the carcass which is expected to be more accurately measured than ultrasound BF (UBF), which is widely used in RFI studies. In our study, 77-79% of σ_p^2 in EI was explained by the models. Cai *et al*. (2008) found that 66% of the σ_p^2 in feed intake was accounted for by the model which included ADG and UBF. At later growth stages, REI resulted in substantially higher σ_p^2, which could be due to lower energy efficiency of animals for maintenance requirements and body growth. REI was highly heritable and the correlations between EBVs suggested favourable associations between REI and BF, ALD, EI and TNE. Based on these correlations, selection for lower REI will result in a decrease in fatness, feed intake and nitrogen pollution in growing pigs.

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An assessment of straw intake by acid insoluble markers in commercial pigs housed in straw based systems

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Introduction Cereal straws have long been used as bedding material for pigs housed in the UK. How much of this is consumed is becoming an increasingly important question with regards to healthy and efficient pig production. Estimates of bedding intake suggest weaned pigs are consuming up to 234g/day (Staals *et al*., 2007), potentially increasing to 2.3Kg in sows left unrestricted (Brouns *et al*., 1995). The subsequent problems associated with straw bedding intake relate (not exclusively) to a reduced nutrient digestibility (Staals *et al*., 2007) and a possible risk of mycotoxicosis (Edwards and Stewart, 2010). Wider implications may include reduced economic performance and increased feed use that may otherwise have been utilised for the growing human population. These effects are particularly detrimental to sustainability in light of increasing cereal prices and rising competition for arable land. The objectives of this current study were to quantify the intake of straw bedding in commercial sows and growers when bedded up with wheat straw either daily or weekly. This summary is part of a larger study into the effects of straw bedding intake on feed digestibility.

Material and methods In the first experiment, commercial sows (n=45) from PIC (LW x LR) and JSR (LW x LR) x D) were housed in pens of 6 to 8, with each pen randomly assigned to one of three treatments. The treatments were zero straw (bare solid concrete), approx. 15 Kg of wheat straw added daily or 100 Kg of wheat straw added at the start of each week. The second experiment used 96 commercial growers (LW x LR x terminal sire) sourced from slatted weaner vans, blocked in pens of 8 and balanced for sex and pen weight (29.7 Kg ± 0.2). Growers received the same treatments as described in experiment 1 but with half the quantity of straw for treatments 2 and 3. Both trials were run over a 3 week period in August where daily temperatures were recorded between 17.5 and 25.5 ^{0}C. A standard dry sow diet (12.4% CP, 4.3% oil & 6.3% fibre) and a standard grower diet (18.7% CP, 4.0% oil & 3.8% fibre) was manufactured by ABN Ltd to include 3g/Kg titanium dioxide (TiO_2). Sows were individually fed at 8 am (approx. 2.2 Kg/sow) in free access stools and growers were fed *ad-lib*. All sow treatments were started at day 0, with TiO_2 diets fed from day 11 and collection of faeces over days 21, 22 and 23 at which point the trial was ended. All grower treatments were started at day 0, with TiO_2 diets fed from day 7 and collection of faeces over days 16, 17 and 18 at which point the trial was ended. Tio_2 was used as a feed marker and the ratio of TiO_2 of feed to faecal output compared with a naturally occurring acid insoluble plant cell wall component (ADL) to estimate ADL originating from feed. Any excess ADL was assumed to be from consumed straw bedding. TiO_2 concentrations were determined based on the method by Myers *et al.,* (2004) and read using a Jenway 6305 spectrophotometer at 408nm. Acid detergent lignin (ADL) in faecal, feed and straw was determined based on the method of Goering and Van Soest (1970). Data was subject to one-way ANOVA (without blocks) and if significant, Tukey's test using GenStat software (version 14) with statistical significance accepted at the 95% level.

Results One result in the sow control group was omitted from the analysis due to being outside the permitted normal range. Results suggest that sows bedded on straw consumed a significant (P=0.002) quantity of bedding material. Although not significant, there were indications that sows on the daily treatment consumed more than those on the weekly treatment. The marker method detected a slight loss of ADL (2 – 4.5g/day) in faecal samples collected from growing pigs. Therefore, no measurable straw intake was possible but calculated values are shown for comparison purposes.

Table 1 Mean daily straw intake of growers and sows expressed as g/Kg of feed

	Control	Weekly	Daily	P - Value	SED
Sow	-4.68^A	178.66^B	273.46^B	0.002	72.13
Grower	-23	-20	-58	0.777	59.1

*Means with the same superscript letters are not significantly different (P=0.05)

Conclusions This trial indicates that sows on a restricted diet consume a significant amount of straw bedding (approx. 179 – 273 g/Kg of feed) regardless of the frequency with which the straw is replenished. The marker method suggested that growing pigs fed *ad-lib* did not consume any measurable bedding material in this case.

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057

Effects of growth rate on meat quality traits in finisher pigs

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Introduction The effect of manipulating the growth rates of pigs on pork quality has worldwide implications. Higher growth rates (HGR) from birth until slaughter at the same live weight produce pigs with enhanced carcass qualities, but similar meat qualities, both in association with an increased slaughter weight (Yang *et al*, 2011; Correa *et al*. 2006). Customers purchase pork in relation to appearance, shelf life and palatability therefore an understanding of how the growth rate affects these traits may result in a higher quality of pork. Tenderness is often considered a very important factor in taste, with the levels of tenderness decreasing with age. Diet does not have a direct affect on this; however it can be used to manipulate the growth rate of pigs, hence younger pigs will reach a specific slaughter weight quicker. The main aim of this study was to establish whether the growth rate of finishing pigs produced a significant change in meat quality and taste. A secondary aim was to evaluate whether sex produced variations in the quality of pork with different growth rates.

Material and methods The experiment was carried out using 64 Hampshire pigs split into 32 for each sex. Pigs were weaned at four weeks of age and slaughtered in 4 weekly batches once they reached 95kg live weight. Pigs were grouped according to their rate of gain throughout the finisher stage; values above 0.950 kg per day were ranked with a High Growth Rate (HGR), those below 0.875kg per day with a Low Growth Rate (LGR) and those in-between 0.875 and 0.95kg per day with a Middle Growth Rate (MGR). For each group carcass cold and hot weight, P2, lean %, pH at 45 minutes and ultimate pH were measured at the abattoir post slaughter. Eleven chops from each pig were transported to Leeds University, held at 4^{o}C in a cooler for meat quality analysis 48 hours post slaughter. Four chops were used to determine drip loss and cook loss in duplicate for each pig. Chops used to determine cook loss were then cored (17x 17mm) for firmness analysis (Model TA-XT2 Texture analysis) with a 2mm probe. A chop from each pig was given a subjective marbling score on a scale of 1-5, 1 having very little marbling and 5 being highly marbled, using photographic standards. Chops were scored on each side. Two way ANOVA statistical analyses using SPSS software were performed on the data.

Results No significant difference in marbling was detected between growth rates, which contrasts with the results produced from an investigation by Yan, *et al*.(2011) however, like this previous article, the ultimate pH was significantly higher in LGR pigs followed by MGR and finally by HGR pigs ($P<0.05$). The reduction in pH from pH 45 to ultimate was greater in HGR pigs compared to LGR and MGR pigs ($P<0.05$). Drip loss, cook loss and carcass pH 45 minutes post slaughter did not show any significant differences between the rates of growth. Firmness was measured to show no significant difference between growth rates. No significant differences in meat quality were found between the Gilts and Boars, however there were more boars in the HGR group than Gilts ($P<0.05$).

Conclusion Growth rate during the finishing period had little effect on the meat quality of pork. Gilt and boar comparisons showed little variation in meat quality for the three growth rates; however boars grew at a faster rate than gilts. A lower ultimate pH is often associated with a decrease in the water retention and tenderness below 5.6; however these results show that the ultimate pH did not decrease to low enough levels to produce a significant change in the drip loss or firmness between growth rates. The taste panel results have yet to confirm a difference in juiciness and tenderness.

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Fatty acid composition of retail organic and non-organic beef in North East England

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Introduction Sales of organic beef is one of the most rapidly growing sectors in the expanding organic food market in UK (Soil Association, 2011). Despite consumers' general belief that organic foods are healthier, more nutritious and taste better, this is largely unproven scientifically (Brenan *et al*., 2003). Beef is an important dietary source of beneficial fatty acids (FA) such as c9t11 C18:2 (CLA9), t11 C18:1 (VA) and omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA: c7c10c13c16c19 C22:5; DPA, c5c8c11c14c17 C20:5; EPA, c4c7c10c13c16c19 C22:6; DHA) as well as proteins and micronutrients. Pasture-based production systems with cattle nutrition based mainly on grazing result in higher concentrations of CLA9, VA and n-3 PUFA in beef than concentrate-based systems (Nuernberg *et al*., 2005; Noci *et al*., 2005). Although extensive research has been performed on the FA profiles of beef produced under different feeding regimes, most studies were conducted under experimental conditions and the FA composition of retail beef, especially organic origin, has not been thoroughly described. The objective of this study was to evaluate differences in FA profiles of UK organic and non-organic beef from retail outlets in North East England.

Material and methods Retail organic (n=10) and non-organic (n=9) beef sirloin steaks, which mainly consist of M. *longissimus* (ML) and subcutaneous fat (SCF) tissue, were purchased from 2 major retail chains in North East England on 5 different occasions during April 2011. Each product was assumed to be originated from different animals based on differences in the EC code of abattoirs, used-by date and date code printed on product labelling and, along with the suppliers' web pages, it was suggested that all beef cattle were reared mainly with grass- or forage-based diets. Fatty acid methyl esters (FAME) were separately prepared from 1g of ML and 50mg of SCF according to the method described by O'Fallon *et al*. (2007). Analysis of FAME was performed with a Gas Chromatography system (Shimadzu, GC-2014, Japan) using a Varian CP-SIL 88 fused silica capillary column (100m x 0.25mmID x 0.2µm film thickness). Peaks were identified using a 52 FAME standard and quantified using C13:0 as internal standard. Analysis of variance (ANOVA) using linear mixed effects model (LME) was used to analyze results in R statistical environment, using "manage-ment system" (organic, non-organic) and "supplier" (supermarket 1,2) as fixed factors and animals (different steaks) as random factor.

Results Total fatty acid content in ML and SCF was not significantly different between the production systems. ML from organic steaks showed significantly lower concentration of omega-6 PUFA (n-6 PUFA) and significantly higher n-3 PUFA and n-3 LCPUFA when compared with ML from non-organic steaks. SCF of organic steaks had significantly lower concentration of c9 C18:1 (OA) and monounsaturated fatty acid (MUFA) and significantly higher c9c12c15 C18:3 (ALN), VA and n-3 PUFA compared with SCF of non-organic steaks (Figure 1).

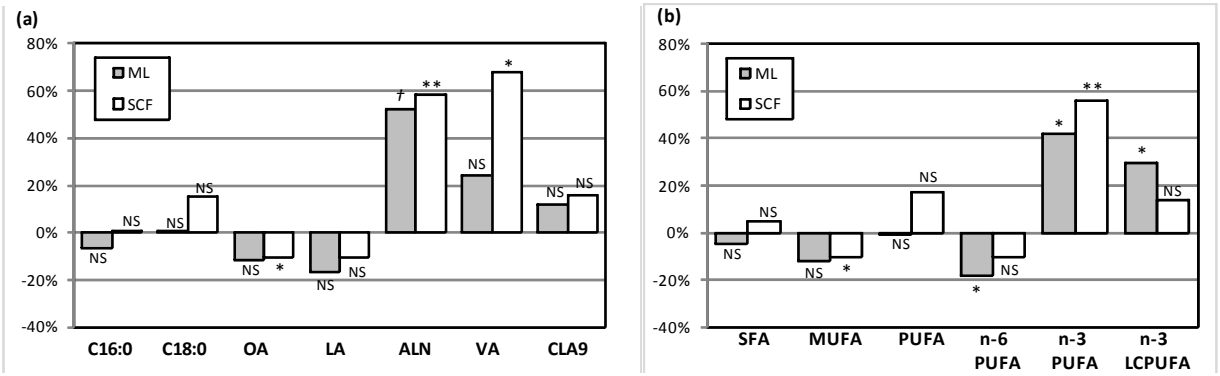

Figure 1 Relative proportions (%) of (a) individual FA and (b) FA group concentrations in ML and SCF of organic sirloin steaks when compared with non-organic
Significances were declared at ***: P<0.001, **: P<0.01, *: P<0.05, †: 0.05<P<0.10, NS: P>0.10

Conclusions Despite the labels of both organic and non-organic steaks implying grass- or forage-based production methods, there were significant differences in FA profiles in both ML and SCF. Since samples were collected in April, the cattle must have been finished over winter, presumably housed, and fed preserved forage and concentrates. Higher n-6 PUFA content in ML of non-organic beef could result from feeds rich in n-6 PUFA, mainly represented by c9c12 C18:2 (LA), such as maize silage and/or concentrates.

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Effect of meat ageing and varying the gas headspace to meat ratio on the oxidative shelf life of beef loin steaks packed in modified atmosphere packs

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Introduction Fresh beef is typically displayed in high oxygen modified atmosphere packs (MAP), containing oxygen and carbon dioxide(80:20). The oxygen gives a desirable cherry red colour to the meat but may also induce lipid and protein oxidation resulting in off flavours (Jakobsen & Bertelsen, 2000) and tougher meat (Rowe *et al*., 2004). The current ratio of headspace to meat is often greater than 3:1 and so MA packs occupy a lot of space during distribution and in retail display. The purpose of this experiment was to examine the effect of varying gas headspace to meat ratios on colour, lipid and protein oxidation of beef sirloin steaks packed in MAP.

Material and methods Beef striploins (n=12) were selected at 72hrs post-slaughter from a commercial abattoir from carcass sides weighing 151-167kg. Each individual loin was vacuum packed and stored to a total of 10 days ageing post-slaughter at 1°C after which they were cut into two equal parts. Alternate head and tail parts were selected as 10 or 21d aged sub-samples. Loin sub-samples were cut into 13, 20mm thick, steaks and trimmed to constant weight. One steak from each 10d aged loin was vacuum packed and frozen at -20°C and served as a day zero control. The remaining 12 steaks were randomised and packed into MA trays which had been variably filled with edible wax to give gas to meat ratios of 4:1, 3:1, 2:1 or 1:1. The remaining half-loins were vacuum packed and stored at 1°C for 11 days (21 days aging in total) when they were cut and packed as for the 10 day aged samples. All the packs were displayed under standard simulated retail display conditions (700lux, 16 h light, 8 h darkness at 3ºC). Colours were measured through the pack lid using a Minolta CR400 chromameter , TBARS (as a measure of lipid oxidation) were measured at day 7 and 10 of display by the method of Tarladgis *et al*. (1960) and thiol concentration (as a measure of protein oxidation) was determined following the methodology of Lund *et al*. (2007). Data was analysed by analysis of variance with ageing and day of display as factors.

Results The 10d aged samples had a longer colour shelf life than 21 day aged meat (Figure 1,13 vs 9 days respectively) and were less prone to lipid oxidation (Table 1). Varying the gas headspace to meat ratio did not significantly affect meat colour or TBARS. Displaying samples for 10 vs 7 days increased lipid oxidation. There was an indication that the 4:1 ratio may confer an extra day's colour shelf life compared to the other ratios, but only after 13 or 10 days display for 10 and 21 day aged meat respectively, which is well beyond the seven days required by retailers. The concentrations of free thiol groups, the disappearance of which are an indicator of protein oxidation, showed that neither ageing nor gas to meat ratio significantly affected protein oxidation. However, display in MAP reduced thiol content to 25% of time zero values (results not shown).

Table 1 Effects of ageing and display time on colour and lipid oxidation

Aged (d)	10		21			
Displayed (d)	7	10	7	10	sed	p
Chroma	24.44[a]	22.77[b]	22.45[b]	15.10[c]	1.03	<0.001
TBARS	1.12[a]	1.73[b]	1.63[b]	2.74[c]	0.433	0.05

[abc]values in a row with a different superscript are significantly different

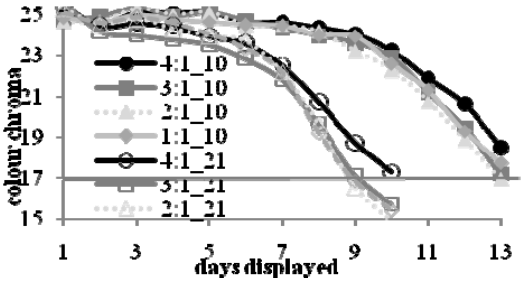

Figure 1 The change in colour chroma with days displayed

Conclusion Varying the gas to meat ratio between 1:1 and 4:1 in high oxygen MAP does not affect chroma or lipid and protein oxidation. Ageing the meat makes it more prone to lipid oxidation and colour deterioration during retail display. Hence, meat should be aged to a minimum time necessary to give a tender product without compromising colour shelf life and hence potential wastage. Reducing the headspace in a pack by 30% would reduce gas and plastic costs by 20% with potentially more packs carried per lorry journey.

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060

The implementation of docility and scrotal circumference genetic evaluations for British Limousin cattle

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Introduction Across-herd genetic evaluations of British beef cattle have been undertaken since the late 1990s for terminal traits (Crump *et al.*, 1997) and 2005 for maternal traits (Roughsedge *et al.*, 2005). In 2011, docility (DOC) and scrotal circumference (SC) EBVs were added to the British Limousin genetic evaluation allowing breeders to select cattle with genetically improved temperament and increased scrotal circumference. Literature has shown both traits to be moderately heritable (Burrow 1997, Cammack *et al*., 2009) indicating that genetic improvement can be made through genetic selection of these traits. Furthermore, favourable genetic correlations with existing performance recorded traits were reported in the literature (e.g. scrotal circumference and daughter age at puberty (Cammack *et al*., 2009) and docility and live weight (Burrow 1997)). The aim of this work was to estimate the genetic parameters from UK Limousin data and develop statistical models required to implement these new traits into the routine UK Limousin genetic evaluations.

Material and methods The British Limousin Cattle Society (BLCS) and Signet have been collecting docility and scrotal circumference records since 2006 with 1,174 and 2,002 records available, respectively for analysis. Both traits were recorded at approximately 400 days of age. Docility was scored using a 1 (docile) – 5 (aggressive) scoring system while scrotal circumference was measured as cm at the widest part of the scrotum. The PROC MIXED procedure in SAS (SAS Institute, 2002) was used to identify significant ($p < 0.05$) non-genetic effects to include in the statistical model. To allow genetic correlations to be estimated the dataset was further expanded to include additional performance recorded traits and animals. Complete contemporary groups (CG) were included in the data if they contained an animal with docility or scrotal circumference recorded or contained a parent, progeny or half/full sib of docility or scrotal circumference recorded animals. Genetic parameters were estimated from an animal model using ASREML (Gilmour *et al*., 2006). The final statistical model fitted a random genetic and residual effect. For both traits, fixed effects were CG based on herd, sex and user defined management group, birth type, birth month, breed status, dam parity and age of measurement. Existing statistical models were used for traits already evaluated. While docility was scored as a 1-5 score, it was transformed into normal scores for analysis and the resulting EBV further transformed and expressed as %docile. Scrotal circumference EBVs were expressed in cm.

Table 1 Genetic correlations (se) of existing performance recorded traits and the new docility and scrotal circumference traits

Trait	Docility (normal scores[+])	Scrotal Circumference (cm)
Birth weight (kg)	-0.12 (0.18)	0.18 (0.14)
200 day weight (kg)	-0.23 (0.20)	0.51 (0.12)
400 day weight (kg)	-0.27 (0.17)	0.44 (0.12)
Ultrasound fat depth (mm)	-0.05 (0.21)	0.23 (0.16)
Ultrasound muscle depth (mm)	-0.43 (0.22)	0.23 (0.16)
Age at First Calf (proportion)		-0.26 (0.31)

[+] Higher scores indicate increased docility

Results Docility was scored at an average of 412 days with 91.7% of animals being scored as docile (scores 1 and 2). Similarly, scrotal circumference was measured on average at 435 days with an average scrotal circumference of 35 cm recorded. Both traits were found to be moderately heritable (docility h^2=0.40 (0.14), phenotypic variance 0.42 normal score^2 (0.03); scrotal circumference h^2=0.33 (0.09), phenotypic variance=3.74 cm^2 (0.15)). The genetic correlations with performance recorded traits are shown in the Table 1 below. A non-significant small genetic correlation between docility and fat depth was estimated with a moderate positive correlation estimated between fat depth and scrotal circumference. A moderate genetic correlation was estimated between age at first calf and scrotal circumference; bulls with larger scrotal circumferences produce daughters which given the opportunity will calve earlier. The average DOC and SC EBV (and accuracy) was 2.2 % (70%) and 0.7 cm (70%).

Conclusions The addition of two new breeding values to the Limousin genetic evaluations provides Limousin breeders the opportunity for the first time to genetically select for more docile animals and provides earlier information (via a scrotal circumference measure) to more accurately identify sires that produce daughters that reach puberty at a younger age.

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061

Breeding structure of the pedigree Limousin cattle population in the UK

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Introduction The majority of genes in the UK suckler herd descend directly from pedigree beef herds, predominantly via breeding bulls used through natural service (NS) (Todd *et al.,* 2011). Previous studies have suggested that an 'elite' core of bull breeding herds is responsible for the majority of gene flow within pedigree populations (Ozkutuk and Bichard, 1977; Robertson and Aker, 1951; Wiener 1952). The concept of the herd as the level at which selection decisions are made is therefore an import one in beef cattle breeding. This study investigates whether such a herd structure exists in Limousin, the UK's most influential beef breed, as well as examining selection intensity for terminal traits in pedigree herds.

Material and methods Pedigree and genetic evaluation records held within the BASCO database were analysed to produce a profile of UK Limousin herds. The 2010 male registration cohort (MC) was used as the base group from which to define herd influence. Herds were ranked according to the proportion of this cohort bred within the herd and the proportions of sires, dams and paternal grand sires (PGS) of the cohort that had been bred within the herd. Imported animals were defined as either directly imported animals or semen. The UK beef value (BV) terminal index was used to determine selection intensity in terminal traits. Since the complete record of historical BV (i.e. available at the time of selection of breeding candidates) was not available, 2010 values were used.

Results Table 1 gives an overview of the influence of the top 5% (63) of UK herds, when ranked by number in MC, compared with the average UK herd. This top 5% bred 50% of the sires of 2010 born males, imported sires bred 21 % of the MC and the remaining 29% had sires bred in other UK herds. Similarly, 39% of the MC had PGS bred in the top 5% of UK herds, 26 % having imported PGS, 21% having non-UK born PGS ancestors and just 14% had PGS that were bred in other UK herds. PGS used in the top 5% of herds had a considerably higher mean BV, equal to the 42nd percentile of the 2010 MC, with only a marginally higher mean accuracy than that of the average PGS, which was equal to the 58th percentile.

Table 1 The Influence of Limousin herds ranked by the numbers of 2010 males registered.

Animal category	Number of individuals	Number of herds of birth of individuals	Mean BV and (accuracy) of individuals	% of 2010 MC imported or bred by imported individual*	(%) of individuals bred by the top 5% of UK herds**	Mean BV and (accuracy) of individuals bred by the top 5% of UK herds
2010 male cohort	8492	1270	23.5 (0.54)	<1%	29%	25.3 (0.62)
Sire	1516	625	24.0 (0.71)	21%	50%	27.5 (0.83)
Dam	8290	1247	19.2 (0.61)	4%	34%	21.1 (0.73)
PGS	705	342	22.4 (0.81)	26%	39%	25.4 (0.91)

* Imported includes directly imported animal or semen only and excludes second generation non-UK born ancestors.
** The top 5% of UK herds were determined by the numbers of 2010 males born in those herds.

Discussion
The influence of the top 5% of herds in Table 1 is evidence that an elite group of bull breeding herds does exist in the UK herd book. These herds are typically large in size and are the most influential group of breeders of sires and PGS. Since the majority of the 2010 MC will become commercial herd sires in suckler and dairy herds (Todd *et al.,* 2011), the top 5% of herds therefore breed a considerable proportion of bulls disseminated to the commercial breeding sector. A classical multiplier group that is distinct from the 'elite' does not exist in the pedigree Limousin population. UK pedigree Limousin breeders continue to select considerable numbers of bulls, mainly from France but now also other European countries and North America to use as sires. Considering the large influence of Limousin genes in the commercial beef herd, selection decisions in elite herds therefore have a major impact on the genetic improvement of terminal traits in the commercial beef herd.

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062

Using home-grown peas and beans to replace soyabean meal does not impair nitrogen balance in pigs.

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Introduction The risk of nitrogen leaching from farming systems is considerable and these concerns should also be viewed within the context of the potential for a governmental imposition of a 'nitrogen tax' to protect the environment; the development of 'nitrate vulnerable zones' is clear evidence of how environmental legislation is impacting on pig production systems. Through continuing to import significant tonnage of protein crops into the UK that is a significant contribution to nitrogen loading of the environment and the energy expenditure associated with these imports. It is for these reasons that there is now much interest in using home-grown legumes in compound pig diets. However, for such interest to lead to actual increased usage there needs to be detailed information on nutritional value. The objective of the current paper is to report N balance of diets based on soyabean meal or home-grown legumes fed to growing / finishing pigs..

Material and methods Details of experimental diets are presented in White *et al*. (2012). Hipro soyabean meal (O) white-flowered peas Prophet (P), spring coloured-flowered faba beans Fuego (F) and white-flowered faba beans Tattoo (T) were added to grower and finisher diets. A four by four Latin Square design using four male pigs over four collection periods was employed to evaluate grower diets (from 35 – 75kg) and a second Latin Square with a new batch of pigs was employed to evaluate finisher diets (from 55 – 95kg). Each collection period consisted of an initial acclimatisation period of six days on experimental diets with animals housed individually in holding pens. Animals were fed twice daily at a rate of 0.90 assumed *ad libitum* intake mixed in the ratio water:feed 2:1. Animals were then transferred to metabolism crates and, on the following day, the dye indigo carmine was added to the evening meal. Quantitative faecal collection commenced on appearance of the dye in the faeces the following day and was stored at -20°C pending N analysis. Quantitative urine collection commenced at 08.30 that day into suitable vessels containing 25ml of 50% sulphuric acid to avoid evaporation by maintaining pH <4.0. A 1% sub-sample was taken and stored at -20°C pending N analysis. Indigo carmine was added to the evening meal of the 6th day; quantitative collection of faeces stopped on re-appearance of the dye the following day and urine collection at 08.30 that morning. Faecal output was thus related directly to 10 meals over five days, and urine output on a timed basis of five days. At the end of each collection period, animals were weighed, allocated to the new experimental diet at a rate based on liveweight and placed back in holding pens. At the end of the two metabolism trials faecal samples were thawed, homogenised two sub-samples were frozen dried to constant weight (allowing the calculation of faecal dry matter). Urine samples were thawed, mixed thoroughly and a 100µl sub-sample pipetted onto N-free potato starch before being analysed in triplicate for N. Diet samples were also analysed for N; all N analyses were undertaken using the Dumas method. Measurements and analyses allowed the calculation of the Coefficient of Total Tract Apparent Nitrogen Digestibility (CTTAD) and the Coefficient of Apparent Nitrogen Metabolisability (CAM).

Results Table 1 presents data for the grower diets and Table 2 data for the finisher diets. There were no significant differences between treatment in CTTAD and CAM. Nitrogen budgets are an important element of environmental impact of pig production systems. The data demonstrate that accurately formulated diets based on home grown peas and faba beans will not make any more contribution to environmental impact than a diet based on hipro soyabean meal.

Table 1 Effect of diet on CTTAD and CAM - Grower

	Diet						
	GO	GF	GP	GT	SED	P	CV%
CTTAD	0.832	0.809	0.785	0.790	0.0246	0.264	4.3
CAM	0.533	0.535	0.535	0.501	0.0540	0.905	14.5
Faecal DM g/kg	312	324	332	332	21.4	0.769	9.3

Faecal dry matter is another important aspect of environmental impact; the data demonstrate that there were no differences between treatments for this particular factor.

Table 2 Effect of diet on CTTAD and CAM - Finisher

	Diet						
	FO	FF	FP	FT	SED	P	CV%
CTTAD	0.795	0.770	0.762	0.787	0.0211	0.416	3.8
CAM	0.512	0.501	0.471	0.511	0.0388	0.704	11.0
Faecal DM g/kg	299	317	317	315	19.6	0.756	8.9

Conclusions Home grown peas and beans can be safely included in diets for growing finishing pigs at a rate of 300g/kg with no additional detrimental effects on environmental impact compared with diets based on soyabean meal, with the proviso that diet formulation is undertaken accurately.

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Reference

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Effects of increasing dietary inclusion levels of peas and faba beans to replace soybean meal on pig growth performance

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Introduction Europe imports over 70% of protein required for animal feed (Crepon, 2006), which is mainly soyabean meal (SBM). This reliance on SBM increases concerns about the sustainability, security and environmental impact of UK pig production. These concerns may be reduced through use of home-grown protein sources such as peas and faba beans as an alternative to SBM in pig diets. Here, we assessed effects of increasing dietary peas and faba bean inclusion levels on grower and finisher pig performance.

Material and methods Faba beans (var. Fuego, coloured-flowered spring beans) or peas (var. Prophet) were included at 75, 150, 225 and 300 g/kg, gradually and completely replacing SBM, included in the control diet at 140 and 120 g/kg for grower and finisher pigs, respectively. Diets were formulated to be iso-energetic (NE 9.3 and 9.0 MJ/kg for growers and finishers, respectively), with the same standard ileal digestible lysine (SID Lys) content (8.1 and 7.1 g/kg), and to meet the minimum requirements of methionine, threonine, tryptophan, calcium and digestible phosphorus (BSAS, 2003) by modifying the inclusion of soya oil, pure amino acids and macro-minerals. Pulses replaced SBM on a SID Lys basis, and wheat was reduced to close the mass balance. Other ingredients were kept constant and included barley, molasses, rapeseed meal, wheatfeed and trace element / vitamin premix. Each diet was fed *ad libitum* to 4 groups of 4 terminal line grower (30-60kg) and finisher (60-100kg) pigs (LW x L, balanced for litter and sex), for 4 weeks, after a 1 week adaptation period, with separate groups of pigs to test the grower and finisher diets. Weekly live weights for individual pigs, and pen intakes were recorded to assess body weight gain (BWG, g/pig/day), average daily feed intake (ADFI, g/pig/day) and feed conversion ratio (FCR as ADFI/BWG). REML with contrast statements was used to locate treatment effects of pulse inclusion *per se*, pulse type, and linear or quadratic pulse inclusion level effects. Group was included as the random effect, as was season nested with groups for finisher BWG and ADFI. Where significant, initial weight and sex were covariates for BWG.

Results There were no significant effects on grower BWG, ADFI and FCR. However, pulse inclusion *per se* reduced finisher BWG by 8.5%, but the associated 4.9% reduction in ADFI and 3.4% increase in FCR were not significant. Faba bean and pea diets resulted in similar finisher growth performance, but there was a significant quadratic effect for finisher BWG, where BWG tended to reduce over initial inclusion levels and then increased again over further and final inclusion levels, without significantly impacting finisher ADFI and FCR.

Table 1 Effect of diet treatment on growth performance of grower and finisher pigs.

		Grower Pigs			Finisher Pigs		
Feeding treatment with pulse inclusion levels (g/kg)		BWG (g/pig/day)	ADFI (g/pig/day)	FCR	BWG (g/pig/day)	ADFI (g/pig/day)	FCR
SBM	-	823	1857	2.25	1083	2697	2.51
Faba bean	75	852	1979	2.34	1024	2529	2.55
	150	858	2000	2.34	1011	2675	2.68
	225	899	2069	2.30	980	2588	2.59
	300	907	2013	2.24	1032	2633	2.57
Pea	75	898	1975	2.20	975	2482	2.51
	150	836	1829	2.25	936	2463	2.59
	225	918	2030	2.26	967	2576	2.65
	300	834	1922	2.34	1003	2580	2.63
SEM		41	81	0.05	40	934	0.06
P-value (contrasts)							
Control vs Pulse *per se*		0.24	0.18	0.51	0.04	0.13	0.19
Beans vs Peas		0.81	0.20	0.23	0.15	0.16	0.97
Linear inclusion level effect		0.24	0.19	0.47	0.15	0.63	0.09
Quadratic inclusion level effect		0.47	0.40	0.72	0.03	0.12	0.27

Conclusions Although our SBM control diets resulted in a greater finisher BWG relative to the pulse diets *per se*, and a biologically unclear quadratic relationship for pulse inclusion level on finisher BWG was found, there was no effect of replacing SBM for peas or faba beans on FCR. This suggests that feeding pigs pea or faba bean based diets during the grower and finisher phase combined is unlikely to affect overall pig performance, indicating that peas and faba beans are a viable home-grown alternatives to SBM in pig diets. Large scale demonstration trials to verify this expectation under practical conditions are underway.

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Effects of low protein diets on performance and fat deposition in pigs

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Introduction High protein levels in pig feeds ensure optimum performance in lean genotypes. Amino acids in excess of requirements are deaminated and excreted, causing N pollution. Feeding low protein diets with the correct amino acid balance would reduce N excretion and should maintain performance. Low protein diets unfortified with key amino acids may increase fat deposition, to the benefit of eating quality.This study compared 3 dietary protein strategies in terms of performance and fat deposition.

Material and methods Four batches of 48 entire male pigs, crosses between a Large White x Landrace dam and a synthetic Pietrain (Titan) boar were reared on 3 protein strategies (Commercial, C; Low protein 1, LP1; and Low protein 2, LP2, Table 1) from 40kg live weight for about 85 days at Dryden (SAC contract farm). LP1 had lower crude protein (CP) but similar lysine (L) concentrations to C and LP2 had both lower CP and L concentrations. All diets were formulated to the same net energy (9.7 MJ/kg). Pigs were reared in groups of 4 on straw and fed *ad libitum*. When the average live weight was about 115kg, they were transported as batches to the Tulip abattoir in Spalding and slaughtered. P_2 fat thickness was measured using an intrascope. Joints were transported to the University of Bristol where samples of *longissimus* muscle were removed from the loin (last rib position). Total fatty acids (TFA) were extracted using methanolic KOH and their methyl esters quantified using gas liquid chromatography. Data were analysed using the general linear model procedure, examining diet and batch as factors. For performance data, pen was the experimental unit but results were calculated on an individual animal basis (averaging over the pen).

Table 1 Diets used

Wt range (kg)	C		LP1		LP2	
	CP[d]	L[e]	CP	L	CP	L
40-60	189	11.1	166	11.3	167	11.3
60-85	171	9.8	147	10.0	145	6.9
85-115	152	8.1	127	8.4	113	5.4

[d] Crude protein (g/kg) [e] Lysine (g/kg)

Results Performance results for the whole growth period are in Table 2. Feed intake was slightly less in C than in LP1 and LP2. Group LP2 had a lower ADG and higher FCR than C or LP1. There was no difference in P_2 backfat thickness between the diet strategies (Table 3), although there was a 46% higher loin muscle fat content in LP2 than in C, with LP1 intermediate. The proportion of the major diet fatty acid linoleic acid (C 18:2n-6) was highest in C and lowest in LP2, indicating that body fat deposition was in the order LP2>LP1>C.

Table 2 Growth performance , 40kg-slaughter

	C	LP1	LP2	P
FI[d] (kg)	189.8[b]	196.8[a]	195.0[ab]	0.077
ADG[e] (kg)	0.98[a]	0.96[a]	0.87[b]	<0.001
FCR[f]	2.46[c]	2.61[b]	2.85[a]	<0.001
Final LW[g] (kg)	117.7[a]	116.2[a]	109.2[b]	<0.001
Hot CW[h] (kg)	91.0[a]	89.5[a]	83.0[b]	<0.001

[d] Feed intake [e] Average daily gain [f] Feed conversion ratio [g] Live weight [h] Carcass weight [abc] Means in rows with a common superscript letter are not significantly different (P>0.05)

Table 3 Fatness measurements in carcasses

	C	LP1	LP2	P
P_2 fat thickness (mm)	13.1	13.8	13.7	0.280
TFA (mg/100g)	1055[c]	1177[b]	1543[a]	<.001
C18:2n-6 (g/100g TFA)	16.6[a]	14.7[b]	11.6[c]	<0.001

[abc] Means in rows with a common superscript letter are not significantly different (P>0.05)

Conclusions The LP1 strategy was designed to reduce dietary protein without affecting performance and this aim was largely achieved although there was evidence of slightly greater fat deposition in muscle. Low lysine levels in LP2 further increased muscle fat, although subcutaneous fat thickness (P_2) was little affected.

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Holo-analysis to determine the factors influencing the efficacy of an enhanced *Escherichia coli* phytase on average daily gain of pigs

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Introduction Holo-analysis describes the integration of all available data on a subject quantifying a dependent response in terms of all available independent management, genetic, environmental, and nutritional variables (Rosen, 2006). Incorporation of holo-analyses in commercial practice allows producers to evaluate effects of various factors including dietary nutrients or ingredients, animal husbandry practices, and enzymes on growth performance of pigs and poultry. The objective of this project was to identify those dietary ingredients, nutrients and animal husbandry factors that may influence phytase efficacy on average daily gain (ADG) of pigs.

Material and methods Nutrient, ingredient, and husbandry data from 43 experiments (5 to 85 kg pigs), conducted at research sites worldwide from 2002 to 2011 were included in the holo-analysis. Most diets were deficient in available P (avP) by approximately 0.05 to 0.20% from recommended levels. Factors included in the holo-analysis, but were not limited to: log dose of phytase (Quantum, AB Vista, Marlborough, UK), concentration of digestible energy, protein, Ca, avP, and the dietary content of corn and soybean meal, presence of antibiotics or ZnO, as well as the weight of the pigs at the start of the experiment, the duration of the trial, and the number of pigs/pen. Data points (n = 351) were used to assess the effects of these parameters on the difference in ADG between pigs fed various doses of phytase. Data were analysed using a step-wise regression in JMP v. 9.0 (SAS Institute, Cary, NC) to determine the effect of specific nutrients, ingredients, phytase dose, and animal husbandry practices on the efficacy of phytase in pigs. The significant factors were then used to calculate the required FTU/kg of phytase in the feed needed to replace 0.10% or 0.13% avP.

Results Factors positively ($P < 0.0001$; $R^2 = 0.82$) influencing ADG of pigs included: the log dose of phytase, the dietary percent of avP and protein, and the weight (kg) of the pigs at the start of the experiment. Due to the nutrient requirement in avP as the pig grows, an avP x start weight interaction ($P < 0.0001$) was also included in the model. Factors negatively influencing ADG included the percent dietary Ca and trial duration (days). Using the regression model to determine the amount of phytase needed to replace avP, the results indicated 250 or 411 FTU/kg phytase was needed to replace 0.10% or 0.13% avP, respectively.

Table 1 Regression analysis factors influencing average daily gain in pigs

Holo-analysis Factors	Unit	Estimate	Mean	Minimum	Maximum	Significance
Phytase dose	Log dose	49.9	2.54	1.70	4.40	< 0.0001
Available P	%	105.4	0.24	0.08	0.58	0.3492
Calcium	%	- 531.1	0.69	0.36	1.00	< 0.0001
Protein	%	15.9	20.05	15.00	25.10	0.0002
Start Weight	Kg	14.8	24.58	5.00	85.00	< 0.0001
Duration of the trial	Days	- 4.0	30.80	7.00	104.00	< 0.0001
Start weight x AvP	n/a	35.0	n/a	n/a	n/a	< 0.0001

Conclusions The model confirmed that several factors including phytase dose and avP influenced ADG. The fact that both avP and phytase had positive estimates enabled a calculation of the avP matrix of phytase at any given dose. The matrix was calculated by trading a reduction in avP for an increase in log dose of phytase. From the holo-analysis it can be concluded that 250 or 411 FTU/kg phytase is needed to release 0.10% or 0.13% avP, respectively. The scale of the response to phytase on ADG is dependent upon many factors and optimization of these factors will result in larger improvements in ADG from this *E. coli* phytase.

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Beta-adrenergic agonist and growth hormone have differential effects on muscle fibre composition in growing gilts

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Introduction According to Foreign Agricultural Services (FAS) and USDA analyses of livestock and poultry markets in April 2011, pork is the most highly consumed meat in the world, making up approximately 43% of total global meat consumption. The predicted increase in world population (reaching 9 billion by 2050) is expected to increase demand for meat, while at the same time there will be increasing demand for land and crops for production of human foods and biofuels, as well as animal feeds. In order to meet the predicted increased demand for pork within a global system where the availability of feed ingredients is limited, the efficiency of utilisation of feed by the animals must be improved. In pigs, growth promoters, including growth hormone (e.g. Reporcin^{TM}) and beta agonists (e.g. Ractopamine^{TM}) are known to improve feed efficiency and are already licensed for use in animals for human consumption in some parts of the world. Within a muscle, differences in proportions of muscle fibre types as well as their number and size affect growth performance and efficiency, as well as meat quality (Maltin *et al*., 2003). Myosin Heavy Chain (MyHC) is a major contractile protein in skeletal muscle and the expression of different MyHC isoforms (MyHC types I, IIa, IIx and IIb) within a muscle fibre generally reflect differences in fibre type and energy metabolism, with fibres often named according to the predominant isoform they contain. This study investigated the effects of a 7 day treatment with Ractopamine or Reporcin on muscle fibre type composition in two muscles of growing gilts.

Material and methods Forty five (45) White Duroc x (Landrance x Large White) gilts weighing about 85(±5)kg were sourced from PIC (Cheshire), acclimatized to the feed and environment for 5days, before being allocated to one of three treatment groups. The Control (n=15) group were fed a standard commercial diet (high energy (14MJ/kg), high protein (16.7% CP)) *ad-libitum*, while the β-adrenergic agonist (BA, n=15) group were also fed *ad-libitum* the standard commercial diet containing Ractopamine (10mg/kg) and the growth hormone (GH, n=15) group were fed the commercial diet *ad-libitum* and administered Reporcin (10mg) intramuscularly on days 0, 2, 4, and 6. After slaughter, samples of *Longissimus Dorsi* (LD) and *Psoas major* (PS) muscles were immediately collected and snap frozen in liquid nitrogen. Total RNA was extracted (Trizol) and first strand cDNA generated using random primers. Using primers specific for the MyHC isoform mRNAs (Wimmers *et al.*, 2008), the relative level of mRNA expression was determined using quantitative RT-PCR analysis (Roche). Data were analysed by ANOVA (Genstat) and *Post hoc* Dunnett's test.

Results Ractopamine (BA) significantly down regulated MyHC IIa mRNA in the LD muscle (p=0.021), and tended to increase MyHC IIb mRNA (p=0.090), suggesting fibre types were switching from IIa to IIb and therefore becoming more glycolytic. A similar effect was also evident in the PS muscle, with a trend for a decrease in MyHC IIa mRNA (p=0.082) and an increase in MyHC IIb mRNA (p=0.097). Interestingly, there was also a consistent trend for a down regulation of MyHC IIb mRNA by the growth hormone (GH) in both the LD (p=0.090) and PS (p=0.097) muscles.

Table 1 Expression of MyHC isoforms in LD and PS muscles of gilts treated with BA or GH for 7 days

Muscle	MyHC Isoform	Control	BA	GH	SED	P-value
		mRNA Expression (Arbitary units)				
LD	MyHC I	0.8378	1.0532	1.0240	0.226	0.590
	MyHC IIa	1.3266	0.7891	1.4595	0.244	0.021
	MyHC IIx	1.5490	1.2906	1.6425	0.262	0.387
	MyHC IIb	1.6475	2.3587	1.3505	0.458	0.090
PS	MyHC I	1.1647	1.0856	1.1359	0.178	0.904
	MyHC IIa	1.1151	0.7934	1.3659	0.252	0.082
	MyHC IIx	1.7284	1.7916	1.6492	0.354	0.920
	MyHC IIb	1.9446	2.4693	1.4007	0.487	0.097

Conclusions While both BA and GH are known to improve growth and feed efficiency in pigs, they appear to have opposite effects on skeletal muscle metabolism as indicated by relative changes in MyHC isoform expression. BA induced changes in MyHC isoform expression are suggestive of a transition to a more glycolytic fibre type whereas the change in MyHC expression mediated by GH indicate a shift to a more oxidative fibre type.

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Effects of dietary potassium diformate in sows during pre-farrowing till weaning on piglet performance and health – a practical approach in Germany

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Introduction Potassium diformate has been shown in numerous trials to improve health and performance in piglets, growing-finishing pigs and sows. It is furthermore currently the only zootechnical additive with EU-approval for use throughout the pig production chain. The effects of potassium diformate (KDF) are often described as strong antimicrobial and digestibility enhancing. Most of the data available on the use of KDF in sows are from trials performed at universities and research institutes and have focused mainly on the effects on sows alone. The objective of the present study was to assess the subsequent effects of KDF, fed to sows, on piglets under practical conditions.

Material and methods The study was carried out with 42 sows (crossbred db Classic, db Naima, db Viktoria) during late gestation. The experiment was conducted on a commercial farm in Hessen, Germany. The sows were randomly allotted to 2 treatment groups. Group 1 served as a control in which sows were fed a complete diet, mainly based on barley, wheat, corn and soy-extraction groats, without supplemented antimicrobial agents. Sows in group 2 were fed the complete diet containing 8 kg/t potassium diformate. The experimental feeding of sows started on day 108 of gestation and finished at weaning (19 days after farrowing). Feeding was done twice a day, while water was available *ad libitum*. Backfat thickness of sows was measured (3-point method) just before farrowing and at weaning. After farrowing, the numbers of born and "live-born" piglets were registered. First weighing was carried out on day 4, while the second weighing was done at weaning. Furthermore all mortalities were recorded. Data on weight and number of piglets as well as backfat thickness reduction in sows from farrowing till weaning were recorded and analysed using the t-test. The results are given as mean ± SD and a confidence level of 95% was defined for these analyses.

Results Sows fed with potassium diformate at a dosage of 8 kg/t under European conditions had a numerically lower backfat reduction during suckling, despite a higher weaning effort. However, due to the low number of sows and the high variance this difference was not statistically significant. Feeding KDF to sows did, however, have significant effects on the new-born piglets (Table 1). Weaning weight tended ($P=0.09$) to be increased, while weight gain during weaning and daily weight gain of piglets were significantly improved ($P<0.05$). Furthermore, the losses during the weaning period were numerically reduced ($P=0.13$).

Table 1 Diet effects of KDF on backfat reduction in sows as well as weight development and mortality in piglets

	Control	8 kg/t KDF	P-level
Piglets, born alive [n]	10.6±1.7	10.5±1.7	0.94
Piglets, weaned [n]	9.9±2.0	10.3±1.6	0.54
Mortality[%]	6.3±10.3	2.3±4.9	0.13
Weaning weight [kg]	5.8±1.4	6.0±1.4	0.09
Weight gain during weaning [kg]	3.8±1.1	4.1±1.1	0.02
Daily weight gain [g]	274±79	293±82	0.01
Backfat loss in sows [mm]	0.9±2.0	0.3±2.0	0.32

Conclusions These result show that the inclusion of potassium diformate into the diet of sows can improve condition and performance in piglets as well as in sows. Similar observations have been made by Øverland *et al*. (2009) and Lückstädt (2011). It is suggested therefore that the 8 kg/t inclusion of KDF will still confer beneficial effects onto sows and piglets under European conditions.

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Utilization of agro-industrial by products by growing pigs in the tropics

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Introduction In Nigeria**,** pig production has been advocated as a short term measure toward alleviating the animal protein and caloric deficit especially in areas where there are no religious edicts preventing their production and consumption (Kalla, *et al.,* 2003). Groundnut cake (GNC) has assumed important feed status as ingredient in livestock feed in Nigeria. Because of high cost of groundnut cake, many pig farmers are finding it increasingly uneconomical to include GNC in the diets. There is an urgent need to improve upon production through the use of locally available cheap sources of feed ingredients that have an acceptable nutrient profile. A substantial amount of slaughter house waste products notably blood and rumen content is generated daily in Nigeria. CBN, (2004) estimated that a potential annual yield of about 3,000 metric tonnes of blood meal is possible in Nigeria from about 1.7 million cattle slaughtered yearly. Adeniji (1996) estimated rumen content of bovine origin output in Nigeria abattoir to be 9,634 metric tons annually. Both blood and rumen content constitute a disposal problem and an environmental pollution menace. Brewers' dried grain (BDG) is a solid waste from the brewery industries which is readily available and cheap. Recycling these agro-industrial by-products possesses advantage of providing alternative feed ingredients for livestock and reduction in environmental pollution. BDG, rumen content (RC) and bovine blood (BB) in Nigeria can be a good source of protein in livestock feed if properly processed. The objective of these studies was to evaluate the effect of inclusion in the diets of these various products (BDG, RC and BB) as a replacement for GNC on the performance of growing pigs in the tropics.

Material and methods Three products were developed. Product 1 was Brewers' dried grain with bovine blood (BDG/BB) mixed in ratio 1:1. Product 2 was rumen content with bovine blood (RC/BB) mixed in ratio 1:1. Product 3 was BDG+RC+BB mixed in ratio 1:1:1. These products were sun-dried after mixing to about 10% moisture content before they were incorporated into the experimental diets to replace GNC. The experimental diets were formulated to meet the nutrient requirements of growing pigs in the tropics. These products had an average of about 35% crude protein. 3 feeding trials were conducted simultaneously with each of these products replacing GNC at 0, 50 and 100%. A total of 36 growing pigs were used in these studies. Each study involved 12 animals with 4 animals per treatment and each animal served as a replicate. The design was completely randomized design. The proximate composition of the test ingredients and the experimental diets were determined as outlined by AOAC (1995). Routine management practices were kept on treatment basis. All data were subjected to analysis of variance using a computer software package (SAS, 2000). There was no physical disability arising from the treatment effect.

Results The diets fed to animal in each study had similar proximate composition except slightly high protein content of the control and slightly higher levels of crude fibre as the inclusion of the test ingredient increases. The performance data is shown in the Tables below:

Table 1 Effects of BDG/BB in diets of growing pigs

	0%BDG+BB	11%BDG+BB	22%BDG+BB
Daily gain (Kg/d)	0.47±0.02	0.44±0.02	0.43±0.05
Feed intake (Kg/d)	1.21±0.09	1.28±0.09	1.30±0.09
Feed/Gain ratio	2.92±0.17	3.24±0.13	3.61±0.23

Table 2 Effects of RC/BB in diets of growing pigs

	0% RC+BB	11%RC+BB	22%RC+BB
Daily gain (Kg/d)	0.47^{a} ±0.02	0.40^{b}±0.01	0.37^{b}±0.01
Feed intake(Kg/d)	1.21 ±0.09	1.23 ±0.09	1.28 ±0.09
Feed/Gain ratio	2.92^{c}±0.17	3.38^{b}±0.08	3.88^{a} ±0.09

Table 3 Effects of BDG/RC/BB in diets of growing pigs

	0% BDG+RC+BB	**11%BDG+RC+BB**	**22%BDG+RC+BB**
Daily gain (Kg/d	0.47^{a} ±0.02	0.42^{b}±0.01	0.40^{b}±0.01
Feed intake(Kg/d)	1.21 ±0.09	1.28 ±0.09	1.29 ±0.09
Feed/Gain ratio	2.92^{b}±0.17	3.42^{a}±0.08	3.63^{a} ±0.09

For all Tables means along same row having different superscripts differ at $p < 0.05$.
In study I, feeding BDG/BB had no significant effect on performance characteristics. In studies II and III the performance characteristics were significantly influenced ($p < 0.05$) as average daily gain decreases with increasing levels of RC/BB and BDG/RC/BB. The Feed: Gain ratio was also significantly affected with the control diets having better values.

Conclusion Findings from the above studies suggest the suitability of BDG/BB mixture to replace GNC completely in the diets of growing pigs. Partially replacement of GNC with RC/BB or BDG/RC/BB gave a satisfactory performance.

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Effects of different additives in diets of growing pigs containing high levels of palm kernel cake and brewers dried grain in sub-humid tropics

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Introduction Inconsistent availability of the conventional energy and protein sources has been identified as one of the major constraints in the growth of the pig industry in Nigeria (Adesehinwa, 2009). Consequently, the use of non-conventional feed resources (NCFR) or agro-industrial by-product (AIBP), which are abundant and cheap has been advocated (Okai *et al*., 2005). However, fibrousness, a feature of most locally available agro-industrial by-products and wastes has limited their use and efficient utilization (Adesehinwa, 2008). Additives in various forms are today used to enhance the nutritional value of many feeds for poultry and pigs. The objective of this study was to determine the efficacy of three of such additives in growing pig diets.

Material and methods Eighty growing pigs (average initial weight ~ 16.63 kg) were used in this study to determine the comparative efficacy of three different animal feed additives in growing pigs' diets. The three animal feed additives used include fossil shell flour (FSF) – a fresh water diatomaceous earth (powder – 10 kg per 100 kg diet), direct-fed microbial (DFM) – a source of live (viable), naturally occurring microorganism (also known as Rumen Enhancer 3 (RE-3)) (liquid – 68 ml per 45 kg diet) and oxytetracycline – an antibiotic (powder – 35g per 500 kg of diet).The control growing pig diet (~17% CP) contained none of the three additives. Each treatment/diet was replicated four times in a completely randomized design with 5pigs/pen representing a replicate. Feed and water were provided *ad libitum* for the 35-day duration of the study. Proximate composition of the test diets were analyzed according to the methods of A.O.A.C. (1990). Blood samples randomly collected from 3 pigs/replicates in each treatment at the end of the feeding trial were used for haematological and biochemical studies as described by Mafuvadze and Erlwanger (2007). Data on the growth performance and hematological parameters of the pigs were subjected to ANOVA using SAS software version 9.2 (SAS Institute. Inc., Cary., N.C. USA).

Results The daily feed intakes, total and daily gains of the growing pigs were significantly influenced by the inclusion of the additives (Table 1). The highest feed intake was recorded with pigs fed FSF and the least (P<0.001) being DFM. The resultant daily and total gains were comparable across the treatment groups (FSF, DFM and OXY-TET), with values significantly higher than that obtained with the control diet. Hence, relatively lower (P<0.001) feed:gain ratio were obtained with the treatment diets indicating more efficient utilization of the test diets than the control. This means that lower quantities of feed will be required per unit kg gain in weight for all the diets containing additives. On the long run, the higher total gains obtained with the additives means the animals will attain market weight faster, hence more economic gains over time. All the haematological and biochemical indices except urea content of the blood of the growing pigs were not significantly (P>0.05) influenced by the inclusion of additives in the diets (Table 1).

Table 1 Growth Performance and some blood parameters of growing pigs fed additive-enhanced diets

Parameters	Control	FSF	DFM	OXY-TET	SEM	P-Value
Daily feed intake (kg)	1.70^{b}	1.80^{a}	1.62^{c}	1.68^{b}	0.028	***
Daily weight gain (kg)	0.42^{b}	0.53^{a}	0.47^{a}	0.51^{a}	0.016	***
Total weight gain (kg)	14.53^{b}	16.79^{a}	16.47^{a}	18.00^{a}	0.430	**
Feed : Gain	4.24^{a}	3.59^{b}	3.56^{b}	3.31^{b}	0.089	***
PCV (%)	31.67	33.50	34.50	31.00	0.818	ns
Hb (g/100ml)	10.52	11.13	11.45	10.30	0.271	ns
Total Protein (g/dl)	4.13	4.56	4.40	4.21	0.101	ns

Significant levels: *=P<0.05, **=P<0.01, ***=P<0.001, ns=not significant (P>0.05).
^{a,b,c}: Within row, means with different superscripts differ significantly at P<0.05.

Conclusion It could therefore be concluded that high levels of palm kernel cake and brewers dried grain in diets of growing pigs could be enhanced with the inclusion of any of these additives, and may have positive effects on the profit margin. However, taking into consideration the resultant side effects and health implications of prolonged use of antibiotics, the use of direct-fed microbial or fossil shell flour as additives is hereby recommended.

Acknowledgement The authors are grateful to Dr K. Oppong-Anane (Director of Basic Environmental Systems and Technology, Accra, Ghana) and Pastor O. Okuneyungbo (Tosin Ventures Ltd, Lagos Nigeria – Representative of Farmaguard Inc USA in Africa) for the supply of DFM and FSF respectively.

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Effects of maternal supplementation with essential fatty acid (Docosahexanoic acid) on neonatal piglet metabolism and vitality

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Introduction The factors which are responsible for the enhanced vitality of piglets from sows fed DHA are poorly understood. In a recent experiment (Adeleye *et al*., 2011), inclusion of DHA in sows' diet in the last four weeks of pregnancy reduced the incidence of stillbirth from 1.2 pigs/litter in controls sows to 0.65 and 0.20 in sows given 0.03% and 0.3%DHA inclusion respectively (P<0.05). Stillbirth occurrence is frequently associated with longer farrowing durations causing intrapartum hypoxia (Edwards, 2002). The objective of this experiment was to obtain a better understanding of the effects of DHA supply on neonatal and maternal physiology in relation to placental oxygen supply and regulation of the parturition process, and on piglet vitality and survival.

Material and methods Eighteen crossbred sows (Landrace x Large White) with a mean parity of 4.0 (sem 0.46) were allocated to treatments according to parity, live weight and previous litter size records in a randomised block design. Diets with different DHA inclusion from Algal Biomass (DHA Gold, Novus Europe) were fed for the last 4 weeks of gestation until 2 weeks *post partum*: 0, 0.03% and 0.3% DHA, delivered by 0, 1.5g/kg and 15g/kg algal biomass, respectively. Sows were individually fed 3 kg/day of gestation diet, then moved to farrowing crates on day 112 of pregnancy. All farrowings were attended and video recorded, and piglet vitality scores (modified from Baxter *et al*, 2008), glucose and lactate concentration in umbilical cord blood, rectal temperature, liveweight and crown-rump length measurements were taken at birth. Rectal temperature was taken again 2 hours later and liveweight change recorded at 2 hours and 24 hours after birth. The birth times for each piglet were noted and the total farrowing duration was calculated. The data collected were, after checking normality, subjected to a one way analysis of variance using the general linear model (GLM) procedure in MINITAB v 16.0. Correlations between individual piglet measurements were also investigated.

Results There were no statistically significant dietary treatment effects on maternal feed intake, weight change and gestation length. The same trend in stillbirth incidence was seen as in the previous large scale production experiment, but was not significant with the smaller animal numbers studied in detail (Table 1). However, neither mean inter-birth interval nor total farrowing duration showed the same trend across treatments. Litter size, birth weight and weight of piglets at 2 weeks were not influenced by the dietary treatments.

Table 1 Effects of DHA supplementation on piglet measures at birth

Parameters/Treatments	0% DHA	0.03% DHA	0.3% DHA	SEM	P value
Birth Weight (kg)	1.47	1.65	1.47	0.07	0.14
Farrowing Duration (mins)	259.3	225.2	259.4*	29.05	0.52
Born alive	13.83	12.50	12.33	1.01	0.65
Still Birth†	0.92	0.40	0.18	0.35	0.34
Blood lactate (mmol/l)#	4.83	4.58	5.68	0.22	0.002
Blood glucose (mmol/l)#	2.67	2.93	2.76	0.11	0.22
Vitality score (1-5 scale)#	3.28	3.85	3.56	0.10	0.00

† with litter size covariate #with cumulative farrowing duration as covariate (using individual piglet data)
*One piglet born dead 24h after farrowing and placental expulsion were apparently completed was not included.

Blood lactate levels showed a significant difference between treatments, but no consistent trend with DHA inclusion, whilst blood glucose levels did not differ. There were significant positive correlations between the cumulative farrowing duration and blood glucose levels (r=0.15, P=0.03) and lactate levels (r=0.21, P=0.001). Rectal temperature at birth and temperature change in the first 2 hours were not affected by treatment, but there was a negative correlation of both blood glucose (r=-0.33, P<0.001) and blood lactate (r=-0.16, P=0.02) with rectal temperature at birth. There was a significant negative correlation between vitality score at birth and blood glucose (r=-0.14, P=0.03) and blood lactate (r=-0.21, P=0.002). Piglets in DHA treatment litters had higher vitality scores than control litters, but mortality of liveborn piglets in the first 3 days was unaffected by treatment.

Conclusion The effect of dietary supplementation with DHA on reduced still birth incidence seen previously, though not significant in the current experiment, cannot be explained by a reduced farrowing duration or by differences in the metabolic measures investigated in this experiment. However, piglets from DHA treatments were more vital at birth compared to the control piglets.

Acknowledgments We thank Novus Europe for provision of test materials.

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071

A platform for genotype imputation and discovery of Mendelian inconsistencies

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Introduction High density genotyping of dairy cattle has enabled the early and relatively accurate genomic prediction of the merit of young animals for selection. Throughput improvements and a reduction in costs have widened the implementation potential of this technique in the commercial population. Additional cost benefits will accrue from the use of lower density DNA arrays comprising fewer genetic markers. It is possible then to use this information, together with accurate pedigree records, to predict the high density genotypes of the animals following a process known as genotype imputation. The objective of this study was to develop of a platform for genotype imputation and confirmation of pedigree accuracy.

Material and methods Data comprised 659 Holstein cows raised on the Crichton Royal experimental farm in Scotland. All cows were genotyped with the Illumina bovine 50K DNA array (Illumina, 2011a) which includes approximately 54,000 Single Nucleotide Polymorphisms (SNP). These cows were daughters of 99 Holstein sires, 64 of which were also genotyped with the same DNA array. Genotype quality was assessed using the criteria described in Banos and Coffey (2010), and SNP that passed these criteria were selected for further processing. For a subset of cows, nearly 3,000 SNP were selected according to the manifest of the Illumina bovine 3K DNA array (Illumina, 2011b). The selected SNP simulated low density genotypes for these cows, which were then used in an imputation exercise to predict the higher density genotypes based on the method of VanRaden (2010). Imputed genotypes were compared with the actual genotypes obtained from the higher density array. At first, the 72 youngest cows (born since 2004) were selected to have their genotypes imputed based on pedigree and the full genotypes of the remaining 587 cows and 64 sires. Subsequently, a subset of 72 cows was selected randomly and had their genotypes imputed; this step was repeated 3 times. During the imputation process, Mendelian inconsistencies were identified. A suite of programme was developed to assign a different sire to cows with pedigree inconsistencies from the pool of known sires used in the herd. The process was then repeated and if no inconsistencies arose after the last sire substitution, it was assumed that this was the correct ancestor. The system was tested by randomly shuffling pedigrees of 10 cows, each one with a different confirmed sire.

Results The full imputation platform is described in Figure 1. After quality checks and edits, 44,276 and 2,812 SNP remained from the high and low density arrays, respectively, for further processing. When inconsistencies were simulated into the pedigrees of 10 cows with known sires, the pedigree checks always picked up the wrong sire. The other 9 sires were then introduced, in turn, as ancestors of this cow. This iterative process eventually identified the correct sire for every single cow. The platform was then used to impute the genotypes of the 72 youngest animals and identified 25 pedigree inconsistencies. After the inconsistencies were dealt with as shown in Figure 1, correlation between imputed and actual genotypes was 0.95, in line with results of VanRaden (2010). When the original pedigree including the inconsistencies was analysed, the correlation was 0.94. When imputation applied to the three random sets of 72 cows, correlations with actual genotypes were 0.88, 0.90 and 0.94, with an average of 0.91 The lower values compared to the 72 youngest animals and the variation of estimates in the random subsets may be attributed to different numbers of genotyped dam-daughter pairs where the dam had a high density genotype. The subset of youngest animals had the highest number of genotyped dams. This is both expected and desirable, as the utility of the platform is more pronounced among younger animals, allowing for a greater number of them to be genotyped and considered in selection programmes.

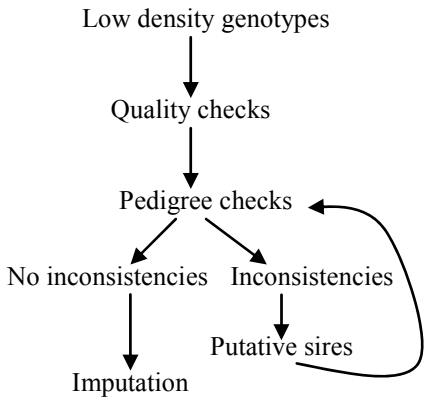

Figure 1 The imputation platform

Conclusions A platform was developed to impute (predict) genotypes based on pedigree and low density genotyping results. The platform was successfully tested for genotype imputation from a 3K to a 50K SNP array and is currently being used in the UK genomic evaluation programme. This procedure can facilitate population-wide application of young cow genotyping at an affordable cost. An additional benefit is the identification and potential remedy of Mendelian inconsistencies in the animal pedigree.

Acknowledgments Paul VanRaden (USDA) for the imputation programmes; Ian Archibald (SAC) for help with the data; the Scottish Government for funding the Langhill lines of dairy cows experiment at Crichton Royal Farm.

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072

Development of a cost effective direct DNA sequencing method for rapid SNP detection and genotyping of candidate genes

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Introduction The search for novel polymorphisms which can either have an impact on animal health, or be utilized for the identification of genotype/phenotype correlations is of paramount importance in the area of animal genetics and genomics. Although high throughput SNP detection and genomic selection tools are now available for production animals such as cattle and sheep (Matukumalli *et al*., 2009), these methods are very expensive mainly due to high instrumentation and operating costs and hence uneconomical for the evaluation of many regional breeds of sheep around the world. The candidate gene approach alone or in combination with data from genome wide association studies however requires DNA sequencing from at least twenty individual animals for SNP identification. Common direct DNA sequencing methodologies used for SNP identification within a gene first require the PCR amplification of the region of interest followed by electrophoresis and gel extraction prior to performing actual DNA sequencing, thereby increasing the processing costs and limiting the sample throughput rate. This study therefore aimed at the development of a rapid and cost effective direct sequenced based genotyping method that would not require gel extraction or PCR clean up kits prior to sequencing. To illustrate the effectiveness of the technique, we have analysed the entire coding region of the ovine Prolactin gene consisting of 5 exons, including both the 5′ and 3′ UTRs

Material and methods Genomic DNA was isolated from Chios sheep breed using standard methods. After designing appropriate primers and optimizing PCR conditions to yield clean, high intensity bands, individual exons were PCR amplified in 25µl reactions from 20 individual animals in a single 96 well PCR plate. The PCR products were purified using isopropanol precipitation in the presence of sodium acetate, collected by centrifugation and washed with 70% ethanol. Following a second centrifugation to remove the ethanol, the wells were briefly dried at 50°C in a PCR block prior to resuspension in 50µl of water. DNA sequencing reactions were then set up in a duplicate plate using $1/16^{th}$ reactions of Big-Dye 3.1 chemistry and cycled according to the manufacturers recommendations. Following cycle sequencing, the termination products were subsequently purified by ethanol precipitation in the presence of EDTA, collected and washed by centrifugation as before, resuspended in 10µl of formamide and read on an ABI 3130 genetic analyzer. The sequence data obtained was confirmed by blast search against both the partial sheep genome database as well as the bovine database.

Results The Prolactin exons sequenced ranged in size from 82-341bp. In a similar fashion to samples processed using conventional techniques, all traces showed an excellent signal to noise ratio with quality values ranging from 48-62 (0.0015% to > 0.0001% read error values). In addition, heterozygosity was easily detected as shown for example in figure 1 (black arrows). In addition to the ovine Prolactin gene sequence presented here as an illustration, we have also used the method to evaluate three other genes from unrelated species, ranging from plants to microbes with reliable read lengths ranging at present up to 500-600bp

Using the suggested method, 96 samples were easily processed from genomic DNA to final sequence data in approximately 7 hours in a microtiter plate format. By circumventing the need for electrophoretic and expensive PCR cleanup or gel extraction procedures and by reducing the amount of sequencing reagents necessary for sufficient quality reads, the cost for genotyping a 96 well plate was reduced approximately 20 times.

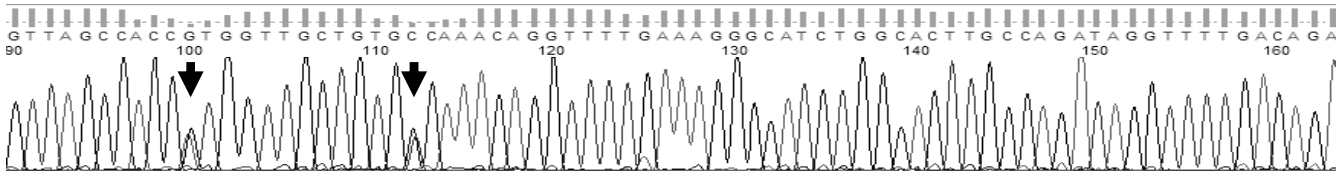

Figure 1 Representative sequencing chromatogram of ovine Prolactin exon 2

Conclusions A rapid and cost effective sequencing protocol supporting large scale sequencing in a 96 well format has been presented which performs with equal efficacy to DNA sequencing performed by conventional methods in terms of reliability and sensitivity. However by circumventing the need to perform gel electrophoresis and subsequent gel purification to prepare the sample for subsequent sequence analysis it provides clear advantages in terms of reduced cost, minimum sample handling and the ability to process multiple samples simultaneously. In addition, the resolution of the reads generated are equal in quality to those generated using conventional techniques thereby facilitating polymorphism detection and subsequent genotyping with a high degree of accuracy.

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073

Effect of soaked and urea treated wheat straw based diets on live weight of wether sheep

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Introduction Wheat straw forms an important component of livestock rations in many countries such as Libya. It is recognised that advanced maturity in cereal straws is associated with high contents of detergent fibres and low N, which cumulatively depress feed intake. While urea treatments are known to improve the straw utilisation for ruminants, the extent of such improvement depended upon the amount of urea, method of its application and the type of a straw. In continuation to our reports about the chemical composition (Shirif *et al*. 2011) and voluntary intake (Shirif *et al*. 2010) of straw, this study tested the effect of the soaked and urea treated straw based diets on the live weights (LW) of wether sheep during 42 days of this trial.

Material and methods Nine bales of about 300 kg of chopped (10-15cm) wheat straw were distributed into 18 polyester silo bags that were individually placed inside galvanized mesh rings. A 2 x 3 factorial design, in triplicate, was used to apply different urea and water levels to prepare treated wheat straws as follows. Water representing 2 water to straw ratios (0.15:1 and 0.50:1; soaking) and 3 urea levels (0, 2.5 and 5% of straw) were sprayed onto these straws in bags which were compressed to exclude air, sealed and left outdoors for ten weeks. A wether sheep trial was conducted to compare the effect of feeding these straws on the LW of 6 similar sheep groups (n = 6 sheep per each treatment) over 42 days. These sheep groups were balanced for initial LW but the sheep were housed individually. The wethers were offered *ad-libtum* the above mentioned straws after their mixing daily with 3% molasses (Shirif *et al*. 2010). The wethers were also fed daily 200g of a concentrate (170g CP and 12 MJ ME /kg DM) plus 20g of a vitamin-mineral premix per head to meet their nutrient requirements (AFRC, (1993). After the adaptation period of 14 days, the weekly straw intake and LW of each wether were recorded for another 6 weeks. The data were statistically analysed by using the Analysis of Variance in Minitab software to compare the effects of soaking, urea and soaking x urea interactions on the weekly LW of these wethers at $P<0.05$.

Results Only the main effects of urea and soaking treatments on the mean weekly LW per weather are shown in figure 1 and 2 respectively. Figure 1 shows that the feeding of urea treated straw at both levels (2.5 and 5%) caused almost no change (*P>0.05*) in LW of wethers for 35 days, but from 35 to 42 days of the trial the sheep LW was improved at both urea levels (Figure 1). The LW of wethers fed with high soaked wheat straw (0.50:1) were always greater than those of the low soaked straw (0.15:1**).**

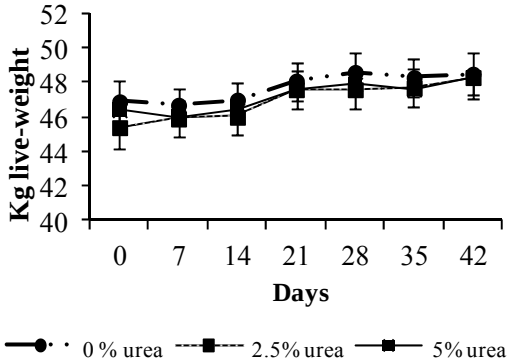

Figure 1 Effect of urea levels on weekly live weight

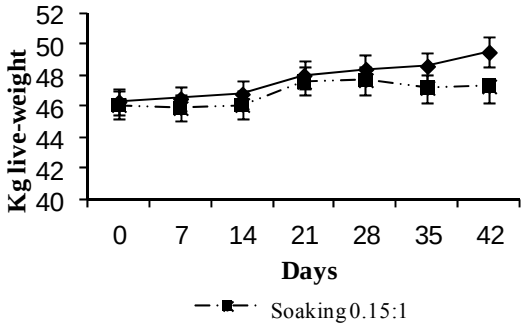

Figure 2 Effect of soaking on weekly live weight

The differences between treatments for live-weights at different days were never statistically significant (*P>0.05*) during the 42 days of the trial. The soaking x urea interaction for LW was not significant during the 42 days of this trial (*P>0.05*).

Conclusion Despite modified chemical compositions (Shirif *et al*, 2011) and less than expected change in the intake by wethers (Shirif *et al*, 2010) of urea treated straw, there was no significant change in live-weight when the wethers were fed either with 2.5 or 5% urea treated straw based diets during 42 days of this trial. However, the LW of the wethers fed with high soaked straw (0.50:1) was slightly increased during this trial. To optimise the combinations of urea treatment and soaking levels further studies are needed especially under warmer conditions. It would also help if relatively younger but growing lambs are used over a much longer period (12 to 14 weeks) to observe the real benefit of similar treatments in combination with an appropriate concentrate supplement.

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Haematological and carcass characteristics of West African Dwarf goats fed with *Moringa oleifera* and *Gliricidia sepium*

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Introduction Goat meat may be lower in fat and cholesterol and is usually considered to be healthier than other animal protein sources, especially other red meats. The quantity and quality of the carcasses, blood characteristics, serum cholesterol and saturated fatty acid contents from goats can be influenced by nutritional status and diets (Olubunmi *et al*., 2005; Hango *et al*., 2007). The aim of this study was to evaluate the haematological and carcass characteristics of West African Dwarf goats fed with *Moringa oleifera* and *Gliricidia sepium.*

Material and methods In a 20-week trial, 40 West African Dwarf (WAD) weaned goats of both sexes, 5-7 months old, (average weight of 6355 ± 8g), were randomly allotted to five treatments of graded mixtures of *Gliricidia sepium* (Gliricidia, G) and *Moringa oleifera* (Moringa, M) (100%G, 75%G25%M, 50%G50%M, 25%G75%M, 100%M) and fed 4% of their body weight on dry matter basis. The experiment was a completely randomized block design conducted at the Goat Unit of the Teaching and Research Farm, Obafemi Awolowo University. At the 1st, 56th, and 100th day of the experiment, 5 ml of jugular blood (using EDTA tubes), were collected from the experimental WAD goats for the determination of parked cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) counts. The concentration of total cholesterol in the serum was assayed according to the enzymatic end point method and the method of assay of triglyceride in the serum was based on a slight modification of the colorimetric method using a commercially available kit. Three goats were chosen from each group based on similarity in weight and slaughtered for carcass evaluation and parameters determined were calculated based on final weight in percentages. Data were statistically analyzed with the general linear model of SAS (2008). Mean values that differs significantly were separated using the Duncan option of the Multiple Range Tests of the same package.

Results The means values of RBC, WBC and PCV from animals fed diet 50G50M were significantly ($p<0.05$) lower than means values of animals fed 75G25M, 25G75M, 100G and 100M diets. Mean serum cholesterol concentrations (mmol/l) progressively decreased ($p>0.05$) as dietary Moringa increased from 0% (2.8) to 100% (2.2). There were significant differences ($p< 0.05$) in dressing percentage, neck percentage and the spleen percentage of the experimental animals.

Table 1 Carcass characteristics and blood analysis of experimental WAD goats

Parameter (Mean)	100G	75G25M	50G50M	25G75M	100M	SEM	PROB
Initial weight (kg)	6.250	6.263	6.263	6.250	6.250	0.4423	1.00
Final weight (kg)	12.533	12.633	12.567	12.600	12.400	0.5231	0.99
Dressing (%)	44.01^{a}	39.20^{c}	38.45^{d}	41.92^{b}	40.97^{bc}	0.650	0.001
Neck (%)	4.50^{b}	5.43^{a}	4.38^{b}	4.98^{ab}	5.31^{a}	0.186	0.01
Kidney (%)	0.31^{c}	0.37^{b}	0.35^{b}	0.41^{a}	0.40^{a}	0.011	0.01
Spleen (%)	0.13^{c}	0.28^{a}	0.19^{b}	0.23^{ab}	0.24^{ab}	0.023	0.01
RBC (10^{6})mm	13.35^{a}	12.31^{ab}	11.91^{b}	13.10^{ab}	13.55^{a}	0.214	0.04
WBC (10^{3})mm	9.03^{ab}	10.10^{a}	8.26^{b}	9.46^{a}	10.40^{a}	0.190	0.02
PCV %	29.50^{ab}	32.50^{a}	26.00^{b}	31.00^{ab}	32.25^{ab}	0.891	0.11

$^{a, b, c, d}$: Means within each row with different superscript are significantly different ($p< 0.05$)

Conclusions It was concluded that *Moringa oleifera* can effectively replace *Gliricidia sepium* in goat's diets without deleterious effect on the hematological parameters and carcass quality of WAD goats. It can therefore be used to develop a novel strategy to produce chevon with lower cholesterol and saturated fatty acid contents.

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Fatter lambs from fostered mums: the benefits of fostering lambs on daily weight gain

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Introduction A strong and positive maternal-neonate relationship is essential for the survival of precocial lambs. The absence of such a relationship can lead to emotional and nutritional stress, a decrease in their immune responses and an increased cortisol response. Maternal separation and artificial rearing of lambs, although sometimes essential to ensure survival, could jeopardise lamb welfare compared to natural maternal care and therefore potentially influence the lambs' growth rates. Previous research has compared meat quality characteristics of artificially reared and maternal-ewe reared lambs in milk production systems. However, lamb growth rates of artificially reared or fostered lambs are more significant in a commercial meat production system. Investigations explored the influence of artificial rearing or the most commonly used fostered rearing systems on the growth rates of lambs within a commercial meat production system in the UK.

Methods Approximately 800 North Country mule ewes housed on a commercial farm were monitored during two lambing seasons (2009 & 2010) in an intensive indoor lambing system. Eighty eight lambs were assigned to one of five treatments with equal male and female lambs; three commonly used foster methods (birth fluids, restraint and cervical stimulation concurrent with birth fluids), artificially reared (bottle fed with a milk replacer) or the control treatment (reared by natural mother). Alien individuals originated from triplets and were fostered onto ewes baring singles. At around 7 days of age, the ewes and lambs were transported to the fields if not being artificially reared. Lambs from each condition were weighed on their date of birth, day 7, day 30, day 90 and at around six months old. Calculations for their daily weight gain were calculated for the first 7 days (DG1), between day 8 and 30 (DG2), between day 31 and 90 (DG3) and between day 91 to 180 days, when they were slaughtered (DG4). Data were analysed using a multivariate ANOVA with foster method treatment and ewe experience as additional variables.

Results The initial three growth periods (DG1, DG2 and DG3) showed significantly lower growth rates for artificially reared lambs compared to other fostered and natal-reared lambs ($p<0.01$, $p<0.01$, $p<0.05$ respectively) however, the final growth period (DG4) prior to slaughter was not affected by the rearing treatment (Figure 1). When the artificially reared lambs were removed from the analysis, the daily weight gain was higher in lambs from multiparous ewes than primiparous ewes throughout DG1, DG2, DG3 and DG4 ($p<0.01$ for all respectively). Results also showed that there were no differences between the growth rates of the fostered lamb and the natal lamb throughout the time period (artificially reared lambs removed from analysis).

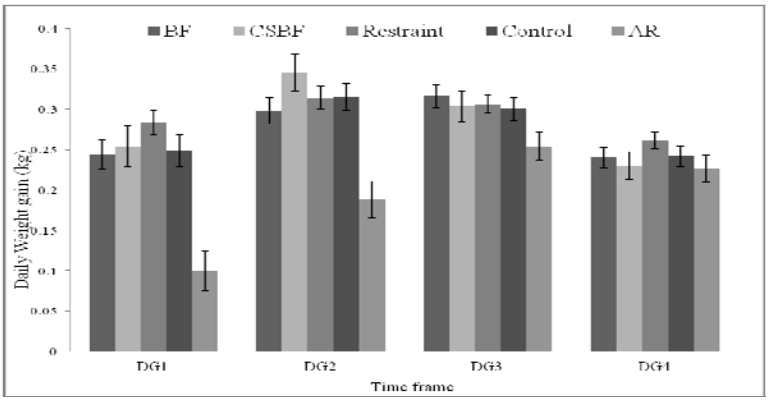

Figure 1 Daily weight gain across the different rearing conditions (where BF = birth fluids, CSBF = cervical stimulation & birth fluids and AR = artificial rearing).

Conclusions Results suggest that fostering lambs using any of these methods is very beneficial for the daily weight gain in the initial 90 days, as lambs were found to gain significantly more weight. Artificial rearing seemed to slow down the lambs' weight gain until at least 3 months of age; this is probably due to time after weaning (around 3 months). All fostered and artificially reared lambs were fed on grass with the same availability so the artificially reared lambs could recover their body condition to match other fostered individuals. It was also found that multiparous ewes were more capable of rearing heavier lambs than primiparous ewes from birth to slaughter. This suggests that when selecting ewes for fostering, an experienced ewe would be more beneficial to ensure a high daily weight gain for each lamb. The daily weight gain between the alien and natal lamb was found to be the same throughout the 180 days. Therefore, if a foster is successful, the lambs are able to feed freely and are not competing for milk from the ewe allowing them to grow naturally. Measuring daily weight gain in fostered lambs could consequently be used as a measure of fostering success.

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Variation in live weight, ultrasonic back-fat and computerised tomography data within a sample of Abermax ram lambs

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Introduction This study is part of a research programme investigating meat eating and nutritional quality of lambs produced, by selecting extreme variations in muscle density as assessed by computer tomography (CT). This study aims to investigate the variation in growth and CT measured carcass traits among the ram lambs. Factors that can affect these traits have been investigated including dam age, birth/rear type and age at measurement.

Material and methods Lambs were bred by mating 340 Texel ewes with 7 high index Charollais rams to create Abermax terminal sire lambs (Innovis bred F1 Texel x Charollais). 198 Abermax ram lambs had traits including live-weight measured at various stages within the first 6 months of production (March to August 2011). Lamb live-weight was recorded at birth, 8 weeks (mean age 63 d ± 0.2 se), weaning weight (mean age 112 d), ultrasonic scanning (mean age 140 d) and CT scanning (mean age 152 d). Pedigree and number of lambs born and reared were recorded. Lambs were reared under their mothers as either a single or a twin, or were artificially reared. The feeding system was grass based with additional concentrates provided in a creep feed system (av. 250g/h/d). Ultrasonic fat and muscle depth and *in-vivo* CT scanning were conducted at week 19 and 22 respectively. Data were analysed via GENSTAT 13 using a model fitting the dam age (2 yr *v.* older) and birth/rear type (i.e. single/twin/artificially reared), with age at measurement fitted as a covariate.

Results Selected live-weight and CT results are given in Table 1. The mean live weight at CT scanning was 46.4kg ± 0.49. Ultrasonic average fat depth had the highest CV=39%. The CV was lowest for muscle density (4%) and had a notably small adj-R^2 =0.05. The highest value of explained variation (adj-r^2) was birth weight (0.37) and the lowest for ultrasonic fat depth (0.03).

Table 1 A summary of the results for the model of growth and CT results within a sample of Abermax ram lambs.

Parameter	Mean (1)	SE	P-Value Dam age (2yr, older)	P-Value Birth/ Rear type	CV%	Residual s.d.	Adjusted R^2
Birth Weight (kg)	5.4	0.20	***	***	17	0.915	0.37
8 Week Weight (kg)	2.5	0.46	*	***	21	5.466	0.24
Weight at Ultrasonic Scanning (kg)	48.1	0.65	**	***	16	7.471	0.24
Ultrasonic Average Fat Depth (mm)	1.4	0.05	ns	*	39	0.577	0.03
Ultrasonic Muscle Depth (mm)	30.9	0.30	***	***	11	3.464	0.17
Predicted Computer Tomography measured parameters							
Fat (kg)	4.0	0.09	ns	***	23	0.960	0.25
Muscle (kg)	13.8	0.16	ns	***	12	1.631	0.16
Bone (kg)	3.4	0.03	ns	***	10	0.341	0.17
Muscle Density	41.7	0.17	*	ns	4	1.766	0.05

(1) = adjusted mean for birth/rear type and ewes older than 2 years

Conclusion The largest proportion of variation explained by the model was for birthweight. In comparison to Karamichou *et al.* (2004), this study had lower variability around the mean for ultrasonic fat depth and a higher mean muscle depth, reflecting differences in breed, recording system and rearing environment of the lambs. Ultrasonic fat depth and muscle density (by CT) were poorly described by the model suggesting other factors such as environment (inc. nutrition) and genetics play an important role. The results indicated that muscle density had a small amount of variation around the mean. This establishes the variance within a sample of Abermax ram lambs to aid selection on extreme muscle density.

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Effect of concentrate feed level and protein source on the performance of ewes in late pregnancy and the performance of their progeny

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Introduction Nutrition in late pregnancy influences lamb weight at birth. Each 1 kg increase in lamb birth weight is expected to increase weaning weight by 3.2 to 3.4 kg (Keady and Hanrahan 2009a, b). During the last 6 weeks of pregnancy, the metabolisable energy and metabolisable protein requirements increase by approximately 60% and 35%, respectively, for twin bearing ewes. Microbial protein synthesis in late gestation is often limited by energy supply. However the shortfall in microbial protein supply can be overcome by increasing the intake of digestible undegradable protein (DUP). Soyabean meal contains a high concentration of DUP but its inclusion in ewe rations is often limited due to cost. The aim of this study was to evaluate the effects of source of concentrate protein, concentrate feed level and their potential interactions on the performance of ewes in late pregnancy and the performance of their progeny.

Material and methods Ninety two 2-tooth ewes (59 Belclare, 23 Belclare x S Blackface, 10 Bluefaced Leicester x S Blackface), which had been mated to Belclare or Suffolk rams and carrying twins or triplets, were allocated to either high (H) or low (L) concentrate feed levels during the last 6 weeks of pregnancy. The ewes were offered grass silage *ad libitum.* The concentrate was formulated using either soyabean meal or other by-products as the protein source. The twin and triplet bearing ewes offered the L and H concentrate feed levels received 16 and 21 kg, and 28 and 32 kg, respectively. The two concentrates were formulated to contain similar crude protein (209 g/kg DM) and ME (12.4 MJ/kg DM) concentrations. The estimated DUP concentration for the soya- and by-product-based concentrates was 68 and 48 g/kg DM, respectively. The soya-based concentrate contained 330, 120, 120, 120, 260, 25 and 25 kg of barley, citrus pulp, soya hulls, sugar-beet pulp, soyabean meal, molasses and minerals and vitamins, respectively, per tonne. The by-product based concentrate contained 165, 70, 70, 70, 120, 200, 250, 5, 25 and 25 kg of barley, citrus pulp, soya hulls, sugar-beet pulp, maize distillers, rapeseed meal, maize gluten, megalac, molasses and minerals and vitamins, respectively, per tonne. The ewes were housed in 3 pens of 4, and 1 pen of 5 and 1 of 6 per treatment. Lambs were blood sampled between 24 and 36 h of birth for the determination of IgG concentration. Post lambing, ewes rearing twins were grazed at pasture and received no concentrate supplement. Ewes rearing triplets received 0.5 kg concentrate daily at pasture for 5 weeks post lambing and their lambs had access to 300 g concentrate/head daily until weaning. The lambs were weaned at 14 weeks of age. The data were analysed as a 2 x 2 factorial using Proc GLM for ewe traits, and Proc MIXED for lamb traits with fixed effects for litter size and sex and ewe as a random term.

Results The pH and concentrations of DM, DMD and ME of the silage were 3.8, 264 g/kg, 738 g/kg DM and 11.6 MJ/kg DM, respectively. The effects of concentrate feed level and protein source on ewe and lamb performance are presented in Table 1. Increasing concentrate feed level increased ewe condition score at lambing ($P<0.05$) and tended to increase ($P=0.08$) live weight immediately post lambing. The mean litter sizes born and reared were 2.16 and 1.93 and were unaffected by dietary treatment ($P<0.05$). Ewes offered the soyabean-based concentrate produced lambs that were heavier at birth and grew faster from birth to 5 weeks ($P<0.05$). Dietary treatment had no effect ($P>0.05$) on blood IgG concentration; IgG concentration was higher for females than males (13.7 and 9.9 mg/ml). Lambs that subsequently died had lower IgG concentrations than those that survived [8.9 and 14.7 mg/ml;($P < 0.05$)]. Lambs born as twins had higher IgG than lambs born as triplets [14.3 and 9.9 mg/ml; ($P < 0.01$)]. The birth weights of Belclare, Suffolk x Belclare and Suffolk x Belclare x S Blackface lambs averaged 3.81, 3.74 and 3.56 kg, respectively; corresponding weaning weights were 30.2, 29.1 and 30.2 kg. These were not affected ($P>0.05$) by breed.

Table 1 Effect of concentrate feed level and protein source on lamb performance

		Crude protein (CP) source x Feed level (FL)				s.e.	Significance		
		Soya		By-product					
		Low	High	Low	High		FL	CP	FL x CP
Post lambing	- condition score	2.74	2.89	2.77	2.91	0.078	*	NS	NS
	- live weight (kg)	52.2	54.1	49.9	52.9	1.52	P=0.08	NS	NS
Lamb weight (kg)	- birth	3.95	4.09	3.77	3.56	0.168	NS	*	NS
	- weaning	30.8	31.0	29.5	30.6	0.79	NS	NS	NS
Lamb gain (g/d)	- 0 to5 weeks	275	286	247	271	11.4	P=0.08	*	NS
	- 0 to14 weeks	267	267	256	267	7.5	NS	NS	NS

Conclusion Altering the source of protein in the concentrate supplement offered in late pregnancy, even when formulated to contain similar ME and crude protein concentrations, had a greater effect on lamb performance than increasing concentrate feed level by 75%.

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Broussonetia papyrifera leaves as supplement increases total feed intake of Djallonké rams offered Napier grass

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Introduction *Broussonetia papyrifera* (Paper mulberry) was introduced into the forest reserves in Ghana to serve as pulp for the establishment of paper manufacturing industry (Bosu and Apetorgbor, 2006). However, the shrub has become a weed, invading forest lands. The leaves of *B. papyrifera* were therefore used as supplements to sheep fed two varieties of Napier grass *(Pennisetum purpureum)* as basal diets to assess other uses of the leaves.

Material and methods Twenty four (24) individually-housed rams (initial live weight 13.9kg and 1 year old) were randomly allocated to the six treatments (described below) in a 2 x 3 factorial in a completely randomized design with four replicates. The factors were two varieties of Napier grass (Local variety and Improved Variety 16798) and three levels of *B. papyrifera* supplement (0g, 100g and 200g/d). The cultivated grasses were harvested each morning, chopped into short lengths (5-10cm) with a cutlass, and offered to each ram at the rate of 50g/kg live weight (sufficient for *ad lib* feeding and allow 15-20% refusal by the rams). Supplements of *B. papyrifera* leaves (air-dried to 905g/kg) were offered in the morning. Feed intake was estimated on dry matter basis. Rams were weighed weekly over a 12-week experimental period. The data from the feeding trial were analysed to account for the supplementation level and grass variety.

Results Final body weight of rams was significantly (P<0.05increased by supplementation. Intake was not affected (P>0.05) by variety of grass offered but total feed intake was significantly higher (P<0.05) as supplement levels increased. Total cholesterol levels in blood were not affected by supplementation. Total blood protein was significant increased as the supplement level increased.

Table 1 Effect of level of supplementation on intake, average daily gain and blood cholesterol of rams

	Local Variety			Improved Variety (16798)				
Supplement Level (g/d)	0	100	200	0	100	200	SE	Sig.
Final live weight (kg)	17.88	18.63	20.25	18.13	18.63	19.00	0.583	*
ADG (kg)	0.05	0.06	0.06	0.05	0.06	0.07	0.005	NS
Intake (gDM/d)								
Grass	586	6046	616	590	622	615	32.0	NS
Supplement	0	99	184	0	97	191	2.3	***
Total intake	585.	703	800.	590	720	806	32.7	*
Total blood cholesterol (mmol/L)	0.783	1.050	0.950	0.800	1.00	0.850	0.1135	NS
Total blood protein (g/L)	41.45	57.73	57.03	39.83	61.35	57.75	5.102	*

NS: not significantly different; *: (P>0.05); **: P<0.01; ***: P<0.001

Conclusion *B. papyrifera* leaves have been identified as having the potential to increase total feed intake and live weights of rams offered Napier grass as basal diets. Total blood protein concentrations were increased by supplementation of leaves of *Broussonetia papyrifera* but blood cholesterol levels were unaffected.

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Supplementation of *Moringa oleifera* leaves and dried *Samanea saman* pods on intake, N digestibility and N balance of Napier grass basal diet in Djallonké sheep

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Introduction The leaves of *Moringa oleifera* and the dry pods (not normally utilized) of *Samanea saman*, could be used to supplement Napier grass (*Pennisetum purpureum*). Although the CP content of Napier grass normally falls below 10 g/kg DM during the dry season it is recommended for smallholder crop-livestock farming systems, for its greater DM yield than other tropical grasses (Nyambati *et al*., 2003). *M. oleifera* leaves in livestock feeding systems could contribute to the reduction of feed protein constraint due to its CP content (19.3 – 26.4 %) and warrants further study (Aregheore, 2002). The purpose of this study was to assess the intake, digestibility and nitrogen (N) balance in sheep offered Napier grass with foliage of *M. oleifera* and dry pods of *S. saman*.

Material and methods The study was carried out at the Livestock Section of the Department of Animal Science, KNUST, Kumasi, Ghana. *M. oleifera* was harvested and shade dried for three days; dry pods of *S. saman* were collected and chopped into 20 mm lengths and Napier grass also harvested and chopped into 60-80 mm lengths before feeding. Three Djallonké rams with an average weight of 21 kg (given Ivermectin (0.2 mg/kg liveweight) to control both internal and external parasites) were used in a 3x3 Latin Square Design to determine the intake and digestibility of nitrogen and DM. The factors were three dietary treatments and three animals. The animals were allocated to three dietary treatments and kept in individual metabolism cages (0.7 x 1.2 m) and allowed *ad libitum* access to water. T_1 (Napier grass alone), T_2 (Napier grass + 166 g *M. oleifera* leaves) and T_3 (Napier grass + 195 g dried *S. saman* pods). The Napier grass was given *ad libitum* in all the three treatments. The study consisted of three 21-day periods (each period comprising two weeks dietary adaptation and one week for measurement of feed intake and collection of faeces and urine). At the start and end of each sampling period the rams were individually weighed. The rams were fed once a day. The N, DM and ash concentrations of the feeds were determined. During the 3 adaptation periods, feed offered and feed refused were weighed daily and random samples collected twice a week for DM analysis using a hot air oven (60 °C). Samples of feed offered on each of the measurement days were divided into two parts, first part was analysed for DM while the second part was pooled at the end of each period and analysed for N. The N content of the faeces and urine were determined for N-digestibility and N-balance respectively. The data obtained for the intake, digestibility and N-balance studies were subjected to analysis of variance (ANOVA) for balanced data.

Results The results showed that the feeds were not dry (DM < 850 g/kg). The CP and ash contents of the three feeds were (Napier; 107.4, 72.0; *M. oleifera*; 254.6, 59.7 and *S. saman* pods; 186.0, 17.2 g/kg DM). Table 1 shows that intake of napier grass was similar for rams on the supplements and was higher ($P<0.05$) for the ram on the Napier grass alone. The digestibility of N increased significantly ($P<0.05$) for rams offered *M. oleifera*; N-balance and average daily gain followed the same trend.

Table 1 Effect of supplements *M. oleifera* and *S. saman* pods on intake, digestibility and N balance in sheep

Parameters	T_1	T_2	T_3	s.e.d	P
Intake (g DM/d)					
Napier grass	1004^a	928^b	949^b	7.2	0.481
M. oleifera	-	166	-	3.3	0.244
S. saman pods	-	-	195	3.3	0.244
Total intake	1004^c	1094^b	1144^a	12.9	0.001
Total intake (g DM/$kg^{0.75}$/ d)	106.2^b	111.5^a	112.6^a	0.63	0.001
N digestibility (%)	58.0^c	69.1^a	62.4^b	1.02	0.003
N Balance (g N/kg)	6.54^c	11.2^a	10.0^b	0.35	0.001
Daily gain (g)	28.6^c	85.7^a	75.0^b	2.19	0.156

Different superscripts within a row indicate significant differences ($P<0.05$)

Conclusion The results show that supplementation of Napier grass during the dry season with *M. oleifera* and dried *S. saman* pods increased total intake, N digestibility and N balance by sheep.

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Upgrading the *in-vitro* dry matter degradability of wheat straw internodes by using soaking, temperature and time treatments

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Introduction Several methods have been tested in the past for upgrading low quality roughages; physical, chemical and biological methods. Pre-soaking of compound feed pellets has been reported to have a large effect on the fermentability characteristics of this feed (Van Laar *et al*., 2007). The application of soaking may also improve the degradation and so utilisation of fibrous feeds including cereal straws. Therefore, the objectives of the current study were to test the effect of different physical pre-treatments (no soaking or soaking with water, or without heating at different times) on the in-vitro dry matter degradability (DMD) of wheat straw by using its only internodes as relatively a less tough part.

Material and methods Factorial experiments 3 x 2 x 2 in duplicate for each incubation time, were conducted to test the effect of three soaking levels; soaking 1 (no water) soaking 2 (one litre of water and one kg straw) and soaking 3 (two litres of water and one kg of wheat straw inter nodes), two soaking temperatures (20˚C and 60˚C) and two soaking times (2 and 16h) on *in-vitro* disappearance of the wheat straw internodes. Approximately 0.5 g of dried and ground samples of wheat straw internodes were transferred individually into 50 ml plastic tubes. The internode samples were incubated in buffered rumen fluid (RF) in water baths at 39ºC for 46 and 92h. The RF was obtained from 2 fistulated sheep each consuming daily 820g grass hay plus 410g of a concentrate. The RF was filtered, through a cheese cloth, pooled & mixed with a buffer (McDougall, 1948) at a ratio of 1:4 to prepare buffered RF. After each incubation time, the un-degraded residues were collected, washed and dried to calculate the DMD of each straw internodes for each treatment combination. The data were statistically analysed by using Minitab programme to study the main effects of soaking level and temperature and time of soaking and their interactions at each of the 2 incubation times. The effects were declared significant if $P<0.05$.

Results The results for the main effects on DM degradation of un-soaked and soaked wheat straw internodes are presented in Table 1. While DMD was improved with increasing soaking levels at both incubation times ($P<0.001$), the increase in temperature has no affect on DMD ($P>0.05$) at both incubation hours (46h and 92h). However, the increase in soaking time show significant reduction in DMD at 46h of incubation ($P<0.001$) while the DMD at 92h of incubation was dramatically improved ($P<0.001$). Predictably, longer incubation time of 92h showed substantially greater DMD for all treatments.

Table 1 Means and SEM for the main effects on DMD (g/kg) of wheat straw internodes at 2 incubations

	Soaking (n=8)		Temperature (n=12)		Time (n =12)	
Treatment level	46h	92h	46h	92h	46h	92h
1	222.5^{c}	303.3^{c}	251.4	349.0	256.0^{a}	337.4^{b}
2	252.0^{b}	377.1^{a}	248.1	343.0	2.43.6^{b}	354.6^{a}
3	274.8^{a}	357.5^{b}	NA	NA	NA	NA
SEM	2.2	2.8	1.8	2.3	1.8	2.3
Significance	***	***	NS	NS	***	***

Values with different superscripts in the same column indicate significance at $P<0.001$ (***); SEM= standard error of means to compare each effect for each incubation time; NS= non significant; NA= Not applicable; Treatment levels= 1,2 and 3 represent soaking levels of 1, 2 & 3 respectively, or Levels 1 & 2 represent either 20 and 60 ºC or 2 and 16h respectively

Conclusion Soaking increased DM degradability of wheat straw internodes but the extent of increase in DM degradability depended upon the incubation time. Therefore, soaking and time could be used as an alternative to chemical methods to increase degradability of straw. Even moderate increase in degradability with soaking would be more desirable because water is readily available and it is easy to use. As soaking with water does not require any expensive equipment or chemicals its use is desirable in low input feeding systems due to its safety for the users and the environment.

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Comprehensive profiling and absolute quantification of equine cartilage extracellular matrix

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Introduction Articular cartilage is composed of a single cell type, the chondrocyte, embedded within an extracellular matrix (ECM) the composition and structure of which provides its properties. Osteoarthritis (OA) is characterised by the slow degeneration of cartilage ECM. Cartilage proteomic studies have allowed the investigation of the functional molecules of cartilage in order to elucidate the pathogenesis of OA (Iliopoulos *et al*., 2010). Although proteomics studies of cartilage and arthritis have increased our understanding of OA and allowed biomarker discovery few studies have quantified the ECM and none in absolute terms. Here we used comprehensive proteomic analysis methodologies on the cartilage ECM in order to identify low abundance proteins. Then we use QconCAT technology (Benyon *et al*., 2005) which allows parallel quantification of large sets of analyte proteins, to absolutely quantify for the first time aggrecan, decorin, biglycan, cartilage oligomeric matrix protein (COMP) and fibromodulin using mass-spectrometry.

Material and methods Full thickness equine articular cartilage was harvested and sequential extractions using NaCl and guanidine hydrochloride according to Wilson 2010 were undertaken (Wilson *et al*., 2010). Mass spectrometric analysis was commenced following reduction, alkylation and trypsin digestion by LC-MS/MS using a NanoAcquity LC coupled to a LTQ Velos. Data produced were searched using Mascot (Matrix Science UK), against the *Equus caballus* database. An equine cartilage QconCAT was designed as a concatenation of tryptic quantotypic peptides using peptides identified here. Between two and four peptides were used for each protein quantified. Genes of these peptides were expressed in *E.coli*, and cultured in media supplemented with $^{13}C_6$ analogues of arginine and lysine. Full thickness equine articular cartilage was harvested from the metacarpophalangeal joints of six skeletally mature horses with grossly normal joints. Cartilage was lyophilized and the soluble proteins extracted using 4M guanidine. Analyte and QconCAT were reduced, alkylated and trypsin-digested in solution on 10000 MWCO centrifugal concentrators. Following desalting using Zip-Tips the digested peptide mixture was assessed using label-free identification and quantification on a LCMS^E using a nanoAcquity coupled to a Synapt G1 using a Hi3 methodology (Silva *et al*., 2006). Next peptides were resolved by LC-MS using a NanoAcquity chromatograph coupled to a Waters Xevo-triple quadropole-mass spectrometer. Quantification was achieved by comparing extracted ion chromatograms of selected y-series ions of heavy (QconCAT) and light (analyte) transitions. The ratios were normalised to dry weight of cartilage.

Results In equine cartilage a total of 585 proteins were identified using sequential extraction techniques including both matrix and chondrocytes proteins. Selected matrix proteins were then quantified using the QconCAT with at least two peptides selected for each protein. To quantify each peptide a minimum of two transitions were used and between transitions variation was minimal. For six donors matrix proteins were quantified as pmol/mg dry weight of cartilage: aggrecan 8.6 ± 1.19 SEM, biglycan 20.1 ± 5.9 SEM, decorin 6.28 ± 1.27 SEM, COMP 10.22 ± 1.6 SEM and fibromodulin 35 ± 5.7 SEM.

Conclusions This study enabled the characterisation and for the first time absolute quantitation, of equine cartilage ECM using QconCAT technology. Quantification data for the proteins studied here will enable baseline parameters to be set for cartilage matrix components and allow the study of conditions relating to arthritic pathology and physiological ageing.

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Assessing therapeutic interventions in the mdx mouse using *in vivo* muscle physiology

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Introduction Muscular dystrophies are a diverse group of conditions characterised by muscle inflammation, necrosis and loss of function. Treatments for these conditions are scarce and mouse models are valuable tools for investigating therapeutic strategies. Many of these diseases are exacerbated by physical activity therefore treatment effects may be overestimated in sedentary mice. While whole body exercise testing can be used, the results may be affected by the motivational state of the mouse, its body composition and other systemic factors. We have used a standardised *in vivo* exercise test of individual muscles *in situ* with an intact blood supply to assess the effect of various treatments in the *mdx* mouse. The *mdx* mouse is a widely used mouse model of Duchenne muscular dystrophy.

Material and methods Under deep anaesthesia the tendon of the *tibialis anterior* (TA) muscle and the common peroneal branch of the sciatic nerve were isolated and the mouse was placed prone on the apparatus. TA muscle contractions were elicited by stimulating this nerve (701A stimulator; Aurora Scientific). Data acquisition and control of the servomotor were conducted using a Lab-View-based DMC program (Dynamic muscle control and Data Acquisition; Aurora Scientific). After the exercise test was completed the mouse was dissected and muscles were collected and frozen for histopathology and western blotting. The exercise test consisted of a warm up followed by measurement of maximum isometric twitch force, the force-frequency relationship and maximum isometric tetanic force. This was followed by an eccentric exercise protocol that consisted of 10 tetanic contractions during which the muscle was stretched by 10% of its optimum length. This protocol causes a sequential drop in the tetanic force generated by the TA muscle in *mdx* mice but not in wild type mice.

Results Beneficial interventions reduce the loss of force towards the wild type response, whereas harmful interventions increase the magnitude and/or speed of this loss of force. For example our recent work on the membrane sealant Poloxomer-188 (P188) demonstrated that a 2 week treatment of either 460mg/kg or 30mg/kg P188 daily via intraperitoneal injection significantly increased the severity of the force drop during eccentric exercise compared to vehicle treated control mice ($P= 0.029$ two way repeated measures ANOVA, $n=6$). However there was no difference in force drop during eccentric exercise between the two doses. Therefore despite published reports that P188 has beneficial cardiac effects in dystrophic animals, this compound may be inherently unsuitable for use in dystrophic patients as it appears to increase contraction induced injury in dystrophic skeletal muscle. In contrast, 10 weeks of oral treatment with the AMP-activated protein kinase (AMPK) agonist, metformin significantly reduced the drop in force following eccentric exercise in male *mdx* mice ($P=0.0042$ two way repeated measures ANOVA, $n=3$). Neither of these drugs produced overt clinical signs in sedentary animals.

Conclusions We feel that subjecting proposed therapeutic strategies to robust *in vivo* physiological testing early in their development will allow resources to be targeted towards those interventions that result in a functional benefit to dystrophic muscle and will facilitate early detection of those that are harmful.

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Complex issues surround the use of hormones to improve dairy cow fertility: an insight into veterinary practitioners' attitudes and prescribing practices

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Introduction In England one way in which hormones are used is to improve fertility in dairy cows that do not have reproductive disease. This pharmaceutical intervention (PI) aims to either (1) increase oestrus detection rates or (2) remove the need for oestrus detection so that cows can be served 'blindly' using Fixed-Time Artificial Insemination (FT-AI). Protocols range from a single prostaglandin injection to multi-injection synchronisation programmes. The key advantage of PI is that submission rates may be quickly improved for relatively low investment and although the costs are on-going, some cattle that may otherwise have been culled, survive; it may also mitigate future health problems related to extended calving intervals in some cattle. However, the routine use of PI may mask underlying management/environmental problems thereby diminishing the need to tackle the root of the problem (which may have health implications for the herd); genetic selection for fertility may also become difficult (Refsdal, 2000). Compared to using PI, correcting underlying issues may involve greater investment and fertility may be slower to improve and/or be less directly attributable to the changes made. When used correctly, there are no public health concerns with PI, yet the potential for mis-use exists. Another potential disadvantage is negative perception of the veterinary profession as providers of hormones, not expertise. Using PI to overcome suboptimal management is a controversial and complex issue that gives rise to ethical/welfare dilemmas and involves multiple stakeholders. In this regard, societal values are important. In 1996 in Sweden, the Farmers' associations decided to stop using PI for FT-AI due to 'fear for consumer reactions based on ethical concerns related to replacement of management with hormones' (Refsdal, 2000). Robust justification of the prescription of PI is important and necessitates accurately recording and monitoring PI outcomes. The aim of this research was to explore the current use, beliefs, concerns and ethical stance of practitioners in England with respect to PI for the purpose of improving lactating dairy cow fertility (in animals without reproductive pathology).

Material and methods A 3 page questionnaire was developed following semi-structured interviews with 3 veterinary academics and 2 private veterinary practitioners. Thereafter, it was piloted on 3 academics and 3 practitioners. Two-stage random cluster sampling (stratified by post-graduate qualifications and location) selected 95 vets in 20 practices in England. Payment for veterinary time aided the response rate= 98% (93 vets). Additional data concerning practitioner characteristics was also gathered.

Results Gender: 58 males to 35 females. Employment: 40 partners, 49 assistants, 4 locums. Years qualified: 0-37 years (median=7 years). Out of the 93 vets, 22 held cattle-specific post-graduate qualifications and 80 carried out dairy cow fertility work at least once per month on 1 or more farms; average number of farms per vet=9.4. In total, routine fertility work was conducted on 753 farms by these 80 vets, 39 of which were organic (5.2%). Of the remaining 714 non-organic farms, 4 farms (0.6%) never used any PI, 56 farms (8%) used FT-AI on the majority of cows immediately after the voluntary waiting period (VWP) had ended, 193 (27%) used FT-AI on the majority of cows by some specified point in lactation, but not immediately after the VWP had ended and the remaining 462 farms (65%) used PI to improve oestrus detection rates (to varying extent) and/or for *ad hoc* FT-AI. In reply to an open question, 48 of the 93 vets said the use of PI gave them cause for concern and the reasons cited are broadly categorized as follows: substitute for good management/failure to address underlying problems (27), genetic selection for infertility (12), public opinion (8), health/welfare issues *per se* (6), artificially improves national herd reproduction performance (2), mis-use by farmers (2),drug residues (1), cost(1). On farms with underlying problems and no efforts taken to address them, long-term routine use of FT-AI immediately after the VWP has ended was unacceptable to 56 vets. PI was considered morally acceptable overall to 75 vets, unacceptable to 8 whilst 10 were unsure. 37 vets (40%) would not use PI if the only stakeholders they had to consider in their decision were themselves and the dairy cow. PI was considered a necessity for the profitability of the UK dairy industry by 60 vets, not a necessity by 19 and 14 were unsure. A preference for total future use to decrease was stated by 69, for it not to alter by 20 and for it to increase by 3. The top 3 areas cited as contributing to poor oestrus expression were: nutrition, poor environment and lameness. If underlying management issues were addressed (relative to using PI instead) then an increase in overall herd fertility performance was expected by 85 vets, in overall cow welfare by 72 vets, in farm businesses' profitability by 83 vets, in veterinary practices' profitability by 31 vets and in genetic selection for fertility by 57 vets. Other issues raised included: (1) ethical/welfare dilemma of 'life *per se*' versus 'a life worth living' and difficulties balancing the issue at the level of an individual animal versus the herd, and over the short versus the long term (2) perceptions that farmers expect vets to provide quick solutions and (3) farmer expectations/preferences arising from historical use.

Conclusions The practical importance of this research rests in (1) reporting the current use of PI by practitioners in England along with their beliefs/concerns (2) raising awareness of the complexity of this difficult issue, including the ethical/welfare dilemmas it generates (3) fostering debate and appraisal of current practices. In this context, the importance of monitoring PI outcomes is clear.

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Deer can become infected with bovine and ovine gastrointestinal nematodes and can transmit anthelmintic resistant nematodes to cattle and sheep

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Introduction Gastrointestinal (GI) nematode parasites are one of the main causes of productivity loss in both cattle and sheep farming, as well as being a threat to animal welfare. Over-reliance on broad spectrum anthelmintics to control these parasites has led to the development of anthelmintic resistance on cattle and sheep farms in the UK. Previous studies have demonstrated that, at least under experimental conditions, deer can become infected with bovine and ovine species of GI nematodes. The present study examined the population of GI nematodes in wild deer in the South West of England and aimed to identify whether wild deer could act as vectors of anthelmintic resistant GI nematodes between cattle and sheep farms.

Material and methods A total of 48 samples, comprising the abomasa and small and large intestines, were collected from fallow (n=24), red (n=14) and roe deer (n=10). Samples were collected from three types of environment, namely, farmed deer with no contact with other wildlife or livestock ("Farmed"), wild deer grazing in areas of extensive cattle farming ("Extensive") and wild deer grazing in areas of intensive cattle and sheep farming ("Intensive").
To study the nematode GI population of these wild deer, the total adult abomasal nematode count was estimated from a 10% aliquot of abomasal contents in individual deer by visual identification of adult male nematodes. Individuals of *Ostertagia ostertagi* and *O. leptospicularis,* for which visual identification was ambiguous, were identified by PCR, using species-specific fixed nucleotide polymorphisms in the internal transcribed spacer 1(Zarlenga *et al*, 1998). In order to identify differences in adult abomasal nematode fauna, principal component analysis (PCA) was undertaken on the number of each nematode species in individual deer and two principal components were used. The effect of deer species and type of environment on nematode fauna was further investigated using a general linear model (GLM) on the first principal component.
To investigate anthelmintic resistance in nematodes of wild deer, adult *Haemonchus contortus* (n=23) were extracted from two wild roe deer and the species confirmed by PCR (Zarlenga *et al*, 2001) (females) or using the discriminant function described by Jacquiet *et al* (1996) (males). Benzimidazole (BZ) resistance was investigated in these nematodes by isolating and sequencing the beta tubulin gene and surveying the resultant sequences for BZ-related mutations, namely F200Y, A198E and F167Y (von Samson-Himmelstjerna *et al*, 2007). In order to confirm the potential of cross transmission, one calf was infected with approximately 10,000 larvae cultured from nematode eggs extracted from wild roe deer (n=10). Finally, one lamb was infected with approximately 4,000 larvae cultured from nematode eggs extracted from the calf. Nematode eggs extracted from the faeces of the lamb were used in an Egg Hatch Test (EHT) (Coles *et al*, 2006) for detection of BZ resistance in *H. contortus* and an EC50 value was calculated to assess BZ resistance status.

Results Wild roe deer grazing in the "Intensive" environment had a significantly different abomasal nematode fauna compared to red and fallow deer (PCA: KMO measure of sample adequacy=0.679, Bartlett's test of sphericity chi-square=111.27, $p<0.0001$). These differences were irrespective of the type of environment in which the latter two grazed. Furthermore, the species of deer was the main influencing factor ($F=53.3$, $p<0.001$) of adult abomasal nematode fauna (as revealed by the GLM, $F=33.9$, $p<0.001$), while the environment had a small effect in roe deer ($F=5.2$, $p=0.01$). The 23 *H. contortus* individuals that were genotyped had BZ resistant allele frequencies of 63% at codon 200, 0% at codon 198 and 15% at codon 167, respectively. The nematode larvae cultured from wild roe deer successfully established an infection in the calf, with 90% of the nematodes established being *H. contortus*. Larvae cultured from this calf successfully established a high level infection (over 1,300 adult *H. contortus* estimated in the abomasum) in the lamb, with *H. contortus* being the only species identified. The EHT undertaken on nematode eggs extracted from the lamb gave an EC50 to thiabendazole of 0.149 µg/ml (CI: 0.136 to 0.162 µg/ml; resistance is indicated by an EHT EC50 over 0.1 µg/ml).

Conclusions This study demonstrates the existence of BZ resistant *H. contortus* in wild roe deer and its potential to infect both cattle and sheep. Although wild deer have the potential to transmit anthelmintic resistant GI nematodes to cattle and sheep, further research is required to determine whether cross-transmission occurs under field conditions and to determine the extent of this cross-infection.

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A proteomic approach for biomarker detection of pancreas disease (PD) in Atlantic salmon (*Salmo salar*)

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Introduction Salmonid Alphaviruses (SAVs) are the aetiological agent of pancreas disease (PD) which infect both farmed Atlantic salmon, *Salmo salar* L., and rainbow trout, *Oncorhynchus mykiss* in the marine environment (McLoughlin and Graham, 2007), having a huge effect on farm production. Whilst the behavioural modifications and the pathology of PD are well understood, and a number of diagnostic tools have been developed for SAV detection, very little is known about the humoral response to the disease or whether serum analytes could be used as non-destructive tools for PD detection. Therefore, this study investigated the alteration of the serum proteome profile of Atlantic salmon to PD, using SAV3 as the aetiological agent, in order to identify serum biomarkers of PD.

Material and methods Seven hundred Atlantic salmon (*Salmo salar* L.) parr of mean weight 30g (<15%CV) were randomly distributed into 1m^3 tanks and allowed to feed and grow for 42 days, after which 60 fish from each tank were bulk weighed and transferred into twelve 0.6m^3 tanks. Additional fish from the tanks were maintained separately to be used as Trojan shedders. These were allowed to acclimatise for 5 days and marked by clipping the adipose fin and injected with SAV 3 infected CHSE cell culture supernatant at ca. 10^5 TCID/fish into their intraperitoneal cavity. Inoculated Trojans were added to each of the twelve challenge tanks at 20% of the total tank population. Cohabitant fish were sampled at 0, 2, 3, 4, 5, 6, 8, 10 and 12 weeks post challenge (wpc). At each time point 9 fish per tank were killed by lethal overdose of anaesthetic (MS-222, Pharmaq) and blood collected in non-heparinised vaccutainers for preparation of serum. Fish sampled at time point 0 were sampled before adding Trojan shedders. For proteomic analysis one microlitre of serum from all serum samples from each tank was taken and one pool for each time point per tank created. Protein concentrations of these were determined and pools diluted to an equal protein loading of 208µg in rehydration buffer for 2-dimension electrophoresis analysis in duplicate (equipment & reagents from Biorad Ltd, Hemel Hempstead UK). Isoelectric focusing was carried out using 11cm immobilized pH Gradient strips with a pH range of 3 to 10. Strips were then run on SDS-PAGE gels with XT MOPS running buffer, stained in Coomassie brilliant blue G-250 dye, de-stained and scanned for gel image analysis using Progenesis SameSpots 2D gel image analysis software (Nonlinear Dynamics Ltd, Newcastle, UK), to identify protein spots which were differentially expressed through time. Initial results were filtered using the programme's statistical analysis function, with only those with a power value of >80% and ANNOVA significance score of <0.05 between replicate gels, being chosen for protein identification. These spots were excised manually by scalpel and subjected to in-gel trypsin digestion prior to identification via ion trap mass-spectrometry and comparison to the MASCOT protein database.

Results A total of 72 proteins were significantly differentially expressed and identified via MS/MS. Among these were a number of well-established biomarkers of viral diseases which, in other species, cause similar damage to tissues, including; creatine kinase, malate dehydrogenase, alpha 2 enolase, and aldolase. These biomarkers of tissue injury peak in spot intensity when pathological damage is at its maximum during PD. In addition, a number of humoral immune system proteins were identified, for instance; complement components, hemopexin, transferrin and both light and heavy chains of immunoglobulin M. A number of unexpected results were also observed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) showing a rapid increase in intensity between wpc 4 and 6 and then rapidly falling to basal levels by 10 wpc and differential transferrin fragment expression.

Conclusion This study has highlighted a number of potential serum biomarkers for PD in Atlantic salmon which includes leaked enzymes from damaged tissues and humoral components of the innate and adaptive immune system. In addition, analysis of the expression profiles of proteins demonstrated the progression of disease from SAV infection to recovery, indicating that it may be possible, in a non-destructive manner, to estimate the disease stage an individual would be in based on its serum protein composition. In addition, the discovery that specific transferrin fragments were increased during PD is interesting as it has been shown that in goldfish (*Carassius auratus*) transferrin fragments are not simply results of full length transferrin degradation but are also secreted as such and can induce the nitric oxide (NO) response of macrophages (Stafford and Belosevic, 2003) in which NO can be both an antiviral-agent and immune system modulator. Furthermore, it is known that NO and GAPDH possess a strong affinity within cells and whilst no extracellular relationship between GAPDH and NO has been discovered it is interesting that transferrin fragments involved in NO macrophage activation and GAPDH both increase in concentration with the latter reaching its peak one week subsequent to the transferrin fragments. GADPH has also previously been reported to not only indicate tissue damage but also to be secreted into circulation which may explain the expression profile in serum found in this study.

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Longitudinal surveys of astrovirus infections of chickens: rapid molecular diagnosis by real time reverse transcription polymerase chain reaction

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Introduction Infectious diseases affecting the digestive tract of commercial poultry probably result in greater economic loss than those affecting other organ systems. Although mortality can be considerable, more typically enteric diseases affect the value of the flock by depressing growth rate, impairing efficient feed utilisation, decreasing flock uniformity, and increasing susceptibility to other diseases. Enteritis and growth depression in poultry are associated with a variety of viruses, the most common being astroviruses. Avian nephritis virus (ANV), which is associated with interstitial nephritis and growth retardation in chickens, is classified as an avian astrovirus. A second astrovirus of chickens, named chicken astrovirus (CAstV), was recovered from growth-retarded broilers. The detection of ANVs and CAstVs in stunted broilers and the ability of some ANV and CAstV isolates to cause growth depression following experimental infection of day-old chickens has led to speculation that both AstVs may be important in causing growth problems in the field. However, the nature and extent of these disease problems has remained largely unknown due to the absence of convenient diagnostic tests. After extensive sequence comparison work, AFBI has developed the first real-time RT-PCR tests for separately detecting ANVs and CAstVs (Smyth *et al*., 2010). These tests are sensitive, specific, fast and convenient in comparison with traditional methods. In addition, when compared with conventional RT-PCR approaches, real-time RT-PCR (RT-qPCR) can be used to quantitatively detect the numbers of virus RNA molecules present in the diagnostic samples. The RT-qPCR tests for ANVs and CAstVs developed by AFBI are the first of their kind and the subjects of separate patent applications.

Methods Kidney and gut content samples from four broiler flocks were tested for the presence of ANV and CAstV using real-time RT-PCR. These flocks were from different sites belonging to the same UK poultry organisation and, based on recent performances, were predicted to exhibit average and below-average performances. Gut contents and kidneys from approximately 12 birds from each flock were sampled at each of 10 time points ranging from day 0 to day 42 for two flocks (Flocks A and B), and for the other two flocks (Flocks C and D) 12 birds from each flock were tested at each of two early time points (day 5 and day 7). The overall performance of each flock was estimated after slaughter by calculating European production efficiency factor (EPEF) values, determined by the following equation:

$$\textbf{EPEF} = \frac{\text{live weight (kg)x liveability (\%) x 100}}{\text{age at depletion (days) x feed conversion rate}}$$

EPEF values are standard measures of flock performance and data relating to all birds in the flock were taken into consideration. RT-qPCR results, for each kidney/gut content sample, Flock A/B and CAstV/ANV combination separately, were analysed in GenStat using a oneway analysis of variance to assess the effect of time point. If any of these were significant, then pair-wise differences were determined by least-significant differences. Prior to analysis all variables were transformed by taking logarithms to the base 10.

Results The ability of the AstV RT-qPCR tests to be used quantitatively has been explored in a recent AFBI investigation involving the testing of longitudinal survey samples collected from 4 broiler flocks with average and below average growth performances. Although ANV and CAstV RNAs were detected in the majority of samples at most timepoints from 4-day-old to 35-day-old, significantly higher levels of ANV RNA were detected at the very early timepoints (days 4 and 5) in flocks that performed poorly when compared to the virus RNA levels detected in corresponding samples from the better-performing flocks.

	Flock A	Flock B	Flock C	Flock D	SEM	P value	Flock A	Flock B	Flock C	Flock D	SEM	P value
	Day 4/5						Day 7					
CAstV gut	4.44	5.00	5.02	5.06	0.193	0.091	3.76	3.98	3.94	4.66	0.261/0.239[a]	0.082
CAstV kidney	4.38	3.94	4.07	4.65	0.283	0.295	2.79	3.02	2.55	3.35	0.565/0.515[a]	0.748
ANV gut	4.67[A]	8.09[B]	4.69[A]	7.69[B]	0.289	0.001	7.27	8.00[A]	8.48	7.89[A]	0.172/0.157[a]	0.001
ANV kidney	1.02	4.71[A]	2.92	5.12[A]	0.383	0.001	3.62[A]	4.94[B]	4.69[B]	3.98[A]	0.226/0.206[a]	0.001
EPEF	327(♂)	308(♂)	315(♂)	238 (♀)								

[a]Standard error of the mean (SEM) presented for minimum/maximum replication as the number of birds from each flock differs.

Conclusions These findings in conjunction with experimental infection data has led AFBI to propose that broiler flocks which experience substantial astrovirus challenges when very young (<4 day-old) are likely to growth-perform less well than flocks in which the majority of birds become infected at older ages. This longitudinal survey investigation and findings with other enteric samples support the view that the vast majority of broiler flocks become infected with both ANV and CAstV at some time in their lives. Although the clinical outcomes of these infections will depend on a range of virus-related factors (eg strain, dose, route of infection) and host-related factors (eg age, maternal antibody status, presence of other pathogens, genetics), the age at which the chick is infected is considered to be very important.

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Evaluation of immature thrombocytes (reticulated platelets) in young calves' peripheral blood

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Introduction Reticulated platelets (RP) are the youngest platelets found in the circulation, having been most recently released from bone marrow megakaryocytes. In analogy to reticulocytes, they are measured as the percentage of cells with detectable RNA and generally increase during the regenerative response of megakaryocytes to thrombocytopenia. A quantification of the percentage of circulating RP can therefore aid the discrimination between destructive and hypoplastic causes of thrombocytopenia (Peterec *et al*, 1996) since decrease in the number of marrow megakaryocytes is often accompanied by decrease in the percentage of RP (%RP). Thrombopoiesis has been classically assessed by bone marrow biopsy; an invasive procedure not often carried out in ruminants. The flow cytometric uptake of a nucleic acid binding dye, Thiazole Orange (TO), has been used in many species, but not cattle, to quantify RP in the peripheral circulation. Presently there is no information on the %RP in ruminants, either evaluated with TO staining or alternative techniques. We have modified the TO staining technique for the evaluation of young calves %RP in peripheral circulation and employed it as part of a larger study on the pathology of Bovine Neonatal Pancytopenia (BNP), with the aim of aiding the characterisation of the bone marrow lesions which underlie this condition.

Material and methods Holstein Friesian male calves (new-borns to 6 months of age) were employed as blood donors for the study. The animals were housed and managed in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986. For the enumeration of reticulated platelets blood was collected by jugular venipuncture using a 20 ml syringe and an 18G -1 inch needle and immediately transferred to a 10 ml EDTA containing tube and processed strictly within one hour of collection. Platelet rich plasma (PRP) was prepared from EDTA blood according to standard procedures. To aid the initial flow cytometric identification of bovine platelets, 2µl of PRP were labelled with a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (mAb) that recognises bovine CD41 cell surface antigen (integrin alpha-IIb, Serotec., UK). For the identification of RP, Thiazole Orange (Sigma-Aldrich, UK) stock solution was prepared in methanol at 1mg/ml and diluted in PBS/EDTA/NaN_3 to 1µg/ml immediately before the staining. 5µl of PRP were then incubated with 1ml of TO working solution at room temperature in the dark for 15 minutes followed by fixation with paraformaldehyde. Sample acquisition at the flow cytometer was performed within 45 minutes from fixation. To further control the specificity of the staining for RNA-containing platelets, samples of PRP were pre-treated with RNAse then subjected to TO staining and compared to untreated controls, allowing the precise localization of the RP fluorescence. For the precise quantification of %RP different analysis strategies were tested (including amongst others, average fluorescence intensity, comparison with RBC fluorescence, and a movable gate strategy).

Results The identification of the PRP-derived platelet population, based on its physical characteristics (forward and side scatter) was confirmed by the use of a specific monoclonal antibody to bovine CD41. Freshness of sample, concentration of TO orange and length of incubation with the dye were identified as important parameters for the evaluation of reticulated platelets. The time elapsing between sample collection and processing resulted in a decrease of the percentage of platelets taking up the dye and storage of samples at 4°C for more than 12 hours led to an alteration of their physical characteristics as detectable by flow cytometry. The total platelet fluorescence showed variable results in relation to the amount of dye employed. Using a TO final concentration of 1µg/ml and an incubation time of 15 minutes the positive population of immature platelets was shown to clearly separate from the main population of cells. On this basis we show that a specific gating strategy, based on the separation between the fluorescence of the large cluster of normal cells and the positive population can be used to quantify the %RP. Samples pre-treated with RNAse before TO staining demonstrated a significant reduction of %RP localised in the chosen gate. Quantification of %RP was performed in 4 calves with experimentally-induced BNP and 4 corresponding controls over the first 10 days of life. Despite the absence of statistically significant mean differences between groups we observed a trend to a higher percentage of RP in the challenged animals. In addition, the data suggest the presence of different proportions %RP in both groups, possibly reflecting responses to the circulatory alterations occurring immediately after birth.

Conclusions In conclusion we demonstrate that the detection of reticulated platelets in young cattle using TO staining and flow cytometry is possible in calves similarly to other species. Further studies should ascertain the validity of such a method in the evaluation of bovine thrombopoiesis.

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Effect of grass silage to maize silage ratio and level of starch in the concentrate on the performance and methane production of lactating dairy cows

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Introduction Reducing enteric methane production by ruminant livestock is of particular interest due to the high global warming potential of this gas. It has been proposed that simple changes in diet composition such as increasing the concentration of starch may reduce enteric methane production due a shift in ruminal volatile fatty acid production from acetate to propionate (Moss *et al.,* 2000). The aim of the current study was to examine the effect of the ratio of grass silage to maize silage when fed with concentrates differing in their fibre and starch concentration on the intake, performance and methane production of mid-lactation dairy cows.

Material and methods Twenty pregnant, mid-lactation, multiparous dairy cows averaging approximately 36 kg milk/d were used in a 4 x 4 Latin square design with four periods each of 4 wk duration. The cows received one of two forage treatments; 30:70 or 70:30 first cut grass silage (GS) to maize silage (MS) respectively (DM basis), and one of two concentrates; high fibre (Fibre; 58 and 346 g/kg DM starch and NDF respectively) or high starch (Starch; 320 and 188 g/kg DM starch and NDF respectively) in each period in a 2 x 2 factorial design. The concentrates were fed at the rate of 7 kg/cow/d in 3 meals using out-of parlour feeders. The forages were mixed fresh daily and fed *ad libitum* through computerised forage intake bins. The two forage diets were balanced for protein content by including 0.7 kg/cow/d sugar beet pulp and 0.3 kg/cow/d soyabean meal (high GS diet) or 1 kg/cow/d soybean meal (high MS diet) in the mix. During the first week of the first period each cow orally received a permeation tube emitting 4.74 mg sulphur hexafluoride (SF_6)/d (SD=0.415). Cows were milked twice daily with yield being recorded at each milking. During the final 7 d of each period subsamples of milk were collected on four occasions and analysed for fat and protein. Additional milk samples were collected for subsequent fatty acid analysis by GC. During the final 5 d of each period the cows were fitted with an evacuated canister connected to a calibrated restriction tube in order to collect subsamples of eructed rumen gases. Canisters were changed daily and then over pressurised with N prior to being analysed by gas chromatography (GC) for methane and SF_6. Daily methane production was calculated according to Johnson *et al.* (1994). Data were analysed by ANOVA using Genstat Version 13 (VSN international), with main effects of forage source (F), concentrate type (C) and their interaction (F x C).

Results Total DM intake was 1.3 kg/d higher ($P < 0.001$) in cows when offered the high compared to the low MS diet, and there was a tendency ($P = 0.070$) for cows consuming the high starch concentrate to have a higher DM intake than those receiving the high fibre concentrate (Table 1). Milk yield was affected by both forage mix ($P = 0.012$) and concentrate type ($P = 0.038$), with cows offered the high MS diet and those offered the high fibre concentrate having higher yields. There was a tendency ($P = 0.068$) for cows when offered the high starch concentrate in combination with the high MS diet to have the lowest methane production (g/d). When corrected for DM intake or gross energy (GE) intake however, methane production was lowest in cows when offered the high MS diet ($P < 0.001$). When corrected for GE intake, cows offered the high starch concentrate had a lower ($P = 0.043$) methane production than those offered the high fibre concentrate. There was a tendency ($P = 0.075$) for cows when offered the high starch concentrate to have a higher milk concentration of t10, c12 conjugated linoleic acid (CLA).

Table 1 Intake, performance and methane production of dairy cows offered diets containing 70:30 or 30:70 GS:MS and 7 kg/d of a starch or fibre based concentrate

	70:30 GS:MS		30:70 GS:MS			Significance (P)		
	Fibre	Starch	Fibre	Starch	s,e.d.	F	C	F x C
Total DM intake, kg/d	20.7	21.3	22.1	22.5	0.37	<0.001	0.070	0.577
Milk yield, kg/d	27.7	26.8	28.4	27.9	0.47	0.012	0.038	0.658
Milk fat, g/kg	48.8	50.6	50.1	49.2	0.82	0.908	0.415	0.355
Milk protein, g/kg	36.3	37.6	36.6	37.6	0.38	0.634	<0.001	0.414
CH_4, g/d	406	412	410	381	13.7	0.168	0.235	0.068
CH_4, g/kg DMI	19.6	19.5	18.6	16.9	0.69	<0.001	0.069	0.123
CH_4, KJ/MJ GE intake	56.9	56.1	53.7	48.6	1.98	<0.001	0.043	0.130
CLA c9 t11 g/100g	0.36	0.38	0.38	0.37	0.016	0.426	0.783	0.255
CLA t10 c12 g/100g	0.02	0.03	0.01	0.02	0.009	0.345	0.075	0.494

Conclusions Feeding a high proportion of maize silage combined with a high starch concentrate reduces methane emissions from lactating dairy cattle

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Postruminal degradation of crude protein, neutral detergent fiber and starch of maize and grass silages in Holstein Friesian dairy cows

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Introduction The current Dutch feed evaluation system uses intestinal data of different *in situ* experiments to derive regression equations for feed value parameters. These data are old since most of these experiments were performed 20 to 50 years ago with different crop varieties, ensiling methods and incubation protocols as compare to current practice. Actually, data on intestinal digestibility of crude protein (CP) are scarce (Cone *et al*., 2006) and even less is known about the intestinal digestibility of neutral detergent fiber (NDF) and starch (CVB, 2007). The current models may not accurately represent the current variations in practice, hampering nutrient evaluation for the maize and grass silages. The aim of this study was to investigate the relationship between the determined intestinal digestibility for CP and NDF in maize and grass silages as well as starch in maize silages, in order to develop regression equations, using the chemical composition.

Material and methods Twenty samples of maize silage and 20 samples of grass silage were selected with a large variation in the chemical composition and silage quality parameters. Three multiparous Holstein Friesian cows fitted with ruminal, duodenal and ileal cannulas were used. Prior to the intestinal incubations, samples were incubated in the rumen for 6h (starch), 12h (CP) and 24h (NDF) using the mobile nylon bag technique. Then the rumen incubated residues were transferred into the small mobile nylon bags. The bags were soaked in a 0.004 M HCl solution at pH 2.4 for one hour. Then the bags were incubated for two hours in a pepsin-HCl solution at 40 ^{0}C in a shaking water bath. After that, 12 bags with 20-30 min intervals were inserted per cow daily into the duodenum by the cannula. Half of the bags were collected from the ileal cannula and the remaining half of the bags was collected from the faeces. For NDF, all the bags were collected from the faeces.

Table 1 Regression equations for rumen degradable (RD), small intestinal digestible (SID), large intestinal digestible (LID) and undegradable fraction (UF) of crude protein (CP), neutral detergent fibre (NDF) and starch based on chemical composition.

Maize silages	R^2	Grass silages	R^2
Crude protein			
RD = 118.76 - 0.14 DM - 0.19 ash - 1.21 ADL	0.688	RD = 94.05 + 0.70 CP - 0.19 NDF	0.865
SID = - 84.40 + 0.11 DM + 0.20 ash + 0.64 CP + 0.98 ADL	0.660	SID = - 281.82 - 0.09 DM + 0.49 ash + 0.36 CP + 0.31 sugar + 0.45 NDF	0.720
UF = 4.44 + 0.15 sugar + 0.01 NDF	0.476	LID = 51.11 + 0.02 DM - 0.07 ash - 0.03CP - 0.08 sugar - 0.07NDF	0.778
Neutral detergent fibre			
RD = - 155.83 + 0.72 NDF	0.808	RD = - 145.70 + 0.39 NDF + 0.60 ADF	0.902
Starch			
SID = -186.99 + 0.88 DM + 1.45 ash - 0.35 ADF	0.867		

DM: Dry matter; ADF: Acid detergent fibre; ADL: Acid detergent lignin

Statistical analysis Regression equations were made to estimate the rumen degradable, intestinal digestible (small and large intestinal) and the undegradable fractions of CP, NDF and starch based on the chemical composition of maize and grass silages by using PROC REG backward stepwise procedure of SAS 9.2. The presented equations were made with significant predictors ($P<0.01$).

Results There was a large variation in the rumen degradability and the intestinal digestibility (small and/or large intestine) of CP, NDF and starch. The rumen degradable fractions, intestinal digestible fractions and the undegradable fractions of CP, NDF and starch were influenced by their proportions in the maize and grass silages. Regression analysis showed that rumen degradability, intestinal digestibility and undegradable fractions were influenced by the variation in the chemical composition of the silages (Table 1).

Conclusions Rumen degradability, intestinal digestibility and undegradable fraction of CP, NDF and starch in maize and grass silage were affected by chemical composition of the silages. The present study proved the assumption of Dutch feed evaluation systems that rumen undegraded starch from maize silage is digested in the small intestine of dairy cows. Regression equations based on chemical composition can give acceptable estimates of the intestinal digestion of CP, NDF and starch.

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Effect of calcium salts of either palm fatty acid distillate or a palmitic acid-rich supplement on the fatty acid profile of cows' milk

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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). In general, enhanced consumption of SFA increases risk factors associated with cardiovascular disease, however individual SFA have markedly different effects. Inclusion of unsaturated lipid supplements in dairy cow diets has been shown to reduce the proportion of SFA in milk fat but there is a paucity of data on the effects of palm-based fat supplements, including those with a high concentration of 16:0 fatty acids (FA), on the FA profile of milk. The objective of this study was to determine whether the inclusion of two different types of rumen-protected palm oil-based fat supplement in dairy cow diets had any effect on milk FA profile, particularly total SFA.

Material and methods Four multiparous Holstein-Friesian cows at a similar stage of post peak lactation were used in a Latin Square design study with 28 day periods. Treatments were a control diet (Control) containing no supplemental fat, or the same diet supplemented with calcium salts of palm fatty acid distillate (CPO; Megalac) or with calcium salts of high-16:0 FA (78g/100 total FA from palm oil (CP), included at 25 g/kg DM. The supplements diluted ingredients in the concentrate portion of the diet. The diet was a total mixed ration consisting of 50:50 forage:concentrate (DM basis), with the forage DM proportion consisting of 75:25 maize silage:grass silage. DM intakes and milk yields were recorded daily throughout the experiment. Milk composition was analysed during the last 7 days of each experimental period for fat, protein, lactose and full FA profile. Data were analysed using a general linear model ANOVA (Minitab), with a model that included fixed effects of treatment and random effect of period. Variables were analysed using Dunnett's test to compare the effects of each supplement with the control treatment. Differences were significant when $P<0.05$.

Results There was no effect ($P>0.05$) of supplement on DM intake, milk yield, milk fat or protein concentration (Table 1). Inclusion of CPO decreased ($P<0.05$) milk fat total SFA concentration. This was mainly due to non-significant reductions in both 12:0 and 14:0 concentrations. Inclusion of either fat supplement had no significant effect on milk fat 16:0 concentration. There was a numerical but non-significant enhancement in MUFA content after CPO supplementation, due in the most part to a numerical increase in *cis*-9 18:1.

Table 1 Effect of palm oil-based supplements on cow performance and milk fatty acid profile

	Treatment[1]				
	Control	CP	CPO	s.e.m.	*P* (diet)
Dry matter intake (kg/d)	23.7	23.5	23.5	0.94	0.974
Milk yield (kg/d)	35.0	36.4	38.7	5.08	0.841
Fat (g/kg)	3.70	3.91	3.58	0.203	0.452
Protein (g/kg)	3.27	3.18	3.07	0.182	0.676
Fatty acid profile (g/100 g fatty acids):					
∑4:0-10:0	12.1	11.0	11.6	0.53	0.361
12:0	4.54	3.97	3.56	0.422	□.244
14:0	12.1	11.1	10.6	0.70	0.248
16:0	30.9	32.7	30.5	1.60	0.539
18:1 *cis*-9	13.7	14.8	16.2	0.89	0.151
∑SFA[2]	70.4[a]	69.5[a]	66.9[b]	0.80	0.029
∑MUFA[2]	17.5	18.5	19.8	0.80	0.139
∑PUFA[2]	2.57	2.55	2.71	0.166	0.696

[1]CP –high-16:0 calcium salts from palm oil; CPO – calcium salts of palm fatty acid distillate.
[2]SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
Means within rows with different superscripts are significantly different (P<0.05).

Conclusions Including a high-16:0 calcium salt in the dairy cow diet did not increase milk SFA concentration, while inclusion of Megalac reduced SFA and increased C18:1 fatty acids. The rumen-protected fat supplements deliver longer chain fatty acids to the mammary gland, perhaps inhibiting de novo synthesis of 12:0, 14:0 and 16:0 (Chilliard *et al*., 2000). It is concluded that the CPO (Megalac) delivers unsaturated fatty acids to the mammary gland and reduces SFA concentration of milk fat, while a supplement of 16:0-rich calcium salts of the type used does not necessarily increase milk fat 16:0 or total SFA.

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Impact of dietary linseed and rapeseed supplementation on milk fatty acid composition from housed cows under organic and conventional management systems

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Introduction The relatively high saturated fatty acid (SFA) content in milk fat has been linked with negative effects on human health, although some monounsaturated fatty acids (MUFA), such as c9 C18:1 (OA) and t11 C18:1 (VA) and polyunsaturated fatty acids (PUFA), such as n-3 and n-6 groups and c9t11 C18:2 conjugated (CLA9) found in milk, are associated with beneficial effects on human health (Haug *et al*., 2007). UK retail milk in winter has lower concentrations of beneficial fatty acids (FA) compared with summer milk while some of the desirable influences of organic management in milk FA profile is diminished in winter (Butler *et al*., 2011). It seems that summer diets based on high intake of fresh grass, in contrast to winter diets of silages and concentrates, increase the concentrations of c9c12c15 C18:3 (ALN), VA, CLA9 and total PUFA and decrease the concentrations of SFA in milk. Milk FA profile can also be improved by oil or oilseeds dietary supplementation (Chilliard *et al*., 2000) and the aim of this study was to evaluate rolled linseed and rapeseed in raising the concentrations of beneficial FA in milk from cows under both organic and conventional management on winter diets.

Material and methods Two herds at Nafferton farm (conventional, organic) were divided into 3 groups of 15 Holstein/Friesian cows, each receiving different diets (linseed, rapeseed, control) for 6 weeks; in all other respects, cows were treated under the routine indoor management of the farm over winter. The control diets were composed of grass silage for conventional cows and grass/clover silage for organic cows, while wheat grain was given to cows in both systems. Conventional diets also included molasses and extracted rapeseed and soyabean meal while organic diets used rolled beans as a protein source. The rapeseed diet provided 1.25 kg rolled rapeseed/cow and the linseed diet provided 1.5kg rolled linseed/cow, adding 600g of oil per cow per day. The control diets for both management systems contained no added oils. Analysis of FA methyl esters was performed with a Gas Chromatography system (Shimadzu, GC-2014, Japan) using a Varian CP-SIL 88 fused silica capillary column (100m x 0.25mmID x 0.2µm film thickness). Peaks were identified using a 39 FAME and c9t11 CLA isomer standard. Analysis of variance (ANOVA) using linear mixed effects model (LME) was used to analyze results in R statistical environment using "management system" (conventional, organic), "dietary treatment" (control, linseed, rapeseed) and "sampling week" (1st, 3rd, 6th) as fixed factors and cow ID as a random factor.

Results Milk SFA and MUFA concentrations were significantly decreased and increased respectively by the addition of both oilseeds in cows' diet. Linseed significantly increased PUFA and n-3 FA concentrations in milk but this was not found in milk from rapeseed-fed cows (Figure 1a). Milk from linseed-fed cows had significantly higher concentrations of OA, VA, CLA9 and ALN and milk from rapeseed-fed cows showed a similar profile although ALN concentrations were not significantly affected when compared with milk from control group (Figure 1b). Organic milk had higher concentrations of VA, ALN, c9c12 C18:2 (LA), PUFA, n-3, n-6 and lower concentrations of OA and MUFA compared with conventional milk (Figures 1a,1b).

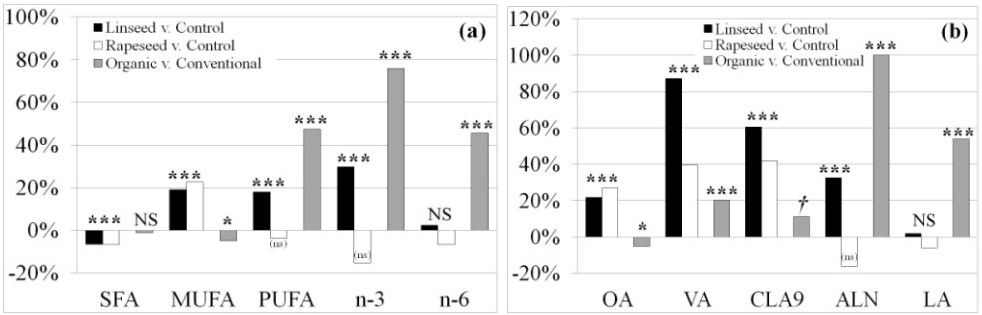

Figure 1 Relative proportions (%) of (a) FA groups and (b) individual FA in milk from cows fed linseed and rapeseed compared with the control group and from organic cows compared with the conventional herd. ANOVA P-values refer to the effect of dietary treatment (up and between linseed and rapeseed bars) and dairy management (on top of organic bar).

Significances were declared at ***: P<0.001, **: P<0.01, *: P<0.05, †: 0.05<P<0.10, NS: P>0.05 while (ns): difference compared to control was not significant despite the overall significant effect of treatment

Conclusions Changes in FA composition of milk after oilseed supplementation reflected the FA composition of the seeds (linseed and rapeseed are rich in ALN and OA respectively) while the increase in unsaturated FA intake elevated the concentrations of intermediates of rumen biohydrogenation and desaturation in the mammary gland, such as VA and CLA9 respectively, secreted into milk. Organic milk showed a more desirable FA profile than conventional milk under winter conditions, possibly due to the clover in silage. In conclusion, winter milk FA composition can be successfully improved by the addition of linseed and rapeseed in cows' diet, with linseed showing more pronounced effects, both under organic and conventional management.

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Effect of forage type and extruded linseed supplementation on milk fatty acid composition

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Introduction Cardiovascular disease (CVD) is the main cause of premature death in the UK and results in over 191,000 mortalities per year (Allender, 2008). As saturated fatty acid (SFA) consumption is an established risk factor for CVD (Mensink *et al*., 2003), the high SFA content of milk and dairy products should be reduced. The dairy cow's diet can be used to alter milk fatty acid (FA) composition, and a decrease in milk SFA concentration can be achieved by feeding a diet high in mono- or poly-unsaturated fatty acids (MUFA or PUFA). Previous research has shown that changing dietary forage type (Ferlay *et al*., 2006), and inclusion of dietary fat supplements (Kliem *et al.,* 2011), can be effective means of decreasing milk SFA concentrations. The present study investigated the effect of an extruded linseed supplement (ELS) on milk FA composition, and whether forage type altered the response to ELS.

Method Four mid-lactation Holstein-Friesian cows were fed one of four dietary treatments according to a 2 x 2 factorial experiment within a 4 x 4 Latin square design. Treatments fed over 28 day periods were total mixed rations with a forage: concentrate ratio of 50:50, the forage portion containing either high proportions of maize silage (MS) or grass silage (GS; 3:1 w/w) both with or without ELS (MS, MSL, GS, GSL). ELS was added to treatment diets at 50g/kg DM, equivalent to approximately 275g oil/cow/day. Diets were fed for *ad libitum* dry matter intake (DMI). Milk yields and DMI were recorded daily, while milk samples were taken at the end of each experimental period for compositional analysis (protein, fat, lactose) and FA analysis by gas-chromatography. Averages for each cow were analysed using mixed model procedures for fixed effects of period and treatment and random effects of cow (SAS Version 9.2, SAS Institute, Cary, NC, USA). Period was treated as a repeated effect, with the compound symmetry covariance structure showing best fit. Least square means are reported and treatment effects were considered significant at $P < 0.05$.

Results Dietary forage type and ELS had no effect on DMI, milk yield (Table 1) or composition. Milk 16:0 concentrations were lower with ELS compared when no ELS was fed. Concentrations of total *trans* FA were higher with MS compared with GS and with ELS compared with no ELS. Levels of α-linolenic acid and total n-3 PUFA were higher when feeding ELS compared with no ELS, while linoleic acid and total n-6 PUFA were higher when feeding MS compared with GS (Table 1).

Table 1 Effect of extruded linseed supplementation and dietary forage type on DMI (kg/day), milk yield (kg/day) and milk fatty acid composition (g/100g total fatty acids)

	Treatment[1]				s.e.m	*P*[2]		
	MS	MSL	GS	GSL		F	L	F*L
DMI	20.3	21.2	19.2	19.7	1.06	0.093	0.309	0.721
Milk yield	36.1	37.4	35.7	35.4	1.13	0.358	0.709	0.519
16:0	29.8	25.7	30.8	28.1	1.657	0.126	0.012	0.503
C18:1c9	17.4	19.4	17.2	17.9	1.402	0.371	0.189	0.482
C18:3c9,12,15	0.44	0.80	0.50	0.78	0.039	0.438	<.0001	0.205
C18:2c9c12	2.26	2.15	1.79	1.74	0.139	0.002	0.377	0.759
∑ SFA[3]	67.5	63.3	69.7	67.1	2.57	0.076	0.055	0.586
∑ trans fatty acids	6.4	7.6	4.7	6.1	0.71	0.011	0.030	0.832
n-3 PUFA	0.73	1.20	0.83	1.20	0.083	0.268	0.0001	0.293
n-6 PUFA	2.6	2.5	2.2	2.1	0.14	0.001	0.187	0.766

[1]MS – maize silage, no ELS; MSL – maize silage and ELS; GS – grass silage, no ELS; GSL – grass silage and ELS.
[2]F – Forage effect; L – linseed effect. F*L – forage x linseed interaction.
[3] SFA – saturated fatty acids; PUFA – polyunsaturated fatty acids

Conclusions Feeding dairy cows ELS and MS compared to no ELS and GS, provide potentially beneficial decreases in SFA without any significant interactions, although with both treatments this was accompanied by increased *trans* FA concentrations. Whilst feeding MS and ELS increased milk PUFA, the effect was small and not likely to be nutritionally meaningful since dairy products make only a very small contribution to total n-3 fatty acid intake in typical UK diets.

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Impact of flavoured water on the performance and health of Jersey calves from birth through weaning

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Introduction Water is essential for calf growth and development and is important to promote solid feed intake. Calves which were not given access to water in a study showed reduced weight gains and a 31 % reduction in starter intake (Kertz *et al*, 1984). Significant correlations between water intake and liveweight gains and calf starter intakes have been demonstrated (Appleman and Owen, 1975). Calf starter intake is related to liveweight gain and has important implications for rumen development. Therefore, methods to promote water intake of calves have the potential to improve starter intake and therefore ease weaning and positively impact calf performance.

Material and methods Thirty six calves were recruited on to the study at birth from a commercial dairy herd. Calves were Jersey heifer replacements (n = 29), Belgium Blue cross Jersey heifers (n = 3) and Belgium Blue cross Jersey Bulls (n = 4). Calves were transferred to calf rearing facility at approximately 24-48 hours post calving having received colostrum. All calves were housed in individual pens with partially solid divides; pens were bedded daily with barley straw. Each calf had free access to two 5-L buckets, one which contained concentrates and the other was used for both milk and water (with corresponding treatment), hayracks containing barley straw were placed between pens thus going two calves access to each rack. Water and calf starter (BOCM QRD pellets, 17 % CP) were provided twice daily for *ad lib* access. Fresh water was provided following milk feeding twice daily, water refusals were recorded prior to milk feeding. Starter feed refusals were measured three times weekly and any milk refusals were recorded twice daily. Calf milk replacer (Premier Performer; Premier Milk Replacers, Lincoln) was offered by bucket feeding twice daily at 0800 and 1700 h by placing it in the bucket that normally contained the water. Whey based milk replacer (22 % CP and 18 % Oil) was reconstituted to 10 % DM and fed at 2 litres per calf twice daily. Calves were weighed on day of birth and thereafter weekly with additional weight recording on day of weaning; for five consecutive days following weaning and 2 weeks after weaning. Calves were randomly allocated to one of four treatment groups which were balanced for birth weight and breed. The treatments were control (unflavoured water), caramel-vanilla flavour (0.1 g/litre; Pancosma Ltd, CH-1218 Le Grand-Saconnex (Genève)), Orange flavour (0.1 g/litre) and a plant extract product "XTract™" (0.15 g/litre). Calf birth weights were (mean ± SE) 26.9 ± 4.4 kg, 27.8 ± 4.4 kg, 26.9 ± 4.8 kg and 28.6 ± 3.2 kg for Caramel, Orange, Control (plain) and XTract treatments respectively. Data were normally distributed and analysed using one way analysis of variance (Genstat, 9th edition) and differences between groups were assessed using a post-hoc Tukey's test.

Results Calves with access to Caramel flavoured water drank significantly more water than the other treatments this was particularly evident in the first 20 days of the trial. This may be a result of novelty and the fact that both the Caramel and Orange flavours had a very strong aroma and may have acted as an attractant to the calves. Weight gain and feed conversion efficiency was similar across the treatments. Calves on the orange treatment very slightly faster to start eating 1kg of concentrate per day although this difference did not reach significance.

Table 1 Feed intake and performance of Jersey calves from birth to weaning

Parameter	Caramel	Orange	Control	Xtract	s.e	p value
Concentrate intake over trial (kg)	30.0	32.7	31.8	27.6	5.52	0.244
Age at which consuming1kg/day conc. (days)	45	43	44	47	6.64	0.513
Average feed intake (kg/day)	0.55	0.59	0.58	0.51	0.10	0.311
Water intake - first 20 days (litres)	7.7 ^{a}	4.1 ^{b}	3.7 ^{b}	1.7 ^{b}	2.27	< 0.001
Water intake over trial (litres)	33.7 ^{cb}	29.2 ^{b}	28.3 ^{ab}	16.0 ^{a}	9.87	0.004
Average water intake (litres/day)	0.62 ^{cb}	0.53 ^{b}	0.52 ^{ab}	0.29 ^{a}	0.06	0.004
Average daily gain (kg/day)	0.41	0.40	0.42	0.40	0.017	0.842
Initial BW (kg)	26.9	27.8	26.9	28.6	5.24	0.898
Final BW (kg)	49.3	50.1	49.7	50.2	5.82	0.988
Feed Conversion Efficiency (kg gain/ kg feed intake)	0.76	0.69	0.73	0.79	0.029	0.093

^{abc} different superscripts indicate significant differences between means within rows $p<0.05$

Conclusion Calves on the caramel treatment drank a large amount of water; this did not correspond to an increase in dry feed intake in this study. However, this increased water intake may be beneficial to producers in particularly hot countries to encourage calves to drink which will aid them to cool down.

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Effect of once or twice per day milk replacer feeding systems on performance of purchased dairy-bred beef calves to 12 weeks

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Introduction As farms and herd sizes expand in the face of low commodity values and an attempt to benefit from economies of scale there is increasing pressure on the time of stockmen with more animals being kept per person. The development of low labour systems is therefore vital in this situation. The introduction of a once-a-day milk feeding system that does not affect performance or health should significantly reduce labour requirements. In a study by Marsh and Collinson (2008) calves were placed onto a once-a-day feeding system at 5 days old. Calves reared on a twice-a-day system gained significantly (P <0.01) more weight than the Once-a-day fed calves from start to 3 weeks, however from birth to 12 weeks of age there were no significant differences in DLWG. The objective of this experiment was to investigate the effect of rearing purchased (approx 3 weeks old) dairy-bred beef calves on a once-a-day milk replacer feeding system.

Material and methods Forty Holstein and beef cross Holstein bull calves were purchased with a mean age of 20.5 days and assigned in a randomised block designed experiment to one of the following treatments: 'Twice', calves fed warm (37^{o}C) milk replacer ('Shine Flying Start', Bonanza Calf Nutrition) mixed at 120g per 880ml of water twice per day at 8.00am and 5.00pm via buckets at 5 litres per day to weaning; 'Once', calves fed warm milk replacer ('Shine Once-a-Day', Bonanza Calf Nutrition) mixed at 200g per 800ml of water and fed at 3 litres per day at 8.00am. Both groups of calves therefore received 600g milk replacer per day. Early weaning concentrates (Primecalf Sprinter Pellets, Carrs Billington) were offered *ad lib* from the start. The calves were initially individually penned on straw and offered *ad lib* straw and water and weaned when eating 1.2kg of concentrates for 3 consecutive days and moved into group pens at weaning. The calves were weighed at 1.00pm. The data were analysed by ANOVA with calves blocked according to age, weight and breed.

Results The Once-a-day and Twice-a-day calves were weaned after 19.1 and 21.9 days, respectively. The Once-a-day calves recorded a significantly higher (P <0.001) daily live weight gain (DLWG) from start to 3 weeks and from 6 to 12 weeks (P = 096). They also recorded a higher (P = *0.141*) last rib girth measurement at 12 weeks.

Table 1 Effect of feeding system on liveweight (kg)

	Once	Twice	s.e.d	Sig
Start weight	56.5	56.7	1.96	NS
6 week weight	84.8	82.2	4.37	NS
12 week weight	132.2	124.4	5.55	0.132

Table 2 Effect of feeding system on DLWG (g) and last rib girth measurement at 12 weeks

	Once	Twice	s.e.d	Sig
Start – 3 weeks	461	289	44.7	***
Start – 6 weeks	674	606	65.8	NS
6-12weeks	1,127	1,003	72.5	0.096
Start - 12 weeks	901	806	59.5	0.121
Last rib girth (cm)	140.6	136.5	2.69	0.141

Table 3 Feed intakes (kg/head) and Feed Conversion Ratio (FCR) from start to 12 weeks

	Once	Twice	s.e.d	Sig
Conc intake (start - weaning) 13.3	12.5	0.769	NS	
Conc intake (wean - 12 weeks) 171.9	161.2			
Milk replacer	16.4	17.5		
FCR (kg feed: kg gain)	2.66	2.82		

There were no differences in coat bloom or faecal scores, or incidence of health (hydration score, cough score, nasal discharge and eye discharge score) between the treatments. Concentrate intakes from start to 12 weeks were higher for the Once-a-day calves and overall consumed an extra 11.5kg more concentrates per head. Feed costs per calf were increased by £4.29 with the Once-a-day system however feed costs per kg gain were reduced by 4.7p based on the costs prevailing at the time of the study. Labour was reduced by 42% with the Once-a-day system resulting in a saving in labour costs of £8.60 per calf. This would negate the increase in overall feeding costs resulting in an increased margin worth £4.31 per calf for the Once-a-day system.

Conclusions Overall performance was very good and both groups of calves exceeded the recognised target for rearing calves to 12 weeks of 115kg. Feeding milk replacer at the rate of 600g per calf per day on a Once-a-day system to purchased 3 week old calves improves calf performance. The saving in manual labour can increase time for stockmanship tasks to enable early identification of disorders such as scour and pneumonia and hence facilitate rapid treatment and minimise mortality.

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Effect of feeding a Yeast Culture (Diamond V XP_{LS}) on the performance of artificially reared dairy-bred bull calves

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Introduction The variable costs for rearing a dairy-bred calf to 12 weeks at Harper Adams University College are currently £99 (October 2011). With increasing interest in improving calf performance and reducing calf rearing costs there is focus on the use of probiotics or yeast cultures to enhance performance. The objective of this experiment was to investigate the effect of feeding Diamond V XP_{LS} in milk and starter concentrates on the performance of dairy-bred bull calves to 12 weeks. Diamond V XP_{LS} Yeast Culture, Diamond V, Cedar Rapids, IA, USA is a *Saccharomyces cerevisiae* fermentation product containing the yeast and the media on which it is grown and fermented.

Material and methods Forty-eight Holstein and Beef cross Holstein artificially reared bull calves were assigned in a randomised block designed experiment to one of the following treatments: Control, calves fed warm (37°C) milk replacer ('Shine', Bonanza Calf Nutrition) mixed at 150g per 850ml of water twice per day via buckets at 4 litres per day to weaning at 46 days. From 3 days prior to weaning the milk replacer was fed at 2 litres per day in one feed. Early weaning concentrates (Start 'n' Wean, Wynnstay Group Plc) were offered *ad lib;* XP_{LS}, identical milk replacer feed rates to the control group to weaning at 46 days. The calves were fed 15g/head/day XP^{LS} mixed with the milk replacer and fed via buckets. At day 28 the XP_{LS} feed rate was reduced to 10g/h/d. From 3 days prior to weaning the XP_{LS} was fed at 5g/h/d and calves were offered *ad lib* early weaning concentrates containing 15kg/t XP_{LS}. The calves were individually penned on straw and offered *ad lib* straw and water. The calves were moved into group pens at weaning. Daily live weight gain (DLWG) was calculated from the difference in weights divided by the intervening number of days. The data were analysed by ANOVA with calves blocked according to weight and breed.

Results The calves fed XP_{LS} recorded a significantly higher ($P<0.05$) pre-weaning concentrate intake, DLWG and feed conversion ratio (FCR) from start to weaning. They also recorded a higher ($P<0.05$) last rib girth measurement at weaning.

Table 1 Effect of XP_{LS} on liveweight (kg)

	Control	XP_{LS}	s.e.d	Sig
Start weight	57.2	57.2	1.23	NS
Weaning weight	76.1	79.6	2.15	NS
12 week weight	122.6	124.8	2.83	NS

Table 2 Effect of XP_{LS} on DLWG (g) and last rib girth measurement at weaning

	Control	XP_{LS}	s.e.d	Sig
Start - 3 weeks	139	205	42.6	NS
3 weeks - weaning	638	721	38.7	*
Start - weaning	410	485	37.1	*
Start - 12 weeks	779	804	41.5	NS
Last rib girth (cm)	107.5	109.8	1.12	*

Table 3 Feed intakes (kg/head) and FCR start to weaning

	Control	XP_{LS}	s.e.d	Sig
Conc intake (start - weaning)	33.8	38.2	2.18	*
Conc intake (wean - 12 weeks)	114.2	117.7		
Milk replacer	24.0	24.0		
FCR start - wean (kg feed: kg gain)	3.06	2.80	0.225	*

Concentrate intakes from start to weaning were significantly higher ($P<0.05$) for the XP_{LS} calves and overall consumed an extra 7.9kg more concentrates per head with an improved ($P<0.05$) FCR.

Conclusions Overall performance was very good and the calves on both treatments exceeded the MLC (1999) target for rearing calves to 12 weeks of 115kg. Feeding XP_{LS} yeast culture was shown to increase pre-weaning concentrate intake, DLWG and FCR from start to weaning however there was no significant difference in weaning or 12 week weight. It could be assumed that the increased concentrate intake resulted in the improved DLWG with the calves. The improved intake with the XP_{LS} yeast culture would minimise growth check at weaning and enhance rumen development. As shown in table 2 the XP_{LS} yeast culture fed calves had a higher last rib girth measurement which is an indication of rumen growth and development. Many commercial calf rearers wean calves when they are eating 1kg of concentrates per head per day and it could therefore be possible to wean earlier with XP_{LS} yeast culture and thus reduce calf rearing costs.

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098

Performance of Holstein calves fed starter containing different levels of fibre and with or without feeding alfalfa hay

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Introduction The intake of solid feed is vital to the calf for making the transition from a pre-ruminant animal to functioning ruminant. Solid feeds especially concentrate or high carbohydrate containing diets, stimulate rumen microbial proliferation and VFA production and subsequently initiates rumen development (Zhang *et al*., 2010). However, there is still much controversy concerning the composition of starter that should be fed to pre-ruminant calves, especially regarding the level of forage those diets should contain. Fibre consumption promotes muscular development of the rumen and stimulates rumination and flow of salvia into the rumen (Hodgson., 1971). The fibre is necessary in the diet of young calves, but type and amount of fibre on starter is unclear. The objective of this study was to determine the effect of feeding alfalfa hay and starter concentrate containing two different levels of fibre on DMI, ADG and FE (feed efficiency) in male Holstein calves**.**

Material and methods Holstein male calves (n=32) born between July to November 2010 were separated from their mothers within 2h of birth, weighed, and moved into individual pens (bedded with wood shaving) where they were fed colostrum at 10% of BW for the first 3 d. The calves enter to trial at age of 3 d and the experiment was ended 3 weeks after weaning. Two experimental main factors were included adding alfalfa hay to diet and the level of starter concentrate fibre, so the 4 experimental treatments were as follow: T_1: starter with low fibre and without alfalfa hay, T_2: starter with low fibre along with alfalfa hay, T_3: starter with high fibre and without alfalfa hay, and T_4: starter with high fibre along with alfalfa hay. The data were analyzed by a linear model in which the effects of starter fibre level, alfalfa addition and interaction between them were included. Statistical comparisons among different means were carried out by Tukey-Cramer test. Significance was declared at $P<0.05$ unless noted otherwise. DMI, ADG and feed efficiency measures were subjected to ANOVA using the MIXED procedure of SAS.

Results Dry matter intake was not affected by treatment effect but ADG was lowest for T4 ($P<0.01$) and FI was lowest for T1 and T4. Addition of alfalfa hay significantly ($P<0.01$) reduced ADG and high starter fibre level significantly lowered ADG ($P<0.01$) and FI ($P<0.05$).

Table 1 Effect of feeding alfalfa hay and starter concentrate containing two different levels of fibre on FI, ADG and FE (ADG/feed intake)

Treatment	DMI (g/d)	ADG (g/d)	Feed efficiency (ADG/DMI)
Low fibre without alfalfa hay (T1)	933.5	622.2[a]	0.52[b]
Low fibre along with alfalfa hay (T2)	952.1	591.3[a]	0.55[ab]
high fibre without alfalfa hay (T3)	908.1	582.9[a]	0.63[a]
high fibre along with alfalfa hay (T4)	779.1	506.1[b]	0.53[b]
SEM	66.11	18.65	0.064
Addition alfalfa hay (A factor)			
Without alfalfa hay	925.8	602.5[a]	0.57
Along with alfalfa hay	865.6	548.7[b]	0.54
SEM	48.24	13.03	0.013
Starter fibre level (B factor)			
Low fibre	942.8	606.7[a]	0.58[a]
High fibre	843.6	544.5[b]	0.54[b]
SEM	46.78	13.05	0.014
Source of variance		p-value	
Addition of alfalfa hay (A)	NS	***	NS
Starter fibre level (B)	NS	***	**
A×B	NS	NS	***

Different superscripts in each column represented the difference is significant. NS = Not Significant.
*P<0.10 **P<0.05 *** P<0.01.

Conclusion The results of this experiment showed adding fibre to dairy calves ration through both starter concentrate and feeding alfalfa hay (before and after weaning) may reduce their performance.

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Effects of anthropogenic activities on some biochemical parameters of *Catla catla*, from the river Ravi, Pakistan

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Introduction Freshwaters are highly vulnerable to different pollutions, since they act as immediate sinks for the consequences of anthropogenic activities. Industrial and municipal effluents are the main culprits for causing undesirable changes in water quality and hence metabolism, biochemistry and physiology of inhabitant fish. As fish are a rich source of essential health promoting nutrients, their meat quality can be affected by the presence of residual pollutants. Therefore it is important to know the impacts of water pollution on the health and growth of fish by using their biochemical profiles as potential stress indicators. This study compared samples of *Catla catla* (Thaila) from an upstream and three downstream sites of the river Ravi receiving untreated wastes around Lahore city for the extent of effluents' stresses by using their muscle biochemistry as a potential indicator of water pollution.

Material and methods A replicated 4 x 2 factorial arrangement comprising 4 sampling sites and 2 flow seasons i.e., high (post monsoon) and low (winter) was used to study the impact of pollution on the muscle biochemistry of *Catla catla* from the river Ravi around Lahore. For the estimation of nucleic acid contents, about 0.5 g of fish wet muscle was boiled in 3 ml ethanol by using test tubes in a water bath at 90ºC for one hour. The test tube contents were then incubated at 37 ºC for overnight. The homogenates were later centrifuged at 2000 rpm for 10 minutes to get a clear ethanol supernatant. The pellets were used for nucleic acid extraction, while the ethanol supernatant was evaporated at 70ºC. The yellowish dried residue containing lipid and cholesterol components was then dissolved in 0.5 ml of chloroform before analysis for cholesterol and total lipids (Henry and Henry 1974). The DNA and RNA contents were estimated according to Schneider (1957). Fish muscle extract was prepared in ice cold saline and each supernatant was used for the determination of total carbohydrates (Dubios *et al.* 1956) and total and soluble protein (Lowry *et al*. 1951). The data were statistically analysed using Minitab software to test if the effects of site, season and site x season interaction on each of the biochemical parameters of fish muscles were significant at $P<0.05$ (*) or $P<0.01$ (**) or $P<0.001$ (***).

Results All parameters showed significant differences between seasons and among downstream sampling sites, except the DNA content. The trend of changes in biochemical parameters appeared to be more responsive to the downstream locations; total and soluble proteins and DNA contents of the muscles showed increases while carbohydrate, total lipids, cholesterol and RNA contents decreased up to site C during both low and high flow seasons. These changes in the biochemical parameters appeared to be stabilized at site D where a recovery trend was observed in these parameters as compared to those for the site C.

Table 1 Mean biochemical components (mg/g) of muscles of *Catla catla* from different upstream (Siphon=A) and downstream (Shahdera=B; Sunder=C; and Head Balloki=D) sites of the river Ravi during two seasons (Low and High flow).

Sites	A		B		C		D		SEM With Significance		
Seasons	Low	High	Low	High	Low	high	Low	High	Site	Season	Site x Season
Biochemical parameters (mg/g)											
Carbohydrates	44.71^{a}	47.32^{a}	26.21^{d}	31.06^{c}	17.07^{e}	25.22^{d}	33.41^{c}	36.33^{b}	0.469^{***}	0.331^{***}	0.663^{***}
Total Protein	81.18^{e}	77.03^{e}	110.76^{d}	107.26^{d}	199.01^{a}	173.69^{b}	155.50^{c}	146.20^{c}	1.986^{***}	1.405^{***}	2.809^{***}
Soluble Protein	39.54^{f}	35.59^{f}	55.64^{d}	49.34^{e}	93.78^{a}	86.05^{b}	63.79^{c}	55.89^{d}	0.904^{***}	0.639^{***}	1.279
Total Lipids	23.96^{ab}	25.70^{a}	$20.23b^{cd}$	22.20^{abc}	16.88^{d}	18.82^{c}	17.87^{cd}	20.85^{bcd}	0.760^{***}	0.537^{**}	1.075
Cholesterol	1.99^{a}	2.05^{a}	1.12^{b}	1.20^{b}	0.33^{d}	0.43^{d}	0.67^{c}	0.77^{c}	0.038^{***}	0.027^{*}	0.054
DNA	1.31^{a}	1.35^{a}	1.42	1.36^{a}	1.46^{a}	1.34^{a}	1.44^{a}	1.36^{a}	0.029	0.020	0.041
RNA	5.91^{a}	5.94^{a}	5.75^{a}	5.73^{ab}	5.33^{c}	5.62^{abc}	5.43^{bc}	5.66^{a}	0.052^{***}	0.037^{*}	0.074

Means within the same row with the same letters did not differ significantly ($P>0.05$)

Conclusion The present study has shown that the ambient toxicants cause significant variation in most of the biochemical parameters of fish muscle from polluted water in relation to control (site A) and these trends might be considered indicative of long term pollution of the anthropogenic activities. The altered biochemical profiles of pollutants exposed fish fillet might have been causing health issues for the fish consuming communities of these areas.

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Effect of industrial pollution on heavy metals, total oxidants and antioxidants of *Labeo rohita* domiciled in the River Chenab

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Introduction In Pakistan, the water pollution has become a serious problem due to discharge of untreated industrial effluents and domestic sewage, containing large quantities of toxic heavy metals, into the river water system that adversely affect the aquatic life. The most affected rivers such as the River Chenab are those that flow through the urban areas having anthropogenic activities that can damage their water quality. This river is affected by the effluents from leather, plastic, textile, printing, soap and sugar industries that are discharged into the main Paharang drain that falls into the river Chenab. Therefore this study explored the effects of heavy metals as pollutants on the metal profile in water and fish kidneys and total oxidants and antioxidants in kidneys of *Labeo rohita* (*L. rohita* =major carp), a valuable fish for its taste and fast growing features for this area of good economic importance.

Material and methods This completely randomised study involved three polluted locations (Thatha Muhammad Shah= Thatta, Pattan Draaj =Draj and Head Trimu= Trimuu) that were about 30 kilometers apart from each other along the stretch of river Chenab in Jhang and a relatively less polluted site of Kot Khera as the control. A total of 108 samples of *L. rohita* were collected by involving nine fish samples of similar size (about 1000g) as replicates from four locations on monthly basis for three months (March to May, 2011). Water samples were also collected from each of these four locations. The fish were dissected to collect kidneys for the estimation of their heavy metal, total oxidant and antioxidant status. Heavy metals in water and fish kidneys were determined by using Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer (AAS, Z-8200, Japan). Total oxidants (TOS) of kidneys were determined by using a novel automated measurement method and the total antioxidants (TAS) were assessed by the automated colorimetric method. The data were statistically analysed by using ANOVA in Minitab software version 16 to test the effect of sampling sites on the metals, total oxidants and antioxidants of fish kidneys. Tukey's post-hoc test was used to compare the means of different analytes for different locations at $P<0.05$.

Results Table 1 shows the significant differences ($P<0.01$) in metal profile of water that were sampled from four selected locations. The concentration of Pb, Cd, Ni and Zn were higher at the three sampling locations than the WHO maximum permissible levels of heavy metals for aquatic life. Conversely, the most metal levels except Ni at the control site were within the safe limits. Table 2 shows significant differences in heavy metal profiles and total oxidants of fish kidneys sampled from selected locations ($P<0.01$). The concentration of heavy metals at sampling sites exceeded the WHO standards of food fish and control fish except Zinc. Consequently the fish of this study area may cause metal related disorders in these fish and their consumers. Moreover the mean metal concentration in fish kidneys was several times higher than the corresponding samples of water. While total oxidants differed significantly ($P<0.05$) for all selected locations, non significant differences ($P>0.05$) were observed for total antioxidants of fish kidneys being sampled from different locations of the same river.

Table 1 Mean± SE of metal concentration (mg/L) at selected locations of the River Chenab and permissible WHO metal levels for water

	Thatta	Draj	Trimu	Control	Maximum permissible WHO levels of heavy metals for aquatic life
Lead (Pb)	0.17 ± 0.05^{a}	0.03 ± 0.005^{b}	0.02 ± 0.0023^{c}	0.01 ± 0.002^{d}	0.025
Cadmium(Cd)	0.05 ± 0.003^{a}	0.02 ± 0.008^{b}	0.02 ± 0.003^{b}	0.01 ± 0.001^{c}	0.002
Chromium(Cr)	0.16 ± 0.03^{a}	0.08 ± 0.01^{b}	0.02 ± 0.005^{c}	0.01 ± 0.001^{d}	0.1
Nickel (Ni)	0.56 ± 0.08^{a}	0.35 ± 0.03^{b}	0.27 ± 0.04^{c}	0.15 ± 0.03^{d}	0.025
Zinc (Zn)	4.72 ± 0.71^{a}	3.95 ± 0.93^{a}	3.84 ± 0.29^{b}	0.45 ± 0.01^{c}	3

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

Table 2 Mean± SE of metal (µg/g), total oxidant and antioxidant (µmol/L) concentration and WHO standards (µg/g) for food fish

	Thatta	Draj	Trimu	Control	Maximum permissible levels of heavy metals in fish (WHO standards)
Cd	0.28 ± 0.01^{a}	0.28 ± 0.01^{a}	0.16 ± 0.02^{b}	0.03 ± 0.005^{c}	0.05
Cr	4.38 ± 0.21^{a}	2.95 ± 0.20^{b}	2.19 ± 0.12^{c}	0.22 ± 0.01^{d}	0.05-0.15
Pb	10.29 ± 1.21^{a}	6.38 ± 0.24^{b}	4.24 ± 0.12^{c}	0.27 ± 0.02^{d}	2.0
Ni	4.97 ± 0.34^{a}	1.90 ± 0.23^{b}	2.09 ± 0.12^{b}	0.30 ± 0.005^{c}	0.5-1
Zn	42.63 ± 2.5^{a}	31.85 ± 1.67^{b}	30.65 ± 1.50^{b}	2.72 ± 0.22^{c}	40
Oxidants	4.29 ± 0.34^{a}	3.28 ± 0.26^{b}	0.97 ± 0.005^{c}	0.23 ± 0.003^{d}	-
Antioxidants	1.95±0.1	2.10±0.12	2.25±0.22	2.42±0.23	-

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

Conclusions It appears that proper measures are required to minimise the contamination of the River Chenab by Main Paharang Drain which could be responsible for the increased levels of metals in water which may damage the fish and other aquatic life and ultimately the fish meat for consumers.

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Detecting molecular features of structural and non-structural carbohydrates in co-Products from bioethanol production using diffuse reflectance infrared Fourier transform (DRIFT)

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Introduction Carbohydrates include structural carbohydrates such as cellulosic and hemicellulosic compounds or neutral and acid detergent fibers in ruminant nutrition and non-structural carbohydrate such as starch. These carbohydrate structural profiles affect nutrient availability or digestive behavior of the bioethanol co-products. The objective of this study was to use DRIFT spectroscopy to detect carbohydrate related molecular features of the new co-products from bioethanol processing.

Material and methods From February 2009 to January 2010, seven different batches of wheat DDGS and blend DDGS (blend1, wheat to corn ratio 70:30 percent; blend2 wheat to corn ratio 50:50 percent) were collected from two bio-ethanol processing plants located in Saskatchewan, Canada. The detailed chemical profiles were reported by Azarfar and Yu (Unpublished data). The experiments were carried out at Saskatchewan Structure Sciences Center (SSSC, Saskatoon, Canada). The DDGS samples were finely ground two times to pass through a 0.25 mm screen (Retsch ZM-1, Brinkmann Instruments LTD, Ontario, Canada). Samples of ground DDGS were mixed with KBr in a ratio of 1 part of co-product with 4 parts of KBr in a 2 mL centrifuge tube and vortexed for 10 s. DRIFT was performed using a Bio-Rad FTS-40 with a ceramic IR source and MCT detector (Bio-Rad laboratories, Hercules, California, USA). Data was collected using Win-IR software. Spectra were generated from the mid-IR (4000-800 cm^{-1}) portion of electromagnetic spectrum with 256 co-added scans and a spectral resolution of 4 cm^{-1}. Molecular spectral analysis was done with OMNIC 7.2 software (Spectra Tech., USA). The carbohydrate related molecular spectral bands studied were: A_Cell (structural CHO, peaks area region and baseline: *ca.* 1485–1188 cm-1)**,** A_1370 (structural CHO, peaks area region and baseline: *ca.* 1485–1188 cm-1.

Results The results of current study indicated that carbohydrate related molecular spectral bands did not differ among the DDGS types (Table 1).

Table 1 Structural Features of Cellulosic compounds: Comparison of Wheat DDGS and Blend DDGS Using DRIFT Molecular Spectroscopy.

	Features of cellulosic compounds (IR KM intensity unit)			
	Cellulose & hemicelluloses (~1420 cm^{-1})		Cellulose & hemicelluloses (~1368 cm^{-1})	
Item	peak area (cm^{-1})	peak height (cm^{-1})	peak area (cm^{-1})	peak height (cm^{-1})
Wheat DDGS	144.88	0.77	38.94	0.63
Blend DDGS (W:C=70:30)	189.52	0.96	50.37	0.87
Blend DDGS (W:C=50:50)	150.24	0.75	41.11	0.71
SEM	26.565	0.150	6.445	0.117

SEM=standard error of mean. Means with the different letters in the same column are significantly different ($P < 0.05$). Multi-treatment comparison method: LSD. W: C, wheat to corn ratio: 70:30 and 50:50 percentage.

Conclusions Since structural and non-structural carbohydrates profiles affect nutrient availability or digestive behaviour it would be expected that carbohydrate utilisation and availability do not differ among the DDGS types used in the current study.

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Effects of bioethanol processing co-products type on estimated ruminal and intestinal availability of protein

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Introduction Bio-ethanol co-products, such as dried distillers' grains with soluble (DDGS) are excellent sources of protein and energy for ruminants. The objective of this study was to evaluate wheat DDGS and blend DDGS for hourly effective rumen degradability of CP, energy and nitrogen (N) release synchrony and intestinal availability of rumen undegraded CP.

Material and methods From February 2009 to January 2010, seven different batches of wheat DDGS and blend DDGS (blend1, wheat to corn ratio 70:30 percent; blend2 wheat to corn ratio 50:50 percent) were collected from two bio-ethanol processing plants located in Saskatchewan, Canada. Protein and carbohydrates were fractionated using an *in situ* approach described in the recently updated DVE/OEB protein evaluation system (van Duinkeren *et al*., 2011). Fractional degradation rates of D for CP, NDF and RNSP (Kd, /h) were calculated by fitting the degradation data to a first-order kinetics model described by Robinson *et al*. (1986). Hourly effective rumen degradability was calculated as (Robinson *et al*. 1986):
In situ fraction × kd/(kd + kp) × $[1 - \exp^{-t(kd+kp)}]$
Hourly as well as cumulative rumen degradable N-to- carbohydrate ratio (N:CHO; g kg^{-1}) and rumen degraded protein balance (OEB) were calculated as described by van Duinkeren *et al*. (2011). The estimation of intestinal digestion was determined by two methods. In the first method intestinal digestibility of rumen undegraded protein (RUP) was determined by a three steps *in vitro* approach as described by Calsamiglia and Stern (1995) was used to estimate intestinal digestibility of rumen undegraded protein (dRUP).

Results The wheat DDGS had a higher effective rumen degraded CP (ERDP) than BDDGS1 and BDDGS2 (201.9, 118.9 and 117.4 g/kg DM in wheat DDGS, blend1 and blend2, respectively) in time frame of 0-12 h. In time frame of 0-12 h, all types of the DDGS had N:CHO ratios much higher (132.5, 89.5 and 90.5g/kg in wheat DDGS, blend1 and blend2, respectively) than the recommended optimum value of 32 g N/kg CHO truly degraded in the rumen. The results showed that blend1 and blend2 were superior to the wheat DDGS with regard to digested rumen undegraded protein (DRUP; 117.8, 151.9 and 161.0 in wheat DDGS, blend1 and blend2, respectively).

Table 1 Ruminal availability of nutrients for microbial protein synthesis in wheat and blend DDGS based on DVE/OEB 2007 protein evaluation system.

Items	2 h				12h			
	Wheat DDGS	Blend1	Blend2	SEM	Wheat DDGS	Blend1	Blend2	SEM
ERDP (g kg^{-1} DM)	129.5a	70.1b	70.3b	3.91	201.9a	118.9b	117.4b	13.51
N:CHO (g kg^{-1})	121.9a	81.3b	83.9b	8.34	132.5a	89.5b	90.5b	10.46

SEM=standard error of mean. [a-c]Means with the different letters in the same row are significantly different ($P < 0.05$). Multi-treatment comparison method: LSD. ND = not determined.
ERDP = Effective rumen degraded protein; N:CHO = rumen degradable nitrogen to carbohydrates.

Conclusions The ruminal availability of nutrients were different in the time frames of 0-6 and 0-12 h among the wheat DDGS, blend1 and blend2. In these time frames, the effective degradability of CP in the DDGS samples increased as the content of wheat feedstock increased. However, increasing the inclusion level of corn from 300 to 500 g kg^{-1} to produce blend2 did have any on effective degradability of CP. Replacing wheat with corn to produce blend DDGS drastically increased digestible rumen undegraded protein. Despite being excellent sources of intestinally available protein, the extremely higher than optimal values of N:CHO and OEB, as indications of potential loss of N, and the variations on nutrient availability among the different batches of the DDGS are factors that may hamper their inclusion as a protein supplement in ruminant rations.

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Reproductive performance of rabbit bucks fed diets containing probiotics

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Introduction Probiotics are preparations or products with defined and viable microorganisms sufficient to alter intestinal micro flora of the host exerting a beneficial health effect (Schrezenmeir and De Verose, 2001). Stanley (2005) noted that probiotic (*Saccharomyces cerevisiae)* can be used to produce certain physiological changes in animals. Probiotics can reduce the supplemental level of antibiotics in animal feed. To further improve rabbit production, a biotechnology approach involving the inclusion of probiotics in the diets of rabbits needs to be evolved. Very little scientific research has been published on efficacy of probiotics on reproductive functions. The objective of the study was to determine the effects of probiotic (*Saccharomyces cerevisiae)* on reproduction in rabbit bucks in order to encourage or discourage its use in animals meant for breeding.

Material and methods The experiment was a CRD with designated treatments T1 (control), T2and T3 containing *Saccharomyces cerevisiae* at 0, 0.1 and 0.16 g/kg diet, respectively. The concentrate in all the treatments contained 16% CP and 2.7MJ dry matter and supplemented with mixed grass and legumes. Six grower rabbit bucks aged 5 months, weighing between 1.8 to 2.2 kg were assigned to each treatment. The bucks were fed for 56 days before data collection and the feeding pattern continued throughout the trial period. Semen was collected twice a week for 6 weeks using Artificial Vagina (AV) designed and constructed for rabbit bucks. To simulate natural vagina, the AV was dipped in warm water at the temperature of 40°C for 15 minutes after which it was dried and lubricated with glycerol. A teaser doe was introduced to the buck's pen. As the buck mounted the teaser, the AV was introduced and ejaculation took place. Semen was collected in a calibrated tube attached to the AV. Sperm motility was determined by dropping an aliquot of semen on a glass slide and viewed under a microscope. Sperm concentration was determined by making the sperm cells immobile using 10% formalin buffer and sperm counting done with the aid of Neubauer chamber haemocytometer. Live sperm cells were assessed by staining with eosin/negrosin dye and counting done under the microscope. The rabbits were slaughtered at the end of semen collection and the testicles harvested and used for histological studies. Data generated were analysed using ANOVA and significant means separated using LSD.

Results and discussion Results of the semen characteristics of the bucks are presented in Table 1. Bucks fed diet containing higher level of *Saccharomyces cerevisiae* (T3 - 0.16 g/kg diet) had $P<0.05$ higher sperm motility, live sperm cells and total sperm count than the T1 (0.00 g/kg diet) and T2 (0.10 g/kg diet). There were $P>0.05$ in sperm concentration between the different treatment groups. The increase in total sperm of T3 is an indication of active state of testes in terms of semen production. The histological studies indicated hyperplasia of germinal epithelium of the testes which resulted to increased number of mature sperm cells observed inT3. This corroborates the higher number of total sperm cells per ejaculate of the T3 group.

Table 1 Semen characteristics of the bucks

Parameters	T2	T2	T3	SEM
Motility (%)	63.33^{b}	63.33^{ab}	78.33^{a}	2.76
Sperm Concentration (x 10^6/ml)	171.67	130.00	253.17	28.00
Live sperm (%)	71.67^{b}	77.50^{b}	86.67^{a}	2.44
Total sperm (%)	119.33^{b}	80.33^{b}	234.70^{a}	27.76

Conclusion Inclusion of *Saccharomyces cerevisiae* at the rate of 0.16 g/kg in the diet of rabbit bucks improved testicular functions and enhanced semen characteristics which are among the attributes of reproductive efficiency. Improvement in semen characteristics and testicular functions implies that more sperm cells will be available to fertilize viable ova produced by the doe leading to increased rabbit production. Rabbit producers are therefore encouraged to include *Saccharomyces cerevisiae* in the diet of rabbit bucks.

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Fatty acid composition of some different forages

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Introduction Green forages represent a high proportion of fatty acids, particularly poly-unsaturated Fatty Acids (PUFA). Recent studies showed dietary PUFA, especially n-3 had positive effects on reproduction and immunity in dairy cows. In developed countries ruminants depending mainly on grazed grass pasture can get adequate amount of PUFA, but in developing countries ruminant animals mostly depend on low quality forages (LQF) such as cereal straws, hay etc, this may be low in unsaturated fatty acid % of total fatty acids content. It is important to know any deficits of various fatty acids in LQF as compared to high quality forages (HQF) so that appropriate strategies for their use in ruminant diets. This study evaluated four different LQF- rice straw (RS), sugarcane bagasse (SB) wheat straw (WS), ryegrass hay (HAY), alongside three HQF- dried ryegrass (RG), ryegrass silage (SIL) and rapeseed plant (RP) for their fatty acids components.

Material and methods Representative samples of RS (Variety, IR50) and SB were collected from, Bangladesh, whereas those of WS (Variety, Einstein), HAY, RG, SIL and RP were collected from the Newcastle University's Farm in the UK. Fatty acid compositions of forages in triplicate were determined by using gas chromatography (GC) of fatty acid methyl ester (FAME) derivatives following the method described by Sukhija and Palmquist (1988) with some modifications. Peaks were identified by using an external standard. The fatty acid results were expressed as percentage of each fatty acid relative to the total fatty acids. General Linear Model of Minitab was used to compare individual fatty acids among the forages. Fatty acids were further analyzed in three different categories based on saturation.

Table 1 Fatty acid composition (% of total fatty acids) of different forages

Fatty Acids	Carbon no.	RS	WS	HAY	SB	SIL	RP	RG
Palmitic	C16:0	35.59	20.08	33.89	18.78	25.43	7.71	13.34
Stearic	C18:0	10.70	7.39	11.78	9.04	5.04	1.47	3.01
Arachidic	C20:0	8.23	6.51	6.66	2.17	1.36	0.56	0.92
Heneicosanoic	C21:0	0.00	0.00	7.08	1.49	0.65	26.91	1.52
Behenic	C22:0	6.74	7.24	5.90	2.07	1.95	0.43	1.46
Others		16.07	14.12	8.40	6.03	4.53	1.24	2.66
Total saturated fatty acids		**77.33**	**55.34**	**73.71**	**39.58**	**38.96**	**38.32**	**22.91**
Oleic	C18:1 Δ^9	7.97	6.49	6.12	16.03	6.33	7.39	1.35
Others		1.55	1.02	0.00	0.49	0.25	3.33	2.41
Total mono unsaturated fatty acids		**9.52**	**7.51**	**6.12**	**16.52**	**6.58**	**10.72**	**3.76**
Linoleic	C18:2 $\Delta^9\Delta^{12}$	3.65	3.42	3.29	37.41	18.5	11.5	10.29
α Linolenic	C18:3 $\Delta^9\Delta^{12}\Delta^{15}$	5.05	17.54	2.72	4.79	34.69	28.42	60.91
Arachidonic	C20:4 $\Delta^5\Delta^8\Delta^{11}\Delta^{14}$	4.45	14.43	8.48	1.51	0.16	1.15	1.00
Others		0.00	1.76	5.69	0.19	1.1	9.89	1.16
Total poly unsaturated fatty acids		**13.15**	**37.15**	**20.18**	**43.9**	**54.45**	**50.96**	**73.36**
		100	100	100	100	100	100	100

Results Individual fatty acid contents of different forages are shown in Table 1. Amongst the two forage groups, the LQF contained more saturated fatty acids (SFA) whereas HQF contained more PUFA. Within LQF, SFA were highest in RS and hay. These two forages contained a large amount of palmitic acid (PA), followed by stearic acid (SA). PA was also higher in wheat straw; however wheat straw also contained large amount of PUFA like α-linolenic (LNA) and arachidonic acid. Among the LQF, sugarcane bagasse contained high amount of linoleic acid (LA) followed by PA. Hay was lower in PUFA content. However, it contained the highest amount of docosahexaenoic acid. Among the PUFA, LNA was most abundant in the HQF. About 61% of fatty acids in RG were represented by LNA. Beside LNA, SIL contained large amount of PA and LA and RP contained large amount of heneicosanoic and LA. RP also contained large amount (9.7%) of an unexpected fatty acid that was not used in the standard. GC analysis of this fatty acid indicated a longer retention time suggesting that this fatty acid had a much longer carbon chain length. The unidentified fatty acid was included in the PUFA. LQF contained more SFA that means during maturation and processing many PUFA were reduced to SFA. PUFA can increase the quality of meat and milk by increasing n-3 PUFA, vaccenic acid and conjugated linoleic acid and may also help improve reproduction and immunity, therefore, PUFA especially LNA containing supplements could help increase the utilization of LQF.

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Fermentation characteristics and chemical evaluation of processed cassava by-products as dry season feed for ruminants

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Introduction A major constraint to small -holder ruminant production is lack of good quality feed throughout the year. Therefore, the use of various agro-industrial by-products is being promoted to augment the quantity and quality of feed available during the dry season. Cassava by-products that is, the peel, foliage or sieviate, have prospects of being used for this purpose. Inclusion of cassava foliage has been reported to increase the protein content and digestibility of cassava based diets for ruminants, however the hydrocyanic acid (HCN) content in cassava products has been a limiting factor to its utilisation (Oduguwa *et al*., 2007). Processing methods like ensiling, pelleting, and sun-drying have been demonstrated to reduce the concentration of HCN in feed. This study was designed to determine the fermentation characteristics and chemical composition of ensiled, pelletized or sundried cassava products (peels, foliage and sieviate) as components of dry season feed for ruminants.

Material and methods Six experimental diets were used as follows: T_1, T_3 and T_5 contained 0g/kg foliage with 600g/kg peels each while T_2, T_4 and T_6 each contained 200g/kg foliage with 400g/kg peels. T_1 and T_2 were ensiled, T_3 and T_4 pelletized while T_5 and T_6 were sun-dried. Other ingredients (g/kg) that made up each of the treatments were as follows: cassava sieviate, 205; molasses, 100; sulphur, 3; corn bran, 80; salt, 7; vit./min. Premix, 5. The ensiling process involved chopping cassava leaves and peels (3-4 cm lengths), loading into air tight polythene bags to facilitate compression and then packing into plastic drums which were also made to be air tight. Ensiling lasted for 45 days. Sun-drying of cassava products was done on concrete slab for seven days while pellets were formed using pelleting machine of 7.5hp. Three representative, homogenous samples were taken from each of the treatments for determination of fermentation characteristics (pH, acetic acid, n-butyric and propionic), HCN, (Poonam and Hahn., 1984), crude protein, ether extract, and ash (AOAC, 2000), ADF and NDF (Van Soest *et al*., 1991). Data generated were subjected to one way ANOVA using completely randomised design. Statistically significant means were seperated using Tukey's multiple comparisons test.

Results HCN content was highest in T_3 (pelletised without foliage) but not higher than T_1 (ensiled without foliage) ($P>0.05$). Improvement in the crude protein content with addition of cassava foliage across the treatments ($P<0.05$) suggested that processing method did not hamper the availability of the nutrient, therefore all the processing methods may be employed in preserving cassava by- products for use in austere periods. Also, the values obtained for NDF and ADF of the silage falls within the range of the fibre fractions recorded by employing other processes. Both cassava peels and foliage T_1 and T_2 can be considered as good silage products with the fermentation characteristics exhibited.

Table Fermentation characteristics and chemical composition (g/kg) of processed cassava by-products

Processing Method	Ensiling		Pelletising		Sun-drying		
Parameters (g/kg	T_1	T_2	T_3	T_4	T_5	T_6	SEM
HCN(g/100kg)	4.96^{a}	4.82^{b}	4.98^{a}	4.78^{c}	4.76^{c}	4.84^{b}	0.038
NDF	508.8^{e}	519.2^{b}	513.3^{d}	514.4^{d}	521.3^{a}	516.6^{c}	0.182
ADF	381.1^{e}	389.7^{ab}	385.1^{c}	382.9^{d}	390.8^{a}	388.8^{b}	0.161
CP	48.6^{c}	77.0^{b}	57.4^{b}	157.2^{a}	68.7^{b}	150.5^{a}	1.956
EE	78.4^{a}	77.9^{ab}	75^{b}	78.1^{a}	78.6^{a}	74.6^{bc}	0.074
Ash	105.9^{b}	132^{a}	96.7^{c}	94.6^{d}	87.3^{e}	112.7^{b}	0.653

^{a,,b,c,d,e}: Means on the same row with different superscript are different ($P<0.05$). Fermentation characteristics (g/kg) for T_1 & T_2 are pH: 3.61; 3.9, acetic acid =390.2; 395.5; n-butyric=104.5; 102.2; Propionic= 412.8; 420.6 in that order.
HCN-Hydrocyanic acid; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; CP-Crude Protein; EE- Ether Extract;

Conclusion The results obtained revealed that preservation methods like ensiling, pelletising, and sun-drying can be used to improve the feeding quality of cassava-based diets because they reduced the HCN contents to levels lower than the recommended maximum safe level (10mg/kg). They also improved the availability of other nutrients contained in cassava peels and foliage for ruminant animals. Inclusion of cassava foliage significantly increased the crude protein in the diets.

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Effects of 3-carene, resorcinol, and *p*-cresol on the metabolism of polyunsaturated fatty acids by rumen microorganisms *in vitro*

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Introduction Ruminant food products (meat and milk) contain high levels of saturated fatty acids (SFA), which are produced as result of biohydrogenation of ingested dietary polyunsaturated fatty acids (PUFA) by rumen microorganisms. High intakes of SFAs are associated with a number of negative effects on human health. Essential oils have been reported to influence several parameters of rumen fermentation such as molar proportions of volatile fatty acids (VFA); methane production and amino acid degradation (Calsamiglia *et al*. 2007 and Hart *et al.* 2008). The objective of this study was to investigate the effects of three essential oil compounds (EOCs), namely, 3-carene, resorcinol and *p*-cresol on the metabolism of PUFA by rumen microorganisms *in vitro*.

Material and methods A 70:30 grass hay (*Lolium perenne)* and concentrate basal feedstock was formulated and milled through a 1 mm screen. The basal substrate was then supplemented with an additional 30 g oil/kg DM (of a 60:40 mixture of fish oil and linseed oil) to give a final lipid content of 50 g/kg DM. The feeds (1 g) were weighed into serum bottles and then 80 ml of buffer plus 20 ml of strained rumen fluid were dispensed to each bottle and incubated (Theodorou *et al.,* 1994). Three, pure EOCs were added to the fermentation bottles to achieve a dosage level of 300 mg of EOC per litre. The four treatments were as follows: control (untreated feedstock), 3-carene, resorcinol and *p*-cresol. Four replicates of each treatment were incubated and gas production was measured at 3, 6, 9, 12, 24 and 48 hours. Another set of extra bottles, treated as above, were incubated alongside and stopped after 3, 6, 12, 24 and 48 hours and their contents analysed for linoleic acid (C18:2*n*-6), linolenic acid (C18:3*n*-3), eicosapentaenoic acid (EPA, C20:5*n*-3), docosahexaenoic acid (DHA, C22:6*n*-3), conjugated linoleic acid (CLA) and trans-vaccenic acid (TVA, C18:1 *trans*-11) content. Treatment effects were analysed using one-way analysis of variance with Genstat statistical software, 11th Edition.

Results The concentrations of PUFA in rumen contents after 48 hour of incubation *in vitro* are shown in Table 1. Compared to the control, concentrations of TVA were significantly reduced by 3-carene and *p*-cresol by 28% and 17% respectively (P<0.001), whilst resorcinol had no effect. 3-Carene decreased CLA concentration in rumen contents by 2.3 times compared to the control (P=0.026), with means of 0.03 and 0.07 g/100g respectively, whilst *p*-cresol and resorcinol had no effect. Relative to the control, both 3-carene and *p*-cresol significantly prevented a decrease in the concentration of C18:2*n*-6 (P<0.001). The mean concentrations of C18:2*n*-6 for 3-carene and *p*-cresol were higher by 200% and 56% respectively, than those on the control. EOCs supplementation also sustained higher concentrations of C18:3*n*-3 relative to the control (P<0.001). 3-Carene supplemented feedstock inhibited linolenic acid biohydogenation by 2.9 fold, whilst *p*-cresol maintained double the amount of C18:3*n*-3 than the control. The effects of the three essential oil compounds on the concentrations of EPA were marginal, with only *p*-cresol inhibiting C20:5*n*-3 disappearance compared to the control (P=0.008). DHA concentration was not affected by any of the three EOCs after 48 hours of incubation.

Table 1 Effects of 3-carene, *p*-cresol and resorcinol on PUFA concentrations (g/100g) after incubation of rumen contents for 48hrs

	Treatments				sed	Significance
	Control	3-Carene	*p*-Cresol	Resorcinol		
C18:1 trans-11	11.21	7.97	9.28	10.69	0.550	P<0.001
CLA	0.07	0.03	0.08	0.08	0.015	P=0.026
C18:2*n*-6	2.23	4.81	3.48	2.48	0.155	P<0.001
C18:3*n*-3	1.87	5.40	3.68	2.14	0.211	P<0.001
C20:5*n*-3	4.03	4.19	4.43	3.75	0.158	P=0.008
C22:6*n*-3	2.52	2.59	2.78	2.65	0.130	NS

Conclusion The results from this study clearly illustrate that both 3-carene and *p*-cresol reduce the biohydrogenation of both C18:3*n*-3 and C18:2*n*-6 by rumen micro-organisms *in vitro*; with 3-carene having the greatest effect. In this study, 3-carene was also demonstrated to inhibit the formation C18:1 *trans*-11 and CLA; the two most important intermediates of rumen biohydrogenation, which is consistent with its observed inhibition of the biohydrogenation of C18:3 *n*-3 and C18:2*n*-6. However, these EOCs do not appear to alter the disappearance of EPA and DHA. Further investigations are required to ascertain whether these effects also occur *in vivo* and translate into increased incorporation of these PUFA into ruminant food products.

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Comparative evaluation of haemagglutination potential of haemolymph from two species of giant African land snails using erythrocytes from cattle, sheep, goat and chicken

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Introduction Snails have long been used by man for food as well as for medicinal purposes. Their products have been used against inflammatory skin diseases, cold and coughs, bronchitis, catarrh and asthma while snail lectin (Helix *pomatia* agglutinin [HPA]) has been applied as a prognostic indicator for some cancers, such as those of the breast, stomach and colon (Dwek *et al*., 2001). Many of the reported medicinal functions are traceable to molluscan immunity. The concept of haemaglutination has been widely used in blood typing ('Serafol' bedside card method), blood donation (cross-matching technique) and disease detection (Brieva *et al.,* 2008). Substances which agglutinate or lyse human and other mammalian erythrocytes occur naturally in the haemolymph and in extracts from varied tissues of molluscs (Michelson and Lorin, 1977). Giant African land snails are rich sources of haemolymph which, we hypothesize, might contain different types of agglutinins that are useful medicinally. These snails are abundant in Nigeria and are mainly slaughtered for the provision of meat, with the haemolymph disposed of as a waste product. This research aims to evaluate the haemagglutination potential of haemolymph from two species of giant African land snails (*Archachatina marginata and Achatina achatina*) with the ultimate aim of harvesting them for economic and medicinal applications.

Material and methods Haemolymph was collected from 72 snails (36 per species) sourced from the Snail Unit of The Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria. The haemolymph was taken from the mantle cavity region and each sample was tested for haemagglutination activity. Five to ten millilitres of cattle, sheep, goat, and chicken blood were obtained aseptically into EDTA bottles and centrifuged at 900g for 5 minutes to harvest erythrocytes. Erythrocytes were washed three times in phosphate buffered saline (PBS), diluted to 2 % v/v and stored at 4°C. Blood from cattle, sheep and goat was taken from the jugular vein, while that of chicken was collected from a wing vein. Serial dilutions of haemolymph were made using 0.85% PBS (pH. 7.2). Diluted haemolymph was aliquoted into microtitre plates (100 µl per well) and equal volumes of 2% cattle, sheep, goat and chicken red-blood cell suspensions were then added. The plates were covered, mixed gently, and incubated for 30, 60, 90, 120 and 150 minutes at room temperature to determine reaction time, after which titre values were recorded. Control wells contained only red blood cell suspensions. Each test consisted of four replicates. Positive controls included pure lectin (*Canavalia ensiformis*) as a source of agglutinin. The titre values recorded are indicators of the strength of agglutination of snail haemolymph in various erythrocytes used. The data were subjected to least-squares analysis. Significant treatment means were separated using Tukey's comparison test (SYSTAT, 1992).

Results Erythrocyte source had a significant (P<0.001) effect on haemagglutination titre (Table 1). Among the four erythrocytes sources used, sheep recorded the highest mean titre, followed by goat and cattle while chicken recorded the lowest titre.

Table 1 Effect of erythrocyte sources on the haemagglutination titre in the haemolymph of giant African land snails

Erythrocyte source	Number of samples (NSP)	Mean Titre (HAU) SEM	
Cattle	330	4.596 ^{c}	
Goat	330	7.175 ^{b}	0.146
Sheep	330	8.306 ^{a}	
Chicken	330	1.727 ^{d}	

^{a,b,c,d}Means in the same column with different superscripts differ significantly (P<0.001) ;SEM: Pooled standard error of mean;
HAU: Haemagglutination Unit/100µl of haemolymph at 150 minutes ; NSP: Total no of observations in micro-titre well used per erythrocyte source

Conclusion Haemaglutination occurred in all four erythrocyte preparations. Sheep erythrocytes had the highest concentration of binding sites for lectin or lectin-like substance present in the snails' haemolymph and suggests that such an agent may have curative ability for inflammatory skin diseases, cold, bronchitis and asthma (in sheep?) as reported in man.

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Effect of different tea-to-water ratios on proximate, fibre and secondary metabolite compositions of spent tea leaves as a potential ruminant feed additive

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Introduction Spent tea leaves (STL), a by-product of the tea industry, are rich in nutrients and bio-active compounds some of which might have potential as additives to manipulate rumen fermentation and methane production in ruminants (Kondo *et al.*, 2007). However, chemical characterization of STL is necessary before testing their potential to modify rumen function and methane by using *in-vitro* and *in-vivo* studies. Hence, this study examined the effect of extracting different amounts of tea leaves in a fixed amount of water on the proximate, fibre and secondary metabolite compositions of green and black STL. The study also tested the hypothesis that a higher tea-to-water ratio would affect the extraction of soluble compounds into water and yield a more nutrient-rich STL.

Material and methods A 3x2 factorial design, in triplicate, was applied to extract 3 different amounts (T1=2.8g, T2=5.6g and T2=11.2 g) of the 2 tea types (green and black) in 300 ml of boiling water for 5 minutes. Both teas were obtained from a tea processing company, located in Bandung, West Java, Indonesia. The black tea was factory graded as 'Broken Orange Pekoe Fanning' and the green tea was of a common grade. Both teas were plucked from *Camellia sinensis* var. Assamica tea plants at the same farm. The AOAC (2005) method was used to determine DM, ash, OM and EE while total N (Nx6.25= CP) was analyzed by Elementar Vario Macro Cube (Elementar, Germany). The NDF content was determined according to van Soest *et al.*, (1991) without sodium sulphite and dekalin. The method of Makkar (2003) was used to determine total phenols (TP), total tannins (TT), condensed tannins (CT) and total saponins (TS) with tannic acid (Fisher scientific, UK), epigallocatechin gallate (Sigma Aldrich, UK) and diosgenin (Molekula Limited, UK) as respective standards. The general linear model on Minitab 16 software was used to examine the statistical effects of tea types and tea-to-water ratios alongside their interaction on the chemical components of the STL from each extraction.

Result Table 1 presents the means for only the main effects of tea types and tea-to-water ratios as these were significant (P<0.05 to P<0.001) but not their interactions (P>0.05). The green STL had significantly higher DM (P<0.01), CP (P<0.001), EE (P<0.001), TP (P<0.001), TT (P<0.001), CT (P<0.01) and TS (P<0.001) but significantly lower OM (P<0.001) and NDF (P<0.001) than Black STL. Increasing tea-to-water ratios from T1 to T3 significantly increased CP (g/kg DM) (from 239.9 to 248.9, P<0.001), TP (from 107.9 to 122.4, P<0.01), TT (from 102.1 to 115.4, P<0.01), CT (from 64.01 to 116.4, P<0.001), and TS (from 46.0 to 65.1, P<0.01) but decreased OM (from 959.0 to 954.6, P<0.01) and NDF (from 439.7 to 412.9, P<0.01) of STL. However, increasing the ratios from T1 to T2 had no significant effect (P>0.05) on most chemical components except OM, CP and CT.

Table 1 The effect of tea types and tea-to-water ratios on mean chemical components of STL (g/kg DM)

	STL		Tea-to-water ratios			SEM with Significances		
	Green	Black	T1	T2	T3	STL	ratios	STLxratios
DM	141.0^{A}	131.1^{B}	130.1^{b}	137.1^{ab}	140.8^{a}	1.72^{**}	2.10^{*}	2.97^{NS}
OM	954.6^{B}	958.6^{A}	959.0^{a}	956.2^{b}	954.6^{b}	0.57^{***}	0.90^{**}	0.99^{NS}
CP	251.7^{A}	239.6^{B}	239.9^{b}	248.1^{a}	248.9^{a}	0.89^{***}	1.09^{***}	1.54^{NS}
EE	22.9^{A}	14.4^{B}	18.3	18.1	19.7	0.56^{***}	0.69^{NS}	0.98^{NS}
NDF	393.9^{B}	461.3^{A}	439.7^{a}	430.1^{a}	412.9^{b}	2.92^{***}	3.57^{**}	5.05^{NS}
TP	130.4^{A}	98.8^{B}	107.9^{b}	113.4^{b}	122.4^{a}	1.76^{***}	2.16^{**}	3.05^{NS}
TT	126.3^{A}	90.2^{B}	102.1^{b}	107.3^{b}	115.4^{a}	1.74^{***}	2.13^{**}	3.01^{NS}
CT	105.4^{A}	77.3^{B}	64.0^{b}	93.8^{a}	116.4^{a}	4.96^{**}	6.07^{***}	8.59^{NS}
TS	70.1^{A}	39.3^{B}	$46.0.^{b}$	53.0^{b}	65.1^{a}	2.13^{***}	2.61^{**}	3.68^{NS}

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

Conclusion Both black and green STL appear to be useful as feed additives in the diets of ruminants, with relatively high secondary metabolite contents especially in green STL. It appears that reducing the amount of water used during extraction yields STL of higher nutritional value which could be used as an additive in ruminant diets to optimise their utilisations and perhaps reduce methane.

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Impact of a mixed chain length omega-3 fatty acid diet on production variables in commercial free-range laying hens

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Introduction Keel fractures in laying hens is a serious welfare issue with up to 70% of birds within free range flocks having keel fractures (Wilkins *et al*., 2004). Their occurrence is likely owed to a combination of factors, including poor calcium retention and housing system. Our group has been researching using ω3-enhanced diets as a means to improve bone strength and reduce fractures following initial experiments indicating short chain ω3-enhanced diets (1.3-ω3:ω6) reduced fractures by 30-50% in commercial settings. Despite this finding, more recent work using a mixture of long and short chain ω3 content (MCD) within a single farm failed to deliver similar benefits to bone health and fracture level while increasing production losses. The current study was undertaken to identify the impact of an MCD ration on flock productivity and mortality across multiple farms and houses rather than localized to the single farm in which our preliminary MCD work was based on. Based on the earlier study using the MCD, we hypothesized that flocks receiving the MCD would have reduced productivity and increased mortality.

Material and methods Production data was obtained from a single, large producer from 12 flocks across 5 separate farms with each farm containing between one and five flocks housed in separate barns where the MCD ration began between 16 and 50 wks and continued for at least 6 wks. The control for each flock fed the MCD (ω3:ω6 = 1.35) was an alternative flock located in the same barn in a different year (± 2 years) and receiving a standard ration (ω3:ω6 = 0.12). The short and long chain contents of the MCD ration were sourced from flaxseed and recovered salmon oil, respectively, while the control ration represented a standard layer diet.
Data collected weekly included: the number of poor quality eggs as recorded at the egg packaging facility, total eggs produced per live hen (hen daily average), mortality, mean egg weight, and daily feed consumption (based on weekly feed consumption). Poor quality eggs were calculated as a percentage of total eggs produced that week and mortality as a percentage of the original flock size. Duration of exposure to the MCD was calculated as the number of weeks from when the flock began the ration. The response variable used for analysis was calculated as the difference between the MCD and control for each week, e.g., response = MCD - control. To account for pre-MCD differences, the difference for the two weeks before the ω3 diet was provided was calculated and averaged to generate a single value that served as a covariate for each particular flock. The generated data set was analyzed using MlwiN (Rasbash *et al.*, 2009), a statistical software package designed for data grouped in hierarchical structures, e.g., repeated measures on a farm over time.

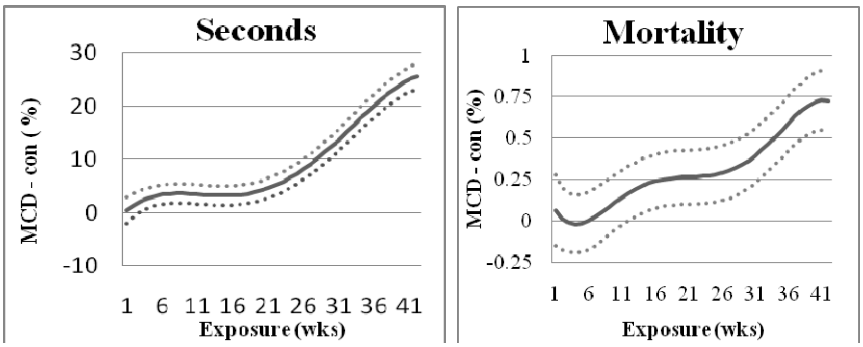

Figure 1ab. Modelled response between seconds and bird mortality against MCD exposure with control.

Results All responses revealed an effect of exposure length to the MCD ($p < 0.05$). As shown in the figures, the immediate period following introduction to the MCD was similar for the two rations based on the responses' inclusion of zero within the confidence interval, e.g., the values between each ration were similar resulting in a difference of zero. However, with increasing duration of exposure the difference between the two rations increased as indicated by the drift of the predicted variable from zero. Specifically, each response became more positive indicating the specific response within MCD ration at a given exposure time to be greater than the control with the exception of HDA where the opposite was seen. For the responses where duration of exposure was found to exhibit a polynomial effect (e.g., HDA, mortality, seconds), following an initial drift between 2-9 wks, the response appeared to stabilize until approximately 20-25 wks, where a drift from zero re-commenced. Farm effects were found for HDA and mean egg weight, while effects of the pre-MCD covariate were found for mean egg weight only. Farm size and age when exposure to the ω3 diet began were ineffective predictors and thus not included in any models ($p > 0.1$).

Conclusion Although fish oil diets may confer a variety of benefits to bird and consumer health, more study is required to insure identify the appropriate ration and duration.

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Quantifying the effect of agricultural co-products inclusion in broiler and layer diets on Global Warming Potential of poultry products

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Introduction Agricultural co-products, including Processed Animal Protein (PAP) from the pig industry and Dried Distillers Grains with Solubles (DDGS) from bioethanol production have been considered as potential alternative feed ingredients in poultry industry. The benefit of these alternative protein sources is that they can partly replace soya, the cultivation of which causes high greenhouse gas emissions, related to land use change. The aim of this study was to apply Life Cycle Assessment (LCA) modelling, "from cradle to gate" to compare the Global Warming Potential (GWP) of broiler meat and eggs with either standard soya-based diets or alternative diets including PAP (broilers only) or DDGS.

Material and methods The calculations were based on typical production and dietary formulation data provided by the broiler and egg industries. The Life Cycle Inventory (LCI) data for PAP and DDGS were derived from WRAP (2011) and Scacchi (2010), while other data and modelling are from Leinonen *et al.* (2012). In the alternative diets, the soya inclusion was reduced and other ingredients were adjusted to maintain the energy and nutrient levels in the diets, most notably essential amino acids (AA) - although non-essential AAs could increase. Two inclusion levels of each co-product were applied: "Realistic" and "Extreme" (Tables 1 and 2). It was assumed that using the alternative diets does not change the animal performance. Economic allocation was used to partition the GWP between co-products (DDGS + bioethanol, PAP + tallow + pig carcass). The relative values of these co-products vary depending on supply and demand. Allowing the use of PAP as poultry feed (currently banned) can be expected to increase its price considerably. So, the results are presented for a range of possible values of the co-products relative to the main product.

Table 1 Inclusion rates (g/kg) of PAP and DDGS in alternative broiler diets

	Starter	Grower	Finisher	Withdrawal
PAP, Realistic	50	50	50	50
PAP, Extreme	75	75	100	100
DDGS, Realistic	50	100	100	100
DDGS, Extreme	100	150	200	200

Table 2 Inclusion rates (g/kg) of DDGS in alternative layer diets

	Starter	Rearer	Developer	Early Lay	Late Lay
DDGS, Realistic	100	150	200	200	200
DDGS, Extreme	150	200	250	300	300

Results The GWP of broiler meat could be reduced by up to 11%, with low relative economic values of PAP (Figure 1). DDGS was only beneficial in the layer diets and only when the economic allocation to DDGS was less than 35% (Figure 1). The negative effect of DDGS in broilers was partly caused by higher N excretion rates causing higher N_2O emissions.

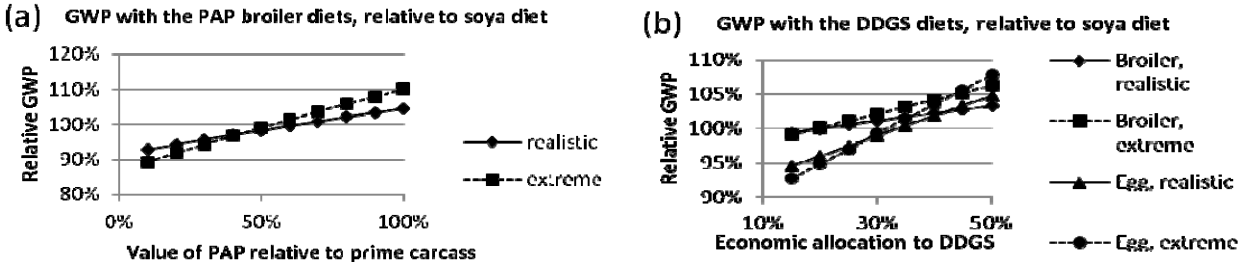

Figure 1 The relative GWP for broiler meat and eggs with different diets and different economic values of PAP (a) and DDGS (b)

Conclusions Using these agricultural co-products in poultry diets has the potential to reduce the total greenhouse gas emissions from poultry production, but only if the relative economic value of these co-products remains low and supplies are adequate. The assumption was made that the alternative ingredients have no effect on bird performance, and quantification of additional environmental criteria is needed to make a holistic judgment on the environmental impacts of these ingredients.

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Effects of feeding distiller's dried grains with soluble in broiler diets on performance and digestibility

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Introduction Distiller's Dried Grains with soluble (DDGS) are increasingly available as a by-product of the beverage and bio-fuel industry worldwide. This raw material is suitable for feeding to many animal species, and offers high levels of crude protein and certain minerals. The increased demand for ethanol therefore, results in mass production of DDGS available for use in livestock feed. Thus, the utilization of these materials can help in solving problems of global importance such as resource recovery, waste utilization, and better environment management (UNDP, 2002). Incorporation of DDGS at higher levels may provide an additional outlet for the increasing amounts available (Noll *et al.,* 2001).One of the concerns about using relatively high levels of this product is the possible effects on performance that might occur (Wang *et al.,* 2007).Therefore, this study aimed to determine the optimum inclusion level of DDGS in broiler diets as well as the effects of feeding DDGS on performance and digestibility.

Material and methods One hundred and fourty four (144) day old Ross broiler chicks (mixed sex) raised together on floor were used for the experiment, which last for 6 weeks. The chicks were individually weighed and assigned to 4 groups (treatments) of 36 chicks each. Each group was further divided into 4 sub-groups (replicates) of 9 birds per replicate. Birds of each replicate were housed in floor pens with the floor covered with wood shavings as litter material. Each pen was equipped with a plastic chick feeder and plastic drinker. The birds were vaccinated at day old against Infectious Bronchitis. Four (4) isonitrogenous with varying metabolizable energy and fibre contents were formulated (one phase) for the study. Diet A, which was the control, contained 0.00 % DDGS while diets B, C, and D contained 10, 20 and 40% respectively. All the diets were analysed for proximate composition according to AOAC, 2005.The wheat DDGs was obtained from a reliable local feed industry. The diets and clean drinking water were provided *ad libitum* throughout the study. Feed intake, weight gain, feed conversion ratio, water intake and dry matter digestibility were measured. Water intake was measured for four days (day 38-42). At the end of the feeding trial, two (2) birds per replicate were randomly selected and transferred to metabolic cages for faeces collection and determination of apparent nutrient digestibility. The digestibility trail lasts 4 days. Polythene sheet were spread underneath the cages for the faeces collection. Feathers were hand- picked and discarded from the faeces before weighing. The total collection for 4 days was dried in a forced air circulation oven at 60^0c.The 4 day samples were ground and then analyzed for dry matter, crude protein, crude, fibre ether extract and total ash according to the method of AOAC (2005). All the data were subject to one way ANOVA analysis using Minitab 16 statistical package. Differences among treatments were tested by using Tukey's test.

Results Significant differences were observed among the treatment groups in feed intake, weight gain, water intake and dry matter digestibility (Table 1).Birds fed a 10% DDGS had significantly ($P < 0.05$) higher body weight gain and higher feed intake than did birds fed control diet and other treatment groups. Significant differences were also observed in water intake where treatment A presented the highest water consumption. Meanwhile, dry matter digestibility was moderate among all treatments with diet A having the highest dry matter digestibility. However, there were no significant differences between treatments in feed conversion ratio.

Table 1 Overall Performance of Broiler Chickens fed varying levels of DDGs (1-42days)

	Treatments				
Parameters	A	B	C	D	SEM
Feed Intake(g/b/d)	85.3^{b}	93.4^{a}	82.4^{b}	34.7^{c}	1.39
Weight Gain(g/b/d	43.0^{ab}	48.6^{a}	40.5^{b}	16.2^{c}	1.44
Feed Conversion Ratio	2.0^{a}	1.9^{a}	2.0^{a}	2.1^{a}	0.07
Water Intake (ml/b/d) *	371.9^{a}	346.7^{a}	322.2^{ab}	258.8^{b}	17.91
Dry Matter Digestibility (%)	69.1^{a}	65.1^{ab}	62.0^{bc}	56.0^{c}	1.46

abc Means bearing different letter superscripts within rows are significantly different ($P<0.05$).
SEM=Standard Error of means.*=Water Intake was measured for 4 days

Conclusions The results of the study showed that inclusion of 10% DDGS supports higher feed intake and body weight gain of broilers, but higher inclusion levels adversely affect performance. These increased levels of DDGS in broiler chickens therefore merit further investigation, especially in relation to treatments which might further improve the nutrient availability to the chickens.

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Dietary supplementation of organic broilers with *Melissa officinalis* L.: effect on meat quality

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Introduction Colour deterioration and lipid oxidation are major factors limiting the shelf life and acceptability of meat. Lately, there has been increasing interest in the effect of phytogenic feed ingredients on animal health, productivity and product quality. Compounds from herbs and spices contain many phytochemicals which are potential sources of natural antioxidants (Windisch *et al.,* 2008). The objectives of this study were to determine the effects of *Melissa officinalis* L. on the sensory characteristics and the fatty acid composition of chicken meat from organically produced broilers.

Material and methods Day old, mixed sex, Ross 308 chicks were fed on a standard commercial diet containing either 0, 2.5, 5 or 10 g/kg feed ground *M. officinalis* denoted as CON, MEL 2.5, MEL 5 and MEL 10 respectively. There were 4 replicates per treatment. Birds were fed the diets for 84 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 and colour changes during the storage period were calculated as colour difference (ΔE*) (Petracci and Baéza, 2011). The oxidative stability was determined as thiobarbituric acid reacting substances (TBARS) on storage days 2 and 5. Fatty acid composition in frozen stored slaughter samples of the same muscles was also determined. Analysis of variance (SPSS version 13.0, 2004) was used to analyse differences between treatments and within muscle type. Linear and quadratic contrasts were performed to determine the response of the supplementation level on lipid oxidation.

Results Inclusion of ground *M. officinalis* in the diet resulted in paler meat colour in the thigh muscle from treatments MEL 5 and MEL 10. The magnitude of colour changes (ΔE*) was more pronounced in the same treatments but only in the breast muscle. Significantly lower TBARS were observed in treatments MEL 5 and MEL 10 in both muscles. Results regarding colour attributes and lipid oxidation were consistent between days 2 and 5; for this reason results are not reported. Feeding increasing amounts of *M. officinalis* had a linear (P<0.001) and a quadratic effect (P<0.01) on lipid oxidation with the lowest TBARS values at 5g/kg feed. The response was constant at supplementation level of 10g/kg feed indicating that tissue saturation with the antioxidant components of *M. officinalis* was reached at the level of 5g/kg feed. Regarding fatty acid composition, there was no effect of *M. officinalis* in the levels of saturated, monounsaturated and polyunsaturated fatty acids and in the levels of the nutritionally important eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. The *n-6:n-3* ratio in all treatments and in both muscles was above the recommended value (<4) according to the nutritional guidelines but within the reported range for poultry meat.

Table 1 Shelf life parameters and fatty acid composition of breast and thigh muscle (n=6)

	Breast						Thigh					
Shelf life parameters	CON	Mel 2.5	Mel 5	Mel 10	s.e.d.	P	CON	Mel 2.5	Mel 5	Mel 10	s.e.d.	P
Redness (a*) Day 5	12.85	11.87	11.85	12.08	0.743	ns	15.78	19.09	13.76	13.55	1.089	***
ΔE* Day 5[1]	1.69	0.89	3.83	3.81	0.814	**	3.24	6.60	4.07	5.29	2.298	ns
TBARS Day 5[2]	0.372	0.343	0.275	0.301	0.1098	***	0.399	0.375	0.291	0.325	0.0135	***
Fatty acid composition (% of total fatty acids)												
∑ SFA	32.27	31.85	32.13	31.52	0.079	ns	32.31	30.16	30.61	30.08	0.797	*
∑ MUFA	28.78	27.61	29.22	31.13	0.957	ns	30.50	30.06	33.20	30.78	2.543	ns
∑ PUFA	30.54	32.13	31.40	30.59	2.046	ns	30.72	34.55	31.16	34.05	2.393	ns
C20:5 (*n-3*) EPA	0.91	1.09	1.06	0.76	0.205	ns	0.68	0.62	0.54	0.66	0.109	ns
C20:6 (*n-3*) DHA	0.81	0.95	0.80	0.74	0.207	ns	0.53	0.40	0.32	0.44	0.119	ns
n-6:n-3	4.72	6.11	6.80	6.99	0.959	ns	7.12	7.61	9.63	9.70	0.805	**

[1]$\Delta E^* = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 - (b_1^* - b_2^*)^2]^{0.5}$ where L_1^*, a_1^* and b_1^* measurements at Day 1 and L_2^*, a_2^* and b_2^* measurements at Day 5; [2]mg malonaldehyde/kg muscle; SFA saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; ns not significant;* P<0.05; ** P<0.01; ***P<0.001

Conclusions Inclusion of ground *M. officinalis* in the diet at the level of 5 g/kg of feed was most effective in limiting lipid oxidation. Supplementation at higher levels may exert a prooxidant effect. In practical terms, lipid oxidation was low in all treatments, not affecting the nutritional and health value of the meat. Sensory evaluation of meat colour is required to examine whether consumers will detect the colour differences. A new line of research would be the investigation of the effect of *M. officinalis* on broiler meat quality characteristics, on diets with different feedstuff composition, considering the various alternative feedstuffs and different feed mixes usually used in organic broiler production.

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Greenhouse gas (GHG) lifecycle assessment of broiler production and the effect of replacing soya bean meal with processed animal protein (PAP)

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Introduction It is estimated that within Europe livestock production contributes 0.1 to total GHG emissions, with the dairy, beef and pig sectors being responsible for 0.29, 0.29 and 0.25 of livestock GHG emissions respectively. Poultry meat production contributes 0.08. In the UK poultry meat, derived from broiler chickens, represents 0.38 of total meat production. However, compared with other livestock sectors, there have been comparatively few studies to quantity GHG emissions. Since the EU ban on meat and bone meal in animal diets, the broiler industry has been heavily reliant on imported protein sources, such as soya-bean meal, that carry a high environmental burden. Processed animal protein (PAP) derived from the rendering of category 3 animal by-products has a similar protein content to soya-bean meal, but a significantly lower environmental burden (Ramirez *et al.* 2012). Following a recent risk assessment, the EU is currently re-considering the use of PAP in non-ruminant diets. The objectives of the study were to quantify GHG emissions from broiler production and to assess the environmental impact of replacing soya-bean meal with PAP in broiler diets.

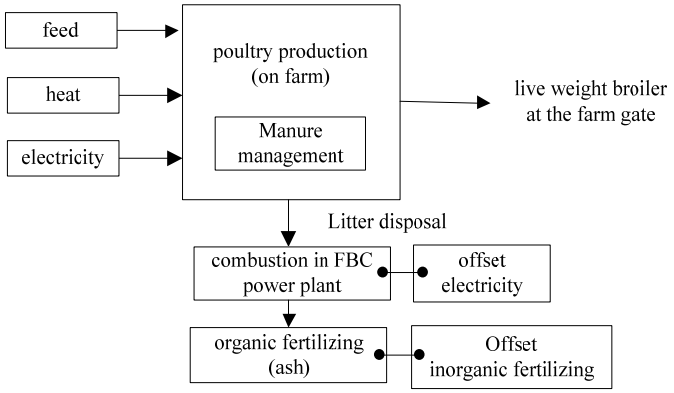

Figure 1 System boundaries for the broiler production system

Material and methods Two product systems (S1 and S2) and two functional units were defined. Product system SI represented broiler feed production and S2 represented broiler production (Figure 1). The functional units for S1 and S2 were one tonne of broiler feed and one tonne of broiler live-weight. Primary and secondary data sources were used to calculate GHG emissions. For SI, primary data on feed formulations (Table 1), raw material inputs, feed outputs, and fuel and electricity use associated with milling and mixing was obtained from a commercial feed mill for 2010 producing 275,300 tonnes broiler feed. For S2, primary data on animal performance, feed, gas, electricity, bedding, water, chemical use, and waste management (wash water and litter) was collected from two commercial broiler farms for 2010 producing 3,872 tonnes broiler live-weight. Wash water was applied to land as a fertiliser and litter was sent in a fluidised bed combustion (FBC) power plant. Secondary data sources for both product systems included published literature and inventory databases. In system S1, economic allocation was used in cases of co-production of raw materials, whereas in system S2, system expansion was used to avoid the use of inorganic fertiliser and British electricity. Simapro® was used for system modelling and calculation of results. Climate change was assessed using the Greenhouse Gas protocol 1.00 impact assessment methods.

Table 1 Aggregated diet formulation used in broiler feed production

Raw material	kg/tonne
Wheat	610
Soya bean meal	215
Field beans	73
Rapeseed meal	59
Soya oil	36
Limestone	5
Monosodium phosphate	1
Fishmeal	1

Results GHG emissions associated with broiler feed production were 923 kg carbon dioxide equivalents (CO_2e)/tonne with soya bean meal and wheat being responsible for 0.30 and 0.27 of total emissions respectively. GHG emissions associated with broiler production were 1798 kg CO_2/tonne live-weight with broiler feed being responsible for 0.79 of total emissions. Assuming that GHG emission for soya bean and PAP are 1.29 kg CO_2e/kg (including land use change, Ecoinvent database) and 0.15 kg CO_2e/kg (Ramirez *et al.* 2012) it is estimated that the direct replacement of soya-bean meal with PAP would reduce GHG emissions associated with broiler feed production by 0.26 and broiler production by 0.23.

Conclusion Assuming 835 million broiler chickens are slaughtered (FAO, 2011), GHG emissions from UK broiler production can be estimated to be 3.5 million tonnes/annum. Replacement of soya-bean meal with PAP could potentially reduce GHG emission from broiler production by 805,000 tonnes/annum.

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Effect of feeding distiller's dried grains with soluble on carcass characteristics and digestive organs of broiler chickens

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Introduction Distillers dried grains with soluble (DDGS) are a by-product of the beverage and bio-ethanol industries with the majority now being derived from ethanol production for fuel. Over the past few years DDGS have became an increasingly available feed ingredient for monogastric rations. The increase in ethanol production results in an excess of DDGS that provide producers a less expensive and a better quality DDGS with a valuable nutritional composition (Shalash *et al.,*2009).Although their nutritive value is quite good for poultry, the high fibre content and presence of some anti nutrients (e.g. phytate) present limitations to optimal use. A high fibre diet affects the size and weight of digestive organs (Sundu *et al.,* 2008) and thus affects the carcass characteristics. Therefore this study was conducted to evaluate the possible effects of varying levels of DDGS on carcass and digestive organs weight of broiler chickens.

Material and methods A total of one hundred and fourty-four- day old male Ross broiler chicks were raised as experimental animals in a completely randomized design. The birds were kept for 6 weeks in a controlled light and temperature room, in floor pens covered with wood shavings as a litter material. The chicks were individually weighed, tagged and assigned to 4 treatments each with four replicate pens of 9 chicks of similar initial live –weight (42.33 ± 3.18). Each pen was equipped with a plastic chick feeder and plastic drinker. Four isonitrogenous diets with varying inclusion levels of DDGS were formulated for the study. Diet A, which was the control, contained 0- % DDGS while diets B, C, and D contained 10, 20 and 40% respectively. The diets and clean drinking water were provided *ad libitum* throughout the study .On day 43; two representative birds from each replicate were randomly taken and processed for determination of carcass and digestive organs characteristics. Digestive organs were measured both with and without digesta and expressed as a percentage of carcass weight. Data were analysed by analysis of variance using Minitab version 16 software. Differences among treatments were tested by using Tukey's test.

Results There were significant differences between dietary treatments ($P < 0.05$) in the percentage weight of eviscerated carcass, cold carcass weight, and percentage weights of the gastro-intestinal tract (GIT), proventriculus, gizzard (both full and empty), thighs, and breast+bone. Results also showed that breast and bone weight decreased linearly with increasing levels of DDGS. Using 20% DDGS did not affect any parameters when compared to the control diet and in most cases where a significant difference was found this related only to the 40% inclusion diet. There were no significant ($P > 0.05$) differences among treatments in the remaining parameters measured, including the major organs.

Table 1 Effect of Feeding varying levels of DDGS on carcass Characteristics and Digestive organs of Broilers (% Carcass weight)

	Treatments				
Parameters	A	B	C	D	SEM
Eviscerated wt (%)	86.6^{a}	83.9^{a}	83.4^{a}	72.8^{b}	1.95
Cold Carcass wt(g)	1368^{a}	1611^{a}	1463^{a}	675^{b}	122
Dressed wt (%)	74^{a}	75.51^{a}	72.5^{a}	70.4^{a}	1.79
GIT (%)	7.51^{b}	6.67^{b}	8.23^{b}	13.53^{a}	1.22
Proventriculus (%)	0.58^{b}	0.57^{b}	0.68^{b}	0.92^{a}	0.05
Empty Gizzard (%)	1.66^{bc}	1.63^{c}	2.36^{ab}	2.85^{a}	0.17
Empty Crop (%)	1.02^{a}	0.66^{a}	0.85^{a}	1.01^{a}	0.12
Liver (%)	3.09^{a}	3.20^{a}	3.28^{a}	4.04^{a}	0.24
Heart (%)	0.76^{a}	0.86^{a}	0.86^{a}	1.02^{a}	0.08
Thighs (%)	13.84^{ab}	13.73^{ab}	14.02^{a}	12.48^{b}	0.33
Gizzard (%)	2.52^{b}	2.46^{b}	3.45^{b}	4.79^{a}	0.31
Drumstick (%)	12.55^{a}	12.70^{a}	13.01^{a}	11.99^{a}	0.49
Breast + Bone (%)	32.54^{a}	29.80^{a}	27.14^{a}	18.74^{b}	1.91
Wings (%)	10.03^{a}	9.96^{a}	9.65^{a}	8.66^{a}	0.41
Neck + Back (%)	17.49^{a}	17.28^{a}	19.45^{a}	20.58^{a}	0.89

abc Means bearing different superscripts within rows are significantly different ($P < 0.05$), *SEM=Standard Error of Means*

Conclusions It appears that inclusion of up to 20% wheat DDGS can favourably be incorporated in broiler diets without any detrimental effects in carcass characteristics.

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The effect of different freezing methods on the concentration of insulin-like growth factor-I and immunoglobulin G in equine colostrum

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Introduction Within the equine industry a foal that is at high risk of failure of passive transfer of immunoglobulins is often supplemented with frozen colostrum (Drogoul *et al.,* 2008; Vivrette, 2001). Whilst colostrum is never supplemented on the basis of IGF-I deficiency, due to the advantages of IGF-I to the neonate an interest should be taken into how this growth factor is affected by the preservation of colostrum in the hope that improvements in preservation methods can be made. Therefore this study aimed to investigate the effect of different freezing methods on the concentration of IGF-I and IgG in equine colostrum.

Material and methods Colostrum samples were collected from 2 thoroughbred and 4 warmblood mares aged between 5 and 16 years old, by hand milking immediately *post partum* prior to the foal suckling as per Csapó-Kiss *et al.,* (1995). Colostral IgG was evaluated immediately using a Bellingham and Stanley Eclipse hand held refractometer. Each sample was divided into 24x1.5ml Eppendorf tubes. All samples were maintained for approximately 15 minutes room temperature until chilling or freezing. One sample group was chilled at +4°C for a minimum of 12 hours before being transferred to a domestic freezer and kept at -18°C until analysis. The second group was placed directly into the freezer at -18°C. The third group was submerged in liquid nitrogen (-196°C) and once frozen solid it was transferred to the domestic freezer (-18°C). The fourth group was suspended in liquid nitrogen vapour at -26°C until the sample had frozen solid and then transferred to the domestic freezer (-18°C). Two tubes from each freezing method were thawed in a water bath at 38°C (Argüello *et al.,* 2002) before IgG concentrations were evaluated using the refractometer. The samples were packaged into a Minitübe transport box (Minitübe, Bayern, Germany) to prevent them from being subjected to temperature variations and transported to Beaufort Cottage Laboratories; Rossdale & Partners Veterinary Surgeons, Newmarket where IgG concentrations were analysed by electrophoresis within 2 hrs. A further two tubes from each group were thawed at +38°C and centrifuged using a Fisher Scientific Microcentrifuge at 5800g for 60 minutes to extract the colostrum serum in which the IGF-I concentrations were evaluated using a human IGF-I ELISA E20 suitable for serum, plasma and other body fluids (Mediagnost, Germany).

Statistical analysis A Paired Samples t test was used to assess if there was any difference in pre freezing refractometer IgG concentrations compared to post freezing IgG concentrations. A Pearson correlation coefficient was used to compare post freezing refractometer and electrophoresis IgG concentrations. A Univariate Analysis Of Variance (2-way ANOVA) was used to discover if there was any difference in colostral IgG or IGF-I between the four freezing methods and the difference between mares. A Bonferroni post hoc was used to evaluate where any differences lay. Finally, a Pearson coefficient was carried out to assess any relationship between post thaw refractometer and electrophoresis IgG concentrations to IGF-I concentrations.

Results There was a very highly significant correlation between refractometer and electrophoresis IgG concentrations ($r=0.929$, $P<0.001$) supporting previous research showing that refractometer measurements allow an accurate estimation of IgG. The paired t test analysis of the refractometer IgG showed no significant difference between the sample prior to freezing and those chilled +4°C and then frozen. The same analysis showed that freezing colostrum at -18°C, -26°C and -196°C does significantly affect colostral IgG concentrations ($P<0.05$). The electrophoresis analysis revealed that there are significant differences between the IgG frozen by each method, and the Bonferroni test showed this difference to lay between the samples frozen by -26°C and those chilled at +4°C prior to freezing. Different freezing methods do not significantly affect the concentrations of IGF-I. The mare had a very highly significant effect ($P<0.001$) on both IgG and IGF-I concentrations. The Pearson coefficient revealed that IGF-I concentrations were significantly correlated ($P<0.05$) to refractometer IgG, but not to electrophoresis IgG concentrations.

Conclusions Refractometer results indicate that chilling colostrum prior to freezing improves recovery of IgG; however discrepancies between refractometer and electrophoresis data do not allow an absolute conclusion to be drawn. Freezing method does not affect post freezing IgG or IGF-I concentrations. There is a possible correlation between IgG and IGF-I concentrations that needs to be further investigated. IgG concentrations measured by refractometry may allow estimation of IGF-I concentrations.

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Heritability of musculoskeletal conditions in a population of Thoroughbred racehorses at the Hong Kong Jockey Club

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Introduction Musculoskeletal disorders are common in racing Thoroughbreds (Parkin, 2008). Many cause a significant loss of revenue to the racing industry, and all are detrimental to equine welfare. Many studies have attempted to quantify the relative importance of certain environmental risk factors for various types of musculoskeletal disorder in equidae, but few have focused on the genetic risk conferred through the pedigree (Jonsson *et al*., 2011; Oki *et al*., 2008). Estimation of heritability is a vital stage in disease investigation, and enables the best use of available resources to maximise impact on the prevalence of deleterious traits. The aim of the present study was to estimate the heritability of important musculoskeletal conditions affecting racing Thoroughbreds in Hong Kong.

Material and methods The data used in this study were provided by the Hong Kong Jockey Club (HKJC). All Thoroughbreds racing in Hong Kong are housed together at Sha Tin Racecourse in the New Territories and managed as a unit. The HKJC employs a team of full-time veterinarians who are the sole clinicians responsible for the clinical care of all horses in Hong Kong. Retirement for medical conditions on the advice of a veterinarian can occur at any time. The HKJC retains detailed horse health information in several ways including records of Official Veterinary Examinations (OVEs) and the reason(s) for retirement of racehorses. For this study OVE records collected between 1995 and 2010 (n=8690) and retirement records collected between 1992 and 2010 (n=5520) were used. Records also contained the identity of the sire and dam, the stakes won over the racing career (in Hong Kong Dollars, HKD), the country of origin, and the date of birth and retirement of each horse. Content analysis software (WordStat, Provalis Research) was used to convert free text health records into a binary numerical profile per horse which described the presence or absence of certain user-defined disease categories. Binary data were analysed using univariate generalized linear mixed sire models with a logit link, constructed in ASREML v3 (VSN International). Wald F statistics, Akaike Information Criteria and likelihood ratio tests were used in a forward selection approach to select significant fixed and random effects for inclusion. Heritability calculations using the binary scale most often produce lower estimates compared with those made on the assumed underlying continuous liability scale, due to a loss of precision when using binary data compared with more graduated scales (Gianola, 1980). Heritability estimates on the observed binary scale were therefore subsequently converted to estimates on the underlying continuous liability scale as per Dempster and Lerner (1950).

Results Inclusion of sire in the final linear model for each reported condition statistically significantly improved the fit of the model (p-value <0.05), suggesting a genetic component underlying susceptibility. The estimated heritability of musculoskeletal injuries was moderate, ranging from 0.08 (tendon injury) to 0.20 (ligament injury). Heritability estimates for fracture and osteoarthritis were 0.09 to 0.1, respectively. A large significant positive genetic correlation was found between fracture and osteoarthritis (0.87, s.e. 0.17). No other positive genetic correlations were identified. A large significant negative genetic correlation was found between osteoarthritis and suspensory ligament injury (-0.53, s.e. 0.35).

Conclusions These results indicate that the incidence of many important musculoskeletal conditions affecting racing Thoroughbreds could possibly be reduced using targeted breeding strategies. Successful breeding against fracture is likely to produce a reduction in cases of osteoarthritis concurrently. Although environmental factors undoubtedly play an important role in the aetiology of musculoskeletal conditions, for completeness, no study of disease should neglect investigation of the potential involvement of genetic susceptibility.

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Phenotypic analyses support investigations of phylogeny in the Skyrian pony and other breeds

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Introduction The Skyrian Pony is unrelated to other horse breeds, as indicated by genetic analyses (Bömcke *et al*, 2011). Proposals that the Skyrian shares the Exmoor pony`s origins from the primitive pony (referred to as Pony Type 1), have been based upon a small number of physical character similarities between the two breeds. However, other phenotypic features of the Skyrian are reminiscent of the Caspian horse, thought to be descended from Horse Type 4. To test the hypothesis that the Skyrian shares the Exmoor`s origin in Pony Type 1, comparisons were made of defined phenotypic characters amongst Skyrians, Exmoors and Caspians.

Material and methods 14 horses of each breed, Skyrian, Caspian and Exmoor (n=42) were selected for a cross-sectional study. Morphometric assessments were made of 18 physical characters including shape of ear, eye, neck, limb, body, cephalic profile, tail placement, parietal crest and ratios of certain bone measurements. A matrix of resemblances was composed and used to calculate the Mean Character Difference (MCD) between each pair of the 42 horses according to the equation MCD = number of unmatched characters divided by sequence length. Average MCDs and standard deviations (SD) were calculated between pairs of Skyrians, Exmoors and Caspians; between Skyrians and Exmoors, Skyrians and Caspians and Caspians and Exmoors. The average MCD values between breeds were used to carry out multiple alignment and construct a phylogenetic tree using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). 15 morphometric measures were taken (Figure 1): Height to withers (HW), croup, elbow (HE), length of body (L) and neck (NL), heart girth (HG), circumferences of knee, cannon and neck (NC), foot diameter, ratios L:HE, NL:NC, HG:HW, L:HW (Khatouf *et al*., 2006).

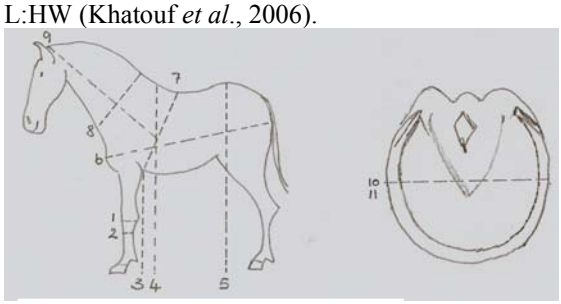


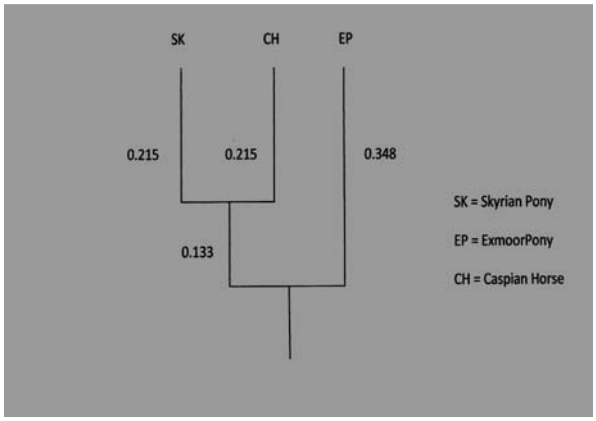
Figure 1 Measurements made

Friedman`s analysis of variance (ANOVA) and, *post hoc,* the Mann-Whitney test were applied to the 10 physical characters yielding ordinal data : cephalic profile, parietal crest, eye shape, ear size, neck width, shoulder angle, height of withers, body shape, limb shape, and tail placement. Morphometric measurements and ratios were analysed by two way analysis of variance (ANOVA). Results were explored further using the *post hoc test,* Fisher`s Least Significant Difference of Means (LSD).

Results The MCD±SD were 0.56 ±0.12 between Skyrians and Exmoors, 0.43±0.15 between Skyrians and Caspians and 0.83± 0.06 between Caspians and Exmoors

The phylogenetic tree constructed using average MCD results (Figure 2) shows that the Skyrian is more closely related to the Caspian than the Exmoor, yet all three are distinct breeds. No differences (P=1.00) were found between Caspians and Skyrians in eye shape, but both differed (P<0.001) from the Exmoor with its prominent “toad” eye. Skyrians shared the Caspians` slender neck, limbs, body and sloping shoulder and there were differences (P<0.001) between them and the Exmoors` stocky proportions (photographic details will be supplied at the presentation).

Figure 2 Phylogenetic tree constructed using average MCD results



Most of the morphometric measurements and the ratio L:HW differed (P<0.001) amongst all breeds. The ratios of L:HE, NL:NC and HG:HW were similar in the Skyrians and Caspians, differing (P<0.001) from the Exmoors, shown in the overall square shape of the Caspian and Skyrian and the rectangular shape of the Exmoor (photographic evidence will be supplied at the presentation).

Conclusions Average MCDs approximate reports between other horse breeds and breeds in other species (Jordana *et al*., 1995), confirming that the Skyrian is a distinct breed. Overall Skyrians appeared phenotypically closer to the Caspian, but unrelated to either breed. To clarify these findings, the study should be repeated with greater numbers of matched horses and with concurrent analyses using single nucletotide polymorphisms (Bömcke *et al*, 2011).

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UK Sport horse market requirements

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Introduction The old adage 'Horses for Courses' is well grounded in its counsel. Not all horses suit all purposes. Horses vary in height, colour, sex, jumping ability, health status and so they vary in their utility and suitability for the various sport horse markets. Understanding what combination of attributes is important within the market place is the key to understanding the utility function for sport horse purchasers and ultimately their willingness to purchase. Previous findings of Hennessy and Quinn (2006) reported a limited influence of catalogue recorded attributes such as height, colour, sex etc, which was subsequently endorsed by the findings of Hennessy (2010) within the Irish sport horse market. It was felt that non-catalogue recorded variables such as soundness, conformation, temperament etc. may be more influential. The objective of this study was to identify the most important attributes that a horse should have from a UK sport horse dealer's perspective.

Material and methods An online survey was conducted (using Surveymonkey) on UK based sport horse dealers. A total of 140 responses were received. A comprehensive list of attributes were considered for inclusion, bearing in mind the findings of Hennessy and Quinn (2006) and Hennessy (2010). Fourteen non-catalogue recorded attributes were examined. An attitudinal scale was used with 'Likert type ratings', each attribute within the list was treated as a separate value with no attribute summation. SPSS version 15 was used for data input and analysis. The attributes were paired and significant differences within the pairing occurred at $p < 0.05$ using the Wilcoxon Signed Rank Test. The attributes were then ranked on level of importance depending on their significant differences from the other attributes (from 1 most important to 5 least important), see Table 1.

Results

Soundness emerged as significantly more important than all other attributes. Temperament and trainability were second in order of importance before those of rideability, conformation and movement. Performance related attributes were of lesser importance.

Table 1 Attribute importance

	Med	Sou	Conf	Move	Pres	Temp	Train	Ride	JTec	Scope	TalJ	TalD	Speed	Stam	Cour	NB
Sou	5	xx	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1
Conf	4		xx	ns	0.00	0.00	0.008	ns	0.002	0.002	0.001	0.00	0.00	0.00	0.013	3
Move	4.5			xx	0.00	0.00	0.002	ns	0.003	0.004	0.002	0.00	0.00	0.00	0.029	3
Pres	4				xx	0.00	0.00	0.00	ns	ns	ns	ns	0.00	0.00	ns	4
Temp	5					xx	0.174	0.002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2
Train	5						xx	0.046	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2
Ride	5							xx	0.00	0.00	0.00	0.00	0.00	0.00	0.001	3
JTec	4								xx	ns	ns	ns	0.00	0.00	ns	4
Scope	4									xx	ns	ns	0.00	0.00	ns	4
Tal J	4										xx	ns	0.00	0.00	ns	4
Tal D	4											xx	0.00	0.00	ns	4
Speed	3												xx	0.00	0.00	5
Stam	3													xx	0.00	5
Cour	4														xx	4

Table abbreviations: sou = soundness, conf = conformation, move = movement, temp = temperament, train = trainability, ride = rideability, JTec = jumping technique, TalJ = talent for jumping, TalD = talent for dressage, NB = level of importance.

Conclusion It is not surprising that soundness was identified as the most important attribute given that the horse's utility and its ultimate career longevity is dependent on its soundness. 'The day you buy is the day you sell' is very apt in these dealer markets. Temperament and trainability (a measure of the horse's willingness and ability to learn) were rated at the second highest level of importance. The horse's ability to cope with the demands of its environment, impact on its health and utility. These findings show some similarity to those of Heydemann and Grobois (2006). Within the study, discipline specific talent attributes were rated lower in significance than soundness, temperament and conformation attributes. It would seem that a decision to buy is based on an assessment of the fundamental health, temperament and conformation attributes and that performance attributes are of secondary importance.

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International brand representation of competition sport horses in the UK

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Introduction "The UK equine industry has not applied or developed the methods of selective breeding and breed improvement schemes implemented by its European neighbours. This has resulted in the UK consistently failing to breed sport horses of international class", Whitaker and Hill (2005, p. 43). Only one of Great Britain's three WBFSH registered studbooks has achieved a top 10 WBFSH ranking for eventing on several occasions but none for show jumping. This is despite British equestrian teams having traditionally been one of the more successful nations in international eventing and show jumping competition, indicating that team GBs success may be built on imported horses rather than homebred horses. The objective of this study was to conduct an analysis of the brand profile of horses registered with the British Show Jumping Association (BSJA) and British Eventing (BE) organisations and also the main brands represented within the yards of UK based dealers (indicating market trends).

Material and methods Two sources of data were used to assess brand representation within the UK competition market place. The first involved data from both the BSJA and BE, on the breed and / or country of origin of horses registered with their respective organizations. The second data source involved a survey of horses owned by UK based sport horse dealers. An Irish based study by Hennessy and Quinn, (2005) reported that 20% of the purchasers bought 51% of the horses at sport horse auction. Hence the dealings and actions of sport horse dealers are very influential within the market place. An on-line questionnaire (using Surveymonkey) was used to survey the UK sport horse dealers. While a highly influential group, the dealer population in the sport horse industry is a minority grouping and hence a total of 140 responses was considered to be sufficient. This sample was tested for representation by comparing results with two previous studies: (1) regional representation of respondents compared well to the findings of Moore-Colyer (2004) and (2) average prices paid by origin of horse compared very well with those of Crossman (2006).

Results The profile of horses owned by the dealers in this study showed very strong similarities with the profile of horses registered with the British Show Jumping Association (BSJA) and British Eventing (BE) respectively. Both sets of results are shown in Table 2. Differences in the nature of data recorded by the respective organizations resulted in differing comparisons (breed / country of origin).

Table 1 Average prices paid

Average price paid	Current study	Crossman (2006)
European	£7,409	£7,652
Irish	£3,743	£3,918

Table 2 Profile of brands owned by respondents (by discipline)

Show jumping horses			Eventing horses		
Breed	Dealers	BSJA	Country of origin	Dealers	BE (2009)
KWPN	12%	12%	Holland	8%	7%
ISH	8%	8%	Ireland	36%	30%
BWP	3%	4%	Belgium	4%	2%
HANN	4%	1%	Germany	4%	3%
HOLST	3%	1%			
SF	8%	1%	France	7%	2%
Other	62%	73%	Other	41%	56%

The Other category for the BSJA (73%) included - Oldenburg, Anglo Arab, Westfalen and TB with 1% of the market each. The Anglo European studbook 6%, breeding unrecorded 47% and the remainder was split between over 100 other breeds. The Other category for BE (56%) included – the UK with over 50%, New Zealand, Australia, Brazil, Italy, Poland and Sweden less than 1% each. Within the study, the other category was an open question to which most of the respondents indicated numbers and not the actual brand, or country of origin of these 'other' horses.

Conclusions Within the show jumping market the two main international brands were the Dutch Warmblood (KWPN) and the Irish Sport Horse (ISH) with similar representation within both the BSJA database and amongst the horses owned by dealers. Potentially indicating that those brands may sustain market share in the near future. Some differences appeared with regard to the Selle Francais (SF), with representation amongst dealers at 8%, which may indicate a potential increase in market share in the future, similarly for both the Hanoverian (HANN) and Holsteiner (HOLST) brands. Within the eventing maket all countries identified show potential for increased market share in the future with higher representation within the dealers herd than in the BE database. This potentially indicates an increased importation and use of horses from European studbook in UK based equestrian sport.

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Brand associations of international sport horse brands within the UK market

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Introduction Europe is the powerhouse of sport horse production, where it produces 80% of the approximate 130,000 sport horse foals registered annually to studbooks affiliated with the World Breeding Federation for Sports Horses (WBFSH), Koenen *et al*. (2004). Each studbook has a brand name and logo that distinguishes it from other studbooks / brands and aids recognition, while EU regulations provide a framework for regulation, quality control and recognition. Hence the studbooks effectively act as collective brands for their small individual producers. Aligning the brand with market place requirements is important for brand success. Brand associations are based on perceptions of performance, on attributes core to the consumers wants and needs, they contain the meaning of the brand for consumers. Brand associations are used by consumers to aid them in making purchase decisions, Aaker (1991). Brand associations are linked with brand sales volume, which ultimately has an impact upon market share, Baldauf *et al*. (2003). Elite competition horses in the UK more often tend to be imported rather than bred domestically. This trend is noted by the British Horse Industry Confederation (2005) in that "competitive dressage and show jumping horse-breeders now purchase a large proportion of their horses from overseas competitors, who market them more effectively". The aim of this study was to capture the strength of brand associations of international brands within the UK market place.

Material and methods An online survey was conducted (using Surveymonkey) of UK based sport horse dealers. A total of 140 responses were received. A comprehensive list of attributes were considered for inclusion, bearing in mind the findings of Hennessy (2010). Fourteen non-catalogue recorded attributes were examined. Respondents were asked to rate each brand for its performance on each of the fourteen attributes. The rating scale went from 1 (very poor) to 5 (very good).

Results

Table 1 below shows the percentage of respondents who rated performance as good / very good.

Attributes	KWPN (Dutch Warmblood)	ISH (Irish Sport Horse)	HANN(Hanoverian)	BWP (Belgium Warmblood)	HOLST (Holsteiner)	SF (Selle Francais)
Soundness	58%	82%	59%	56%	62%	63%
Conformation	70%	61%	70%	66%	68%	56%
Movement	84%	51%	90%	55%	67%	59%
Presence	77%	51%	82%	59%	65%	58%
Temperament	67%	86%	67%	60%	66%	58%
Trainability	81%	84%	67%	67%	71%	59%
Rideability	64%	77%	60%	59%	67%	49%
Jump Tech	88%	76%	79%	80%	78%	76%
Scope	90%	88%	79%	84%	78%	79%
Talent Jump	87%	87%	82%	83%	80%	74%
Talent Dress	86%	40%	91%	62%	73%	44%
Speed	17%	68%	26%	28%	37%	60%
Stamina	31%	80%	32%	37%	46%	60%
Courage	51%	92%	56%	53%	57%	66%

Note: The results that are shaded indicate the brand with the highest rated performance for that attribute.

Conclusions Only three of the six brands achieved a highest rating performance for any attribute (ISH, KWPN and HAN), of which the ISH and KWPN were the most prominent. Such brand perceptions are reflected in the market place in that the ISH and KWPN brands were the highest share internationals brands in the UK sport horse market, Hennessy (2010). A higher percentage of respondents rated the ISH as good or very good across a number of attributes in comparison to the other brands, the most notable of these were soundness and temperament, which were identified as the two most important attributes to the UK sport horse dealers, Hennessy (2010). The HAN had the highest rating of the brands on talent for dressage and conformation and the KWPN had the highest rating of the brands on jump technique, conformation and scope. It would seem that achieving the highest ratings on important market place requirements is related to high market share for the respective brand.

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The effects of two endocrine treatments on the size of ovulatory follicles in Lusitano mares in Portugal

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Introduction The Lusitano is an equine breed in Portugal, with approximately 4000 registered brood mares in Portugal and 5600 worldwide. The Lusitano is the product of thousands of years of selection, steeped in the tradition of its historical military links, bullfighting and the classical training methods of the "Haute École". Previous studies of the Lusitano focus on the stallion and enhancement of his competition and breeding performance, however breeding programmes may also benefit from an understanding of the breed specific reproductive physiology of the mare.
Modern breeding management in Portugal routinely use trans-rectal ultrasonography to monitor the size of developing follicles in order to estimate the time of ovulation and ensure covering or insemination close to ovulation. The size of pre-ovulatory follicles and cyclic activity has been found to vary greatly between breeds. Previous research using a variety of breeds comment on the use of either human chorionic gonadotrophin (hCG) to induce ovulation, or, Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), where insemination has not resulted in a successful pregnancy, to shorten the mare's cycle and return the mare to oestrous more quickly. Little information exists to document the use of these hormone treatments in Lusitano mares therefore this study aims to investigate the influence of the use of hCG and $PGF_{2\alpha}$ on the time of ovulation and the size of follicle during ovulation in Lusitano mares.

Methods Veterinary breeding records from approximately 170 Lusitano breeding mares in the breeding seasons 2006 through to and including 2011 were used to identify the diameter of the pre-ovulatory follicles. A number of mares did not require hormonal treatment, but animals subject of hormonal treatment with human chorionic gonadotropin (hcG) and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were identified. An unbalanced two-way type III ANOVA, was applied to the data to ascertain whether differences in follicle size could be established for mares that received hormonal treatments and those that did not.

Results Follicle sizes were smaller for mares that had been treated with both hCG and $PGF_{2\alpha}$ ($F=2.836$, $p<0.05$). There are significant differences ($p<0.05$) in pre-ovulatory follicle size between Lusitano mares with and without hcG treatment. There is a no significant difference ($p>0.05$) in pre-ovulatory follicle size between Lusitano mares with and without $PGF_{2\alpha}$ treatment.

Conclusion Results suggest that treatment with prostaglandin $F_{2\alpha}$ decreases the size of the follicle at ovulation, in comparison with mares that were not treated. This could be due to reduced time for follicular development due to the quicker return to oestrus, resulting in shorter cycle duration following administration of prostaglandin. Although a difference in follicle size at time of ovulation was apparent following treatment with human-chorionic gonadotrophin these results were significant, as treatment may not reduce the duration of follicular development to the same extent as $PGF_{2\alpha}$. As in other breeds, a reduction in follicle size has been linked to decreased pregnancy rates, further investigations would be warranted in this breed to establish the effects of treatments on pregnancy rates.

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Equine fore limb and hind limb stride kinematics: A response to dynamic stretching

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Introduction Obstacle exercises are commonly integrated throughout training and rehabilitation of the equine athlete with ground pole and caveletti (raised poles) involvement regularly employed (Paulekas and Haussler, 2009). Incorporating dynamic stretching this technique is suggested to hold many positive benefits primarily stimulation of proprioceptive awareness, enhanced neuromotor responses, core muscle strengthening, improved coordination and agility (Goff, 2009). Dynamic stretching is also applied to flexibility training for athletic horses and in restoration of locomotor function following injury or immobilisation (Goff and Stubbs, 2007). Much of the evidence in support of these findings is anecdotal in nature and thus the focus of this study was to provide objective analysis pertaining to the effects of dynamic stretching on the horse.

Material and methods A dynamic stretching protocol was developed incorporating raised poles on a sand/rubber surface of an outdoor arena. Five poles were placed at 2.74 metre intervals and raised at alternating ends with a centre height of 32cm. 8 healthy horses between the age of 4 and 6 years were used to carry out a three week progressive treatment protocol 3 times a week that encouraged dynamic stretching in walk. Prior to data collection self adhesive markers were placed bilaterally in accordance with Clayton and Schamhardt (2001) at the centre of rotation of joints for video capture and to enable subsequent motion analysis. Saggital plane video data (50Hz) was collected using a Panasonic 3CCD digital camcorder (NV-GS180) prior to treatment (T1), directly after completion of the three week protocol (T2) and two weeks after the treatment ceased (T3). Kinematic software (SIMI motion analysis©) was used to measure velocity, stride length, maximal carpal flexion, maximal tarsal flexion and carpal and tarsal joint range of motion. Parametric data was analysed using the matched pairs t test.

Results Velocity before treatment was recorded at 3.28m/s (± 0.06 s.e.m) before treatment, 3.34m/s (± 0.08 s.e.m) after treatment and 3.23m/s (± 0.065 s.e.m) 2 weeks post treatment. There was no significant difference between T1, T2 or T3. There was a significant difference in forelimb stride and hind limb stride length ($p<0.01$) after treatment (T2). No significant difference was noted in hind limb stride length before treatment (T1) (2.28 m ± 0.077 s.e.m) and two weeks post treatment (T3) (2.24m ± 0.065 s.e.m.). Maximal carpal flexion measured 179° (± 3.81 s.e.m) following dynamic exercise carpal angle reduced to 169° (± 14.32 s.e.m). No significant difference was shown but there was a trend ($P<0.08$) towards increase carpal flexion. Tarsal angle demonstrated increased flexion pre treatment (119° ± 2.73 s.e.m) when compared directly post treatment (116° ± 2.82 s.e.m) no significant difference was shown. Maximal carpal angle (joint extension) demonstrated a highly significant difference before (244.82° ± 2.085 s.e.m) and directly after treatment (250.49° ± 2,60 s.e.m) ($p<0.01$). Tarsal range of motion showed a significant difference ($p<0.05$) pre (44.37° ± 2.73 s.e.m) and post treatment (54.19° ± 3.81 s.e.m) and returned to 44.80° (± 2.28 s.e.m) two weeks after stretching was discontinued.

Conclusion Dynamic stretching has positive biomechanical effects on kinematic gait variables of the equine athlete improving stride length, carpal angle and tarsal range of motion and thus have the potential to provide performance enhancement and support rehabilitative processes. However, these adaptations were not sustained two weeks after the trial concluded which suggests to enhance positive performance regular application of dynamic stretching must be applied.

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Habituation of horses to the equine hydrotherapy spa unit

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Introduction Hydrotherapy is becoming increasingly popular within the equine industry. Previous research has demonstrated the beneficial effects of using cold spa hydrotherapy on horses with limb injuries (Hunt, 2001). Equine spa units represent a novel environment for the horse and yet the habitation of horses to spas appears to have been given little attention within the industry or scientific research. The first aim was to gain an insight into industry uses of equine spas and methods used to introduce horses to the units. The second aim was to monitor naive horses' responses upon exposure to the spa for five consecutive days.

Material and methods Fifteen industry spa users were surveyed to ascertain current uses of the spa and relevant concerns when introducing horses to the equine hydrotherapy spa unit. Three geldings and three mares (mean age 11.8±1.51; mixed breeds) were introduced to the equine spa following a standardised four phase procedure and received a 15 minute hydrotherapy treatment. This was repeated on five consecutive days. Heart rate and behaviour were measured on each day of the study. Behaviours were categorised into communication and agonistic behaviours for the analysis. Differences in stress responses within each phase and on each day of the study were compared using a one way ANOVA test or Kruskal Wallis test where appropriate.

Results Industry survey results suggested that the spa was used most commonly for injury treatment and performance enhancement; 53% of spa users reported some difficulty in introducing horses to the spa; 60% felt horses habituated within 1-2 days and 86.67% reported seeing behaviours related to stress in the horses. Highly significant differences were found in peak heart rates and behaviour during the different phases of habituation (table 1; $p<0.001$), but these did not differ between days. A highly significant reduction was found in mean heart rate over the five days of the study ($p<0.01$).

Table 1 Heart rate and behavioural responses during the different phases of the spa introduction protocol. Differences between phases are denoted by differing letters ($p<0.001$).

Phase	Mean Peak Heart Rate (beats per minute)	Total Communication Behaviour (median)	Total Agonistic Behaviours (median)
Entry to room	101.07^{a}	n/a	n/a
Walk through spa	98.81^{ac}	n/a	n/a
Securing into spa	83.81^{b}	2.00^{a}	1.00^{a}
Water introduction	82.56^{b}	6.50^{b}	3.00^{a}
Jets turned on	82.04^{b}	5.00^{b}	2.50^{a}
Jets turned off	86.07^{bc}	16.50^{c}	10.00^{b}

Conclusion Spa users within the industry recognised behaviours associated with stress in association with spa use, but did not perceive this to be a problem related to habitation. This indicates that scientific interpretation of equine behaviour does not match the opinions of industry professionals. Scientific investigation of habitation to the spa and dissemination of the results was therefore warranted. Reduction in mean heart rate over the course of the trial suggested that the horses were becoming habituated to the procedure. The initial stages of the treatment resulted in the highest heart rates, suggesting these were the most stressful periods. Behavioural responses contradicted heart rate responses, indicating a greater response to the turning off of the jets, which may have been associated with the change in noise levels. This demonstrates that behaviours demonstrated by the horse are not always accurate in predicting the level of stress experienced. Further research could concentrate on other methods of quantifying stress, such as the use of cortisol measurements. In conclusion, the initial exposure to the equine spa can increase stress levels of horses, but repeated exposure leads to some level of habitation. Spa users should consider methods to limit the stress imposes on naïve horses to improve welfare.

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The physiological and behavioural effect on the equine species of four housing designs allowing differing levels of physical and social contact with con-specifics

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Introduction Isolation is one of the main causes of stress in social species therefore certain restrictive housing designs may have detrimental effects upon the welfare of the horse. Research suggests that a primary cause of stereotypical behaviour in horses is limited social contact and prolonged isolation through individual housing (McGreevy *et al.,* 1995). Results of both epidemiological and empirical studies show that enhancing a horse's social environment can reduce the incidence of such behaviour (Cooper *et al*., 2000).

Material and methods Horses (n=16) were divided equally into four groups and exposed to four housing treatments for a period of five days. The four housing treatments used were single housed no physical contact (SHNC), single housed semi contact (SHSC), paired housing full contact (PHFC) and group housing full contact (GHFC). Horses were brought from their paddocks where they were turned out in their experimental groups of four by the same handler at 0800h every day and walked the short distance to the relevant housing treatment. Horses remained in the housing treatment until 1600h at which point they were walked back to their paddock where they spent the night. At the end of the five day period they were turned out in their paddocks in their experimental group for two days and then the groups rotated to the next housing treatment. Faeces were collected for assessment of the stress hormone corticosterone from two randomly chosen horses in each group. One sample per day was collected from each horse on an opportunistic basis during the first three days of each rotation. A camera system installed throughout the equestrian centre was utilised to record the behaviour of the horses in each housing treatment. The video footage was used to form a time budget of the horses' activity in each housing treatment. An ease of handling score was also assigned to each horse using a pre-determined scale of 1-5 with one being easy to handle and 5 being difficult to handle. A one way repeated measures ANOVA was conducted to examine any difference in faecal corticosterone levels and any difference in ease of handling between housing treatments.

Results There was a significant effect of housing treatment on faecal corticosterone (p=0.01) with higher levels displayed in the most restrictive SHNC treatment (Figure 1). Significantly more stereotypical behaviour was observed in the SHNC housing treatment. The stereotypies performed were box-walking, head nodding, weaving and crib-biting. Stereotypical behaviour was observed in seven of the ten study horses used for time budget assessment. No stereotypical behaviour was observed in the GHFC housing treatment in any of the horses. There was a significant difference (p=0.003) in ease of handling between housing treatment. As the level of isolation increased the difficulty of handling the horses due to evasive behaviour also increased (Figure 2).

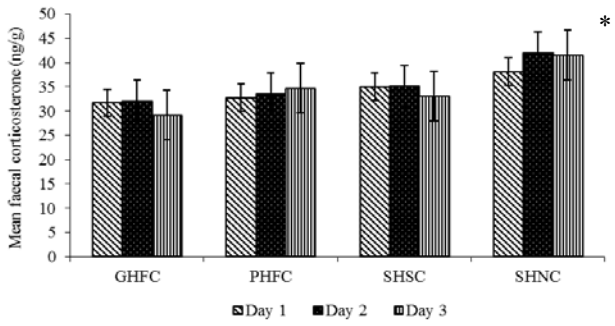

Figure 1 Mean (±SD) faecal corticosterone concentrations during the first three days of each housing treatment.

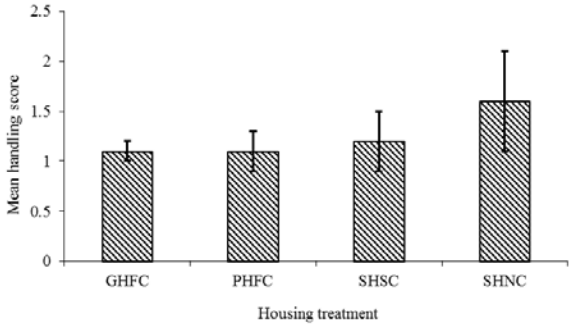

Figure 2 Mean (±SD) ease of handling score in each housing treatment

Conclusion During this study horses showed a decreased incidence or absence of stereotypic behaviour when in the social housing treatments. These treatments provided an environment where horses were able to display natural behaviour and allowed contact with con-specifics. The behavioural findings imply that the social housing treatments were less aversive than the single housing and provided an improved standard of equine welfare. In addition horses were easier to handle in these treatments. Faceal corticosterone results support the behavioural findings with significantly higher levels reported in the isolated treatment

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An *in vitro* study into the degradation of fat sources fed to equines using the gas production technique

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Introduction Providing the equine athlete with sufficient energy, without supplying an overload of starch is a significant problem for equine producers, trainers and breeders. Recently fat has been used to increase calorific value, without using high starch cereal feeds. Oil has been traditionally used, and more recently 'encased' fats such as rice bran and fibre impregnated with oil have been advocated. However much of the support for such encased fats is anecdotal. The aim of the present study was to assess the degradation of three types of fat sources, using the gas production technique over two experiments.

Material and methods Experiment 1 used the manual pressure gas production technique of Theodorou *et al.* (1994) to measure the pressure and gas produced from the fermentation of five feed substrates Alfalfa (AA), Alfa Oil (AO), Alfalfa with 20% added linseed oil (AA20) and Alfalfa with 40% added linseed oil (AA40) and a high fat rice bran product (EJ). The feeds were incubated for 60 hours with faecal inoculum, and pressure and volume readings were taken at *ca.* 5, 11, 19, 24, 30, 37, 46, 54 and 60 hours post inoculation. Apparent dry matter (DM) loss was calculated from the feed residue. Pressure readings were corrected using linear regression, and mean cumulative gas volumes were calculated for each substrate. Corrected cumulative volumes were processed using the maximum likelihood programme (Ross, 1987), which was used to fit curves to the gas production profiles using parameters derived from the France *et al.* (1993) model. The France *et al.* (1993) parameters were used to calculate the percentage of DM loss, the extent of degradation, the lag time, time to reach 50% (t_{50}) and 95% (t_{95}) of gas production, and the fractional rate of gas production (FRGP). The data was analysed for significant differences using a one way analysis of variance statistical test (ANOVA), and comparisons between the treatments were then made using least significant difference equations.
Experiment 2 followed the same protocol as Experiment 1, and the same feed substrates from the first trial were also used. In Experiment 2 half of the samples were subjected to a predigest treatment prior to fermentation, which was designed to replicate precaecal digestion in the horse. All the feed substrates were fermented with equine faecal inoculum for *ca.* 63 hours, and the data was analysed using the same process outlined in Experiment 1. The data was analysed for significant differences using a two way ANOVA, which calculated potential differences between the feed substrates, and between predigest and non-predigest samples to show any effect of the treatment. Predigest and non-predigest samples were analysed respectively using a one way ANOVA to demonstrate any statistical differences between the feed samples.

Results Experiment 1: The rice bran substrate (EJ) had the highest FRGP, and this was significantly ($P<0.05$) greater than the other substrates. AO had the lowest mean value for FRGP, and this was significantly ($P<0.05$) lower than EJ, AA20 and AA40. EJ had the highest DM loss, and this was significantly ($P<0.05$) higher than AO, AA20, AA40. AO had the lowest DM loss, and this was significantly ($P<0.05$) lower than the other feed substrates. A significantly ($P<0.05$) higher extent of degradation was found for EJ, whilst a significantly ($P<0.05$) lower extent of degradation for AO was noted compared to the other feed samples.
Experiment 2: EJ had the greatest lag time, and this was significantly ($P<0.05$) higher than the other feeds. EJ also took the shortest time to reach 95% of gas production (t_{95}), significantly ($P<0.05$) different from all the other feed substrates. EJ had the greatest extent of degradation, significantly ($P<0.05$) higher than AO, AA20 and AA40. The predigest treatment had a considerable effect on the cumulative gas production profile for EJ, AA20 and AA40, with a marked decrease in cumulative gas production for sample given the predigest samples.

Conclusions The results of Experiment 1 indicated that degradation of high fat rice bran (EJ) was significantly higher than the degradation of Alfalfa with linseed oil (AA20, AA40), or the degradation of a high fat forage (AO). This was quantified by significantly ($P<0.05$) higher DM loss, higher FRGP, and a greater extent of degradation compared to AO and AA20 and AA40. The high fat forage was not found to be well degraded with significantly ($P<0.05$) lower DM loss and extent of degradation compared to the other fat sources. Experiment 2 demonstrated that the use of a predigest treatment was found to have a significant ($P<0.05$) effect on degradation, with significantly ($P<0.05$) higher values for FRGP, and the extent of degradation for non-treated samples, and a significantly shorter time to reach t_{50} and t_{95}.
In both experiments EJ had a higher FRGP and a greater extent of degradation when fermented with microbial inoculum compared to a high fat forage, or oil added as a top dressing to a Lucerne chaff. This could imply that under *in vivo* conditions that the feed would have an increased digestibility to other sources of fat.

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The effect of freezing on the fermentative activity of equine faecal inocula for use in an *in vitro* gas production technique

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Introduction The *in vitro* gas production technique (IVGPT) is used to evaluate the nutritional quality of feedstuffs. The methodology for the IVGPT uses freshly collected microbial inoculum as an alternative to caecal fluid, but freshly voided faeces cannot always be obtained. Using frozen faeces as inocula for the IVGPT would provide flexibility to this technique. However, information on using frozen inocula in gas production (GP) studies is limited. Consequently, the aim of the experiments reported here was to examine the effect of freezing on the fermentative activity of equine faecal inocula for use in the *in vitro* gas production technique of Theodorou *et al*. (1994).

Material and methods Two *in vitro* experiments were conducted. In experiment 1, high-temperature dried alfalfa (HTA) and mature grass hay (GH) were incubated with a faecal inoculum prepared either fresh faeces (Fr) or following storage at -20°C for 7 days (F7). In experiment 2, HTA, GH, High-temperature dried grass (HTG), and unmolassed sugar beet pulp (SB) were incubated with an inoculum prepared either fresh faeces (Fr) or following storage at -20°C for 24 hours (F24). Substrate/inocula combinations were fermented using the IVGPT of Theodorou *et al.* (1994). *In vitro* fermentations were conducted over a period of 80 hours, with GP measured at frequent intervals throughout. Cumulative GP values at 80 hours were analysed for significant differences using two-way analysis of variance in Genstat Release 9.1. Comparisons between treatment groups were made by least significant difference equations.

Results In both experiments, total GP values revealed an interaction (P<0.001) between inocula and substrate (Tables 1 and 2). GP from HTA was less affected by inocula source compared to all other substrates. In experiment 1, total GP was reduced by 33 percent for GH inoculated with F7, compared to 3 percent for HTA, whilst in experiment 2, total GP was reduced by 47, 37, 14 and 8 percent for GH, HTG, SB and HTA, respectively when inoculated with F24 compared to fresh faeces.

Table 1 Total gas production values (ml/g DM) at 80 hours following incubation of grass hay (GH) and high-temperature dried grass (HTG) with either fresh (Fr), or frozen equine faeces stored for 7 days (F7) at -20°C prior to inoculation (n = 3).

	Substrate			
Inocula	GH	HTA	Inocula Mean	Inocula s.e.d. (P)
Fr	145.4[b]	142.1[b]	143.8	
F7	97.6[a]	137.2[b]	117.4	
Substrate Mean	121.5	139.7		
Substrate s.e.d. (P)	2.63 (<0.001)			2.63 (<0.001)
S x I s.e.d. (P)	3.72 (<0.001)			

Table 2 Total gas production values (ml/g DM) at 80 hours following incubation of grass hay (GH), high-temperature dried grass (HTG), high-temperature dried alfalfa (HTA), and unmolassed sugar beet pulp (SB) with either fresh (Fr), or frozen equine faeces stored for 24 hours (F24) at -20°C prior to inoculation (n = 3).

	Substrate					
Inocula	GH	HTG	HTA	SB	Inocula Mean	Inocula s.e.d (P)
Fr	172.8[d]	214.4[e]	175.6[d]	283.6[g]	211.6	
F24	91.9[a]	135.3[b]	161.5[cd]	244.9[f]	158.4	
Substrate Mean	132.4	174.9	168.6	264.2		
Substrate s.e.d (P)	5.52 <0.001)					3.90 (<0.001)
S x I s.e.d. (P)	7.81 (<0.001)					

Conclusion Using frozen equine faecal inocula affect the extent and rate of substrate fermentation *in vitro*, which appears to be dependent upon the nature of substrate assessed.

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Palatability and ingestion behaviour of 6 Polo ponies offered a choice of dry, soaked and steamed hay for 1 hour on three separate occasions

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Introduction Dust in the stable environment is known to cause the debilitating condition Recurrent Airway Obstruction (RAO) in horses. Steaming has been shown to be an effective alternative to soaking for reducing respirable particles in fodder (Stockdale and Moore-Colyer, 2010). However, to date no information is available on the palatability of steamed vs soaked vs dry hay when offered free choice to horses. The current trial used 6 Polo ponies, previously fed haylage in a repeated measures design experiment, where dry hay, hay steamed in the Haygain steamer - HG 600 (Propress Equine Ltd, Hungerford, UK) for 40 minutes and hay soaked for 30 minutes in water were offered simultaneously on 3 separated occasions.

Methodology Replicate bales of hay were taken from first cut Timothy and Meadow Fescue hay which had been barn-stored for 6 months. Bales were divided into 3. One half was steamed in the HG 600; the second section was soaked for 30 minutes in fresh tap water and the third section was left dry. One kg (on a 95% DM basis) was then taken from each of the three treated hays and placed on the floor in 3 different corners of a rubber-matted stable, where water was available *ad libitum.* The horses were given free access to the fodder for 1 hour and observations of their first choice of fodder recorded. In order to eliminate positional preferences, and determine the preferred choice of fodder, the experiment was repeated 3 times for each of the 6 Polo ponies, with the position of each of the hays being rotated between the 3 corners of the stable. Data was collected on amount of hay eaten in kg which was then subjected to a repeated measures analysis of variance (Genstat, 12). Observations were recorded on the first choice of forage eaten for a consecutive 5 minutes.

Results

Table 1 Average amount of forage consumed in kg (on 95% DM basis) when offered to 6 polo ponies for 1 hour, on 3 separate occasions.

	Steamed	Soaked	Dry	s.e.d	Sig
Kg of Hay consumed	0.867^{c}	0.050^{a}	0.183^{b}	0.0246	***

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

Conclusions The results from this experiment clearly demonstrate that horses preferred to consume steamed hay to dry or soaked hay when offered free-choice in a stable environment. Observations of choice of feed revealed that steamed hay once tasted was always the first consumed. Some horses did nibble some dry hay, but quickly returned to the steamed hay until it was all consumed whereupon they then chose to eat the dry hay.

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Evaluating the impact of different levels of residual polygenic effect on genomic evaluations in the United Kingdom

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Introduction Recent developments in molecular biology have resulted in the emergence of low cost genotyping technology for Single Nucleotide Polymorphism (SNP). Due to linkage disequilibrium between SNPs and quantitative trait loci for traits of economic value, genomic breeding values can now be computed directly for animals on the basis of SNP effects. The genomic BLUP model used to estimate SNP effects in most dairy populations are based on the Illumina Bovine 54K chips and it is usually assumed that these SNPs explain all the genetic variation for the traits analysed. However, fitting a residual polygenic effect (RP) may account for the fact that SNPs may not explain all the genetic variance and has also been found to render SNP effects less biased (Solberg, et. al. 2009). The level of RP may differ for traits of different heritabilities. This study examines the impact of including different levels of RP on genomic evaluations for milk yield (MY) and $\log_e$ somatic cell count (SCC) in Holstein/Friesian, representing traits of high and low heritabilities respectively.

Material and methods Data from 11480 bulls with 50k genotypes were available for the analysis. However, 600 of these bulls were genotyped with the Illumina 800K chip but only the corresponding SNPs on the 50k chip were extracted and used for these bulls. These genotypes are a combination of the North American Cooperative Dairy DNA Repository (CDDR), UK AI industry and SAC genotypes. Minor allele frequency was set to 0.05 and call rate for animals at 95%. In total 41866 SNPs were selected for genomic evaluations after these various edits. Deregressed sire proofs (DRP) from the UK official April 2011 run and MACE proofs were used as input variables in the genomic evaluations. A linear model was used for the estimation of SNP effects with fixed mean effect and random polygenic and SNP effects. Genotypes were coded as 0 and 2 for the homozygotes and 1 for the heterozygotes. The RP levels were set at 0, 5, 10, 15, 20 and 25% of the total genetic variance. Genotyped bulls born before 2004 were used as the reference population and were used to estimate the SNP effects. Bulls born after 2004 were used for the purposes of validation. The effects of different levels of RP were examined by computing correlations between direct genomic values (DGV) and DRP for bulls in the reference and validation sets. In addition correlations between SNP and polygenic solutions with DGV were computed. The changes in SNP solutions for SNPs of different allele frequencies were calculated for different levels of RP.

Results The slope coefficients from the regression of DGV on DRP were generally similarly for MY in the reference data set but correlations increased until an RP of 10% was reached and thereafter became constant (Table 1). However, in the validation data set, highest regression was obtained at RP of 10% but the correlations generally decreased with increasing levels of RP. For SCC, regressions and correlations increased with levels of RP in the reference data set. A similar pattern was observed for regressions in the validation set but correlations were constant across levels of RP. While correlations of SNP solutions with DGV decreased in a similar way for both traits with increasing levels of RP, the correlations of the polygenic solutions were always higher for SCC at every level of RP. This would indicate the higher impact of the RP for traits of lower heritability. Results also indicate the polygenic solutions for SCC for bulls with few daughters were more influenced by parent contributions at low levels of RP. The mean SNP solutions for SNPs with alleles of medium (0.4 – 0.6), and high (> 0.6) frequencies decreased by up to 15 and 23% for MY, while SNPs of low (<0.4) frequencies increased by 25% with increasing levels of RP. For SCC, SNPs of low and high frequencies increased by up to 8 and 53% with increasing levels of RP but SNP with medium allele frequencies decreased by up to 32%.

Table 1 Regressions (Reg) and correlations (Corr) between DGV and DRP

Polygenic level (%)	Milk yield				Somatic cell count			
	Reference		Validation		Reference		Validation	
	Reg	Corr	Reg	Corr	Reg	Corr	Reg	Corr
0	1.053	0.96	0.802	0.67	1.154	0.90	0.913	0.74
5	1.063	0.98	0.807	0.66	1.175	0.92	0.937	0.74
10	1.063	0.99	0.816	0.65	1.186	0.93	0.952	0.74
15	1.061	0.99	0.815	0.64	1.193	0.94	0.964	0.74
20	1.059	0.99	0.815	0.64	1.197	0.95	0.974	0.74
25	1.057	0.99	0.811	0.63	1.199	0.95	0.982	0.73

Conclusions Considering the estimates of regressions and correlations in both the reference and validation data sets a RP of about 10% and 20% seems more appropriate for MY and SCC respectively.

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Reliability of genomic selection for different reference population designs

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Introduction In genomic selection animals are evaluated using genetic markers (Meuwissen *et al*., 2001). Effects of these markers are estimated using a so-called reference population. Estimated marker effects are used to predict animals' breeding values. Reliability of breeding values, expressed on a scale from 0 to 1, measures how reliable these estimates are. The reliability of genomic selection depends on the accuracy of estimated SNP effects and the proportion of the genetic variance explained by the markers (Daetwyler *et al*., 2008; Goddard, 2009). The accuracy of estimated SNP effects is influenced by the size of the reference population and the heritability of the considered trait. The proportion of genetic variance explained by the markers is influenced by the effective size of the considered population and the density at which the SNP chip covers the genome. Additionally, the design of the reference population in terms of the family structure of the reference population may influence the reliability of genomic selection. For traits difficult or expensive to measure, such as methane emission or fertility traits the number of phenotypic observations may be limited. The design of such small reference populations may have an important effect on the reliability of the genomic breeding values. The objective of this study was to investigate the effect of various relationship levels within the reference population and level of relationship of evaluated animals to the reference population on the reliability of direct genomic breeding values (DGV) for evaluated animals.

Material and methods A population reflecting a dairy cattle population structure was simulated with a trait with a heritability of 0.3. Four reference populations with different family structure (2,000 cows each) consisted of highly (HR), moderately (MR) lowly (LR), and randomly related animals (RND), which was achieved by choosing paternal half-sib families of different sizes. The evaluated animals (1,000 cows) were chosen randomly from one generation after the reference populations. Reliabilities of DGV predictions were calculated deterministically based on selection index theory, using genomic relationships and phenotypic information as in VanRaden (2008).

Results The average relationship of animals in the reference population, calculated using at least 5 generations pedigree, decreased along with weakening of the family structure (Table 1). The reliabilities increased when the average relationship within the reference population decreased (Table 1). Individual reliability strongly increased with increased relationship to the reference population.

Table 1 Average pedigree-based relationship within the reference population and average reliabilities of all the evaluated animals across highly (HR), moderately (MR) lowly (LR), and randomly related (RND) reference populations.

	Reference population			
	HR	LR	MR	RND
Relationship	0.095	0.056	0.050	0.049
Reliability	0.442	0.490	0.521	0.531

Conclusions This study shows an importance of the reference population design. To achieve maximum possible reliability of DGV, relationships among animals in the reference population should be minimized and at the same time the relationships of the evaluation animals with the reference population should be maximized. Results of this study can be potentially used to compose optimal reference populations to enable genomic selection to mitigate methane emission from farm animals or improve difficult to measure fertility traits.

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Genomic selection in UK beef cattle terminal traits using commercial phenotypes

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Introduction Genomic selection (GS) has been readily adopted by the dairy AI industry worldwide, and the opportunity exists to implement this technology in UK beef breeding and exploit this novel approach to identify animals of high genetic merit. In contrast with the dominance of AI usage in dairy breeding, the beef sector relies predominantly on natural service bulls for the dissemination of improved genetics in the suckler herd. Furthermore, there is currently no collection and exploitation of commercial offspring performance information in the genetic evaluation of elite breeding herds. These considerations make it clear that the model for implementing GS in beef breeding is not the same as that adopted for dairy breeding, where the primary aim is to reduce the generation interval within the selection programme. The primary breeding goal adopted by the UK beef industry is terminal selection, where growth and carcass traits are key. This study quantifies the potential selection response in terminal traits from the use of GS and the availability of commercial beef carcass phenotypes (CP).

Material and methods The UK Beef Value (BV) terminal index (Amer *et al.,* 1998) was used in a deterministic simulation to predict genetic gain in the UK pedigree Limousin (LIM) population when using GS to incorporate CP. The goal traits include 3 carcass traits and 2 calving traits. The selection criteria, which consist of live animal measurements was augmented by 3 CP traits evaluated through GS. It was assumed that carcass phenotypes of commercial progeny of Limousin bulls were available to create a SNP key to genomically evaluate bulls for these traits. In this model, which considers only additive genetics, genomic breeding values (GBV) for commercial carcass traits were incorporated as correlated traits within a conventional selection index framework as per Dekkers (2007), using index software developed by Abacus Biotech. Within this framework, conceptually the conventional BLUP EBV is combined with GBV to produce GEBV for greater accuracy when SNP markers do not capture all the additive genetic variation. The approach of Daetwyler *et al*., (2008) was used to predict GBV accuracy as a function of the number of animals in the training population (TP), the heritability of the trait and the effective number of haplotypes. In this case the TP refers to pedigree NS Limousin bulls which in practice would be dense-chip genotyped and have phenotypes from CP of their progeny. Another variable considered in this simulation was Rpc, the genetic correlation between pure-bred and commercial performance, which is not currently taken into account in UK beef evaluations. Population statistics used in the model were estimated from the actual LIM population through analysis of records in the BASCO database. Three scenarios was considered; 1) GBV + EBV for existing animal selection criteria (2) GBV for 3 CP traits + EBV in existing traits (3) GBV for new and existing traits plus EBV for existing traits. Each scenario was simulated for three variations of Rpc.

Results Scenarios 2 and 3 in Table 1 produced similar and substantial percentage increases in gain over current selection (TP = 0). A TP of 2000 sires assuming an Rpc 0.7 is predicted to increase response by 69 %.The percentage increase in response over current selection increased with decreasing Rpc; however the effect of varying Rpc was reduced at high TP levels for scenarios 2 and 3.

Table 1 Beef Value selection response (£/animal/year) with varying TP size in 3 scenarios each for 3 variations of Rpc.

	Scenario								
	Rpc = 1.0			Rpc = 0.7			Rpc = 0.4		
TP size	1	2	3	1	2	3	1	2	3
0[1]	0.93			0.62			0.36		
1000	0.99	1.08	1.12	0.67	0.88	0.90	0.38	0.76	0.76
2000	1.02	1.18	1.23	0.69	1.03	1.05	0.40	0.94	0.94
5000	1.08	1.36	1.41	0.73	1.25	1.25	0.42	1.20	1.20
20000	1.17	1.61	1.73	0.79	1.54	1.54	0.46	1.49	1.49

[1] TP = 0 (no genomic selection) is the equivalent of current selection

Conclusion Scenario 2 predicts faster gain than scenario 1 as correlations between commercial traits and goal traits are higher than those between pure-bred traits and goal traits. Scenario 3 improves on scenario 2 (at high TP and with Rpc = 1.0), as the two calving traits (which only have pure-bred phenotypes) have genomic information in the third scenario. GS using CP does therefore offer UK beef breeding the potential of a large improvement in genetic gain in terminal traits without a requirement for structural change in UK beef breeding practices. Predicted increases in gain are similar to those observed after the implementation BLUP in the UK.

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Comparison between BayesC and GBLUP methods to estimate genomic breeding values in threshold phenotypes

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Introduction Accurate estimation of breeding values is one of the main aims in breeding programs. There are two main approaches in genomic selection for estimation of breeding values. The first approach assumes that all single-nucleotide polymorphisms (SNPs) have effects on the trait variance such as GBLUP and the second approach assumes that just some SNPs contribute to the trait variance such as BayesC. The objective of present study was to compare the accuracy in estimating genomic estimated breeding values (GEBVs) using two diverse methods, GBLUP and BayesC in threshold simulated traits.

Material and methods A genome of four chromosomes each of 100 cM length was simulated with 4000 SNPs equally spaced and 40 or 200 QTL randomly positioned on the genome and their effects sampled from a normal distribution. Linkage disequilibrium between the SNPs was generated by allowing a population of 50 mating pairs to undergo 50 generations of random mating. Thereafter, the population was expanded to 1000 per generation and 5 extra more generations were created. Given the QTL effects and the individuals' genotypes, breeding values were calculated and rescaled to have a variance of 0.3. Phenotypes were, then, simulated by adding an environmental effect sampled from a normal distribution with variance equal to 0.7 (i.e. h^2 =0.3). This continuous phenotype (CON) was, then used to calculate three threshold traits: TH1, TH2, TH3 by setting a threshold value and assigning individuals to be a "case" if their phenotype was above the threshold, or "control" otherwise. The threshold points for TH1, TH2, TH3 were at the mean, mean + SD and mean + 2SD, representing an incidence rate of approximately 50%, 16% and 2% respectively. A study was then carried out to compare the GBLUP and the BayesC method in term of the accuracy of their GEBVs. A set of 500, 1000 or 3000 phenotyped individuals (training population) was used to calculate the GEBV of 1000 unphenotyped individuals from last generation (testing population). The correlation between estimated and true genomic breeding values was used as measure of accuracy. T-test was used to compare BayesC and GBLUP estimations for all cases. A total of 10 replicates were used in the simulation.

Results The average accuracies for the different methods are shown in Table 1. As expected the accuracy of all methods and traits increased with the size of the training population. Additionally, the accuracy was greater for the continuous trait and decreased in the threshold traits when the incidence rate decreased. Decreasing the incidence rate from 50% (TH1) to 2% (TH3) resulted in approximately a loss of 30-40% in the GEBV accuracy. Compared with the results from the continuous trait, the accuracies when assuming an incidence rate of 2% were around 40-50% lower.
For the scenario assuming 40 QTL, in general the accuracy of the BayesC method was better than or at least the same as those obtained with GBLUP. On the other hand when assuming 200 QTL, the slight advantage of BayesC disappeared with GBLUP having slightly better accuracy for some scenarios. This diminishing advantage of BayesC over GBLUP has being previously reported when comparing them in scenarios considering continuous traits (Daetwyler *et al*., 2010).

Table 1 Average accuracies for BayesC and GBLUP for different numbers of QTL and training population sizes

# QTL	Trait	Training population					
		500		1000		3000	
		BC	GB	BC	GB	BC	GB
40	TH1	0.648	0.624	0.725	0.719	0.846	0.802
	TH2	0.557	0.543	0.652	0.624	0.784	0.748
	TH3	0.363	0.361	0.471	0.470	0.578	0.565
	CON	0.736	0.731	0.813	0.797	0.892	0.858
200	TH1	0.650	0.650	0.739	0.720	0.797	0.796
	TH2	0.605	0.607	0.667	0.675	0.734	0.747
	TH3	0.414	0.418	0.463	0.484	0.534	0.574
	CON	0.743	0.740	0.802	0799	0.859	0.852

BC: BayesC, GB: GBLUP

Conclusions The results from this study showed the advantage of genomic selection methods in threshold traits. Even for the least favourable scenario assuming an incidence rate of 2%, the accuracy of those methods were at least half those observed when assuming a continuous trait. Comparing both methods, BayesC tended to show slightly better accuracy when the trait was controlled by 40 QTL, but they disappeared when the number of QTL affecting the trait rose to 200. For the later situation, GBLUP had better accuracy for some scenarios.

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Genetic factors controlling wool shedding in an Easycare composite sheep flock

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Introduction Several sheep breeds are known to shed their fleece in summer. They range from British breeds (e.g. Wiltshire Horn) and tropical breeds (e.g. Sabi) to composites such as the newly created Easycare breed. Wool shedding is a complex trait which has been reported to be influenced by a dominant major gene (Pollott, 2011). This study reports results of a study to dissect the genetic control of wool shedding in an Easycare sheep flock, using both quantitative genetic and DNA analyses.

Material and methods A total of 565 sheep with wool shedding scores and 1474 pedigree records were available from a commercial flock of Easycare sheep, which also included introgressions of Lleyn, Meatlinc and Blackface genotypes. Animals were scored for wool shedding in their second year of life, with the scoring system based on a 10 point (0-9) scale. Animals which retained all their wool were scored zero and those which completely shed their wool were scored nine. DNA sampling was done using a non-invasive commercial nasal collection kit. Forty eight animals with extreme phenotypes (non shedding/shedding) were genotyped using the Illumina® Ovine 50SNP BeadChip (50k SNP chip). After quality control, 45133SNPs were available for analyses. Three genetic analyses were performed: (i) a standard heritability analysis; (ii) a segregation analysis to determine if a major gene affecting wool shedding was segregating; and (iii) a genome wide association study (GWAS) to map regions in the genome affecting the trait. For heritability estimation, the data were analysed using linear mixed models with phenotypes fitted as a continuous trait, or as a binary trait (full fleece *vs*. the rest) using logit and probit link functions, using ASReml (Gilmour *et al*., 2009). Year, sex, type of birth and age of dam were fitted as fixed effects. Random effects fitted included animal, permanent environmental effects due to dam and litter. It was not possible to correctly partition the breed component. For the segregation analysis, data were fitted as a continuous variable and additive and dominance effects were estimated for the putative major gene. The GWAS was conducted using GenABEL (Aulchenko *et al*., 2007) fitting the kinship matrix to account for genetic background effects. SNPs identified as significant ($p<0.05$ at the genome-wide level) were further analysed as fixed effects in mixed models using ASReml. The genetic effects were calculated as follows: additive effect, a = (AA - BB)/2; dominance effect, d = AB - [(AA + BB)/2]; and proportion of genetic variance due to SNP = $[2pq\ (a + d(q - p))2]/VA$, where AA, BB and AB are the predicted trait values for each genotype class, p and q are the SNP allele frequencies and VA is the total additive genetic variance of the trait obtained when no SNP effects are included in the model.

Results High heritability estimates (0.80±0.06 to 0.83±0.07) were obtained for wool shedding as a continuous trait either with direct additive or permanent environmental effects due to the dam as random effects, whereas moderate heritability estimates (0.37±0.10 to 0.59±0.10) were obtained when wool shedding was treated as a binary trait (approaching unit on the liability scale). A similar heritability estimate (0.76±0.06) was obtained using complex segregation analysis fitting direct additive effects. However, in addition, the segregation analysis revealed the locus for fleece shedding to be dominant, in agreement with Pollott (2011) with an allele frequency of 0.38 for wool shedding. The association analysis revealed four possible significant SNPs ($p<0.05$) as shown in Table 1, on two chromosomes, and a high estimated heritability (0.87) using the genomic kinship matrix. Within each chromosome, the SNPs are closely linked, giving two regions. However, these SNPs can only be in partial linkage disequilibrium with the putative causative mutation as they only explain a proportion of the observed genetic variation and they do not display the expected dominance effect.

Table 1 Summary of additive and dominance effects for genome-wide ($p<0.05$) significant SNPs

Description	SNP1	(±S.E.)	SNP2	(±S.E.)	SNP3[1]	(±S.E.)	SNP4	(±S.E.)
Chromosome	2		2		5		5	
Additive effect	1.835	0.469	1.713	0.504	2.323	1.155	1.853	0.888
Dominance effect	-1.098	0.823	-0.26	0.807	NA		-1.078	1.033
Proportion of VA due to SNP	0.28		0.19				0.29	

[1] SNP3 had only two genotypes

Conclusions High estimates of heritability for wool shedding in Easycare sheep conform that wool shedding has a strong genetic component. These high estimates were confirmed using both segregation analysis and analyses with the genomic kinship matrix. GWAS results have identified two putative regions for sequencing in subsequent studies which aim to identify the causal mutation(s).

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Regional Genomic Relationship Mapping to indentify loci underlying nematodes resistance variation in Scottish Blackface sheep

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Introduction Several quantitative trait loci (QTL) associated with nematode resistance have been reported in sheep using linkage analyses (e.g., Dominik, 2005; Davies *et al*., 2006). Recently, however, genome-wide association studies (GWAS) have become more common, following the release of the Illumina® Ovine 50SNP BeadChip (50k SNP chip) in January 2009. Unfortunately, GWAS seems to localise a relatively small proportion of the total genetic variation in the traits of interest (e.g., Kemper *et al*., 2011). An alternate approach proposed by Nagamine *et al*. (2011), known as Regional Genomic Relationship Mapping (RGRM), may better capture genetic effects. This method provides heritability estimates attributable to small genomic regions, and it has the power to detect regions containing multiple alleles that individually contribute too little variance to be detected by GWAS. The aim of this study, therefore, was to use RGRM to identify loci underlying faecal egg count (FEC) variation, as an indicator of nematode resistance, in Scottish Blackface sheep genotyped with the 50k SNP chip.

Material and methods The population comprised 752 Scottish Blackface lambs, bred over a 3-year period (2001–2003). Lambs were continually exposed to natural mixed nematode infection by grazing. FEC were collected at *ca*. 16, 20 and 24 weeks of age for two groups of nematodes, i.e. *Nematodirus* spp. or other nematode genera collectively termed *Strongyles*. An average animal effect for both *Nematodirus* and *Strongyles* FEC, i.e. the average weighted FEC across the three age points calculated with a repeatability model, was also used as a phenotype. FEC measurements were log transformed as $ln(\text{trait} + x)$, where x is a constant used to avoid zero values. After quality control, 42,841 SNPs were available for the analysis. In the RGRM approach each chromosome is divided into windows of a pre-defined number of SNPs. In our case, the window size was 100 adjacent SNPs, and the window was shifted every 50 SNPs. A mixed model was used for the analysis. The fixed effects considered were sex, year of birth, management group, litter size and age of dam, with day of birth as covariate. The residual and the additive genetic (both regional genomic and whole genomic) effects were fitted as random. The whole genomic additive effect was estimated using a genomic relationship matrix constructed from all SNPs, whereas the regional genomic additive effect was estimated from a genomic relationship matrix constructed from the SNPs within each window, i.e. region. The whole genomic relationship matrix was also used in a mixed model analysis, assuming that there was no regional variance (null hypothesis). To test for variance in each region, a likelihood ratio test (LRT) was calculated and assumed to follow a $\frac{1}{2}\chi^2_{(1)}$ distribution (Self and Liang, 1987). In total, 868 windows were tested. Hence, after Bonferroni correction to account for multiple testing, the LRT threshold for genome-wide ($p<0.05$) and suggestive (i.e., one false positive per genome scan) significance levels were 13.56 and 9.29, respectively.

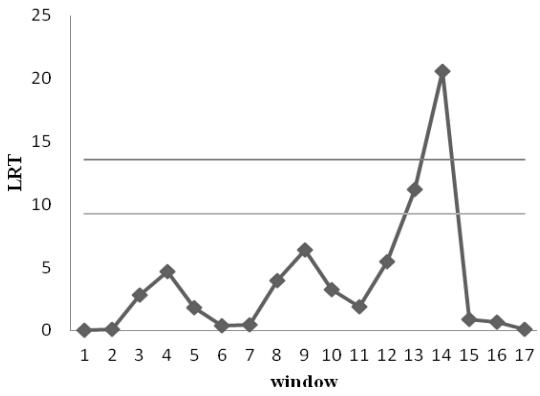

Figure 1 Plot of the LRT across chromosome 14 for FEC for the *Nematodirus* average animal effect (genome-wide $p<0.05$ and suggestive thresholds are also shown in plot)

Results Whole genome heritabilities for *Nematodirus* and *Strongyles* average animal effect FEC were 0.27 and 0.22. One region (Figure 1) was significant at the genome-wide level ($p<0.05$), this being for the *Nematodirus* average animal effect (LRT=20.54, window 14 on chromosome 14). Seven other regions were significant at a suggestive level (on chromosomes 2 for FEC at 16 weeks, 4, 9 and 14 for FEC at 24 weeks, and 14 for the average animal effect), with LRT from 9.32 to 11.41. For *Strongyles*, three regions (on chromosomes 6 for FEC at 16 weeks and 3 and 21 for the average animal effect) reached suggestive significance (LRT from 9.41 to 13.44), but no region reached the genome-wide significance level.

Conclusions Use of 100 SNP windows allowed identification of significant regions for FEC traits. Improved mapping resolution and definition of the genetic architecture of these regions will be facilitated through exploration of variable window sizes within each region.

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Ruminomics: Connecting the animal genome, gastrointestinal microbiomes and nutrition to improve digestion efficiency and the environmental impacts of ruminant livestock production

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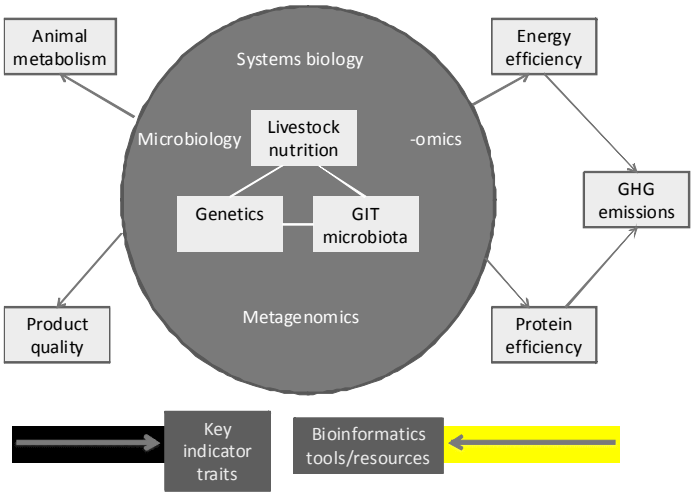
A new collaborative, large-scale integrating project named 'Ruminomics' (project no. 289319) has been commissioned under the EC's Seventh Framework Programme: Food, Agriculture, Fisheries and Biotechnology. The call was KBBE.2011.1.1-03: Efficiency of ruminant digestive systems and reduction of the ecological footprint through a combination of systems biology, 'omics' and nutrition. The intention of the project is to integrate expertise and technologies to increase the efficiency and decrease the environmental footprint of ruminant production, significantly advancing current knowledge in this sector. The project will exploit state-of-the-art –omics technologies to understand how ruminant gastrointestinal microbial ecosystems, or microbiomes, are controlled by the host animal and by the diet consumed, and how this impacts on greenhouse gas emissions, efficiency and product quality. New models and tools will be developed to enable the livestock industry to decrease environmental damage from methane and nitrogen emissions, and to improve efficiency of feed utilisation. A large-scale genetic association study involving 1000 dairy cows will relate feed intake, digestion efficiency, milk production/composition and methane emissions to the ruminal microbiome and host genome, leading to new indicator traits and tools for use in both traditional and genomic selection. Cow-reindeer metagenomic studies will establish how host species influence ruminal microbiology and function. Bovine twins studies will define how the rumen microbiome varies in an identical host genetic background. Nutrition work will assess how dietary oils, nitrogen and carbohydrates affect the ruminal microbiome and product quality in terms of milk composition. A meta-barcoding 16S rRNA analysis protocol will be developed to investigate ruminal microbiomes more accurately, rapidly and cheaply. Saliva and faeces will be analysed as possible tools for non-invasive assessment of ruminal microbiome and function. A novel method for on-farm methane analysis will be refined for easy application. Results will be publicly available through an online data warehouse that will provide tools to build new queries and create novel information. Transversal work packages include dissemination and industrial liaison, targeted towards the enlarged EU, and candidate and developing countries.



The concept driving the project is that there is a three-way interdependence that determines the efficiency of rumen function and therefore the emissions from ruminant livestock production (Fig. 1). Evidence that the animal itself controls its own microbiome is almost entirely anecdotal. One of the main aims of the project is to establish to what extent such host control of ruminal microbial ecology occurs, because if it is significant, and the characteristic is heritable, it should be possible to identify genotypes that produce lower emissions. The impact of nutrition on these interactions will be a major theme of the project. The technical driver is that DNA sequencing costs have fallen to such an extent, and speed has increased so massively, that bioinformatic investigations that were inconceivable only 2-3 years ago are now affordable and achievable.

Figure 1 An outline of the Ruminomics concept

The project partners and senior contacts are as follows:-

1. Rowett Institute of Nutrition and Health, University of Aberdeen UK. R.J. Wallace (coordinator)
2. Parco Tecnologico Padano, Lodi, Italy: J. L. Williams
3. Agrifood Research Finland, Jokioinen, Finland: K. J. Shingfield
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More information can be found at www.ruminomics.eu.

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Vendeen sheep prolificacy: no evidence for role of known major genes with large effects on ovulation rate

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Introduction Mutations with large effects on ovulation rate have been invoked to explain the exceptional prolificacy observed in many sheep populations and in some of these cases the causative mutations have been identified (Table 1). Vendeen sheep are a well known highly prolific sheep breed originating from the Vendee region of western France. While Vendeen sheep have been favoured due to increased performance including fecundity and high fertility no evidence has been provided to suggest a role for mutations with a large effect on ovulation rate in the prolificacy of Vendeen sheep. The objective of this study was to ascertain if any of the 10 established mutations with large effects on ovulation rate in sheep, or any other variations in these genes, are involved in the high prolificacy of Vendeen sheep.

Table 1 Known mutations with large effects on ovulation rate in sheep.

Gene	Breeds involved	Alleles	Chr.	Reference
BMP15	Romney, Lacaune, Cambridge, Belclare, Rasa-Argonesa	$FecX^{I}$, $FecX^{H}$,$FecX^{L}$, $FecX^{G}$,$FecX^{B}$, $FecX^{R}$	X	(Galloway *et al.* 2000; Hanrahan *et al.* 2004; Bodin *et al.* 2007; Monteagudo *et al.* 2009)
GDF9	Cambridge, Belclare, Thoka, Santa-Ines	$FecG^{H}$,$FecT^{T}$, $FecG^{E}$	*5*	(Hanrahan *et al.* 2004; Nicol *et al.* 2009; Silva *et al.* 2011)
BMPR1B	Merino	$FecB^{B}$	*6*	(Wilson *et al.* 2001)

Material and methods DNA, extracted from the whole blood using a modified detergent based method (Hanrahan *et al.* 2004), was obtained from 29 ewes born between 2004 to 2009. Litter size records (n=72) were available from 2006 to 2011 with over 74 % of the litter records showing twin, triple or quadruplet births (Figure 1). Genotyping of the 10 mutations, $FecX^{G}$, $FecT^{T}$, $FecG^{E}$, $FecX^{B}$, $FecG^{H}$, $FecX^{R}$, $FecX^{L}$, $FecX^{I}$, $FecX^{H}$ and $FecB^{B}$, were carried out via DNA sequence analysis (Eurofins-Medigenomix, Ebersberg, Germany) of the entire coding regions of all three genes, *BMPR1B*, *BMP15* and *GDF9*.

Results None of the ten mutations listed in Table 1 were detected in the set of sheep tested. Three previously identified mutations in *GDF9*, G2, G3 and G4 (Hanrahan *et al.* 2004), and a novel SNP resulting in a non-synonymous substitution, cysteine to arginine, in *BMP15* were identified.

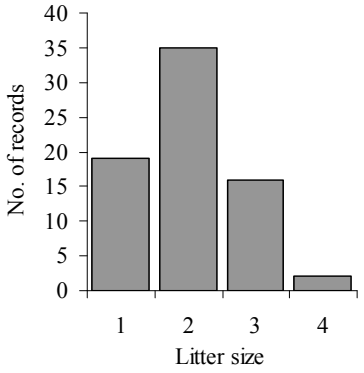

Figure 1 Vendeen litter size records

Conclusions The variation in litter size observed in this sample of Vendeen sheep was not due to any of the known mutations listed in Table 1. Thus there was no evidence that known mutations with large effects on ovulation rate are involved in Vendeen sheep prolificacy. Although a novel SNP resulting in a non synonymous substitution in *BMP15* was identified, larger sample sizes would be required to ascertain if this SNP is associated with litter size. Future analysis, on a larger sample set, involving genome wide association analysis of data generated using dense genome-wide SNP arrays or the rapidly developing high throughput sequencing technology would determine the genomic regions associated with prolificacy in Vendeen sheep and indicate whether members of the TGFß superfamily are involved.

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Do genotype by environment interactions (GxE) exist in Scottish Blackface ewes?

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Introduction Current breeding indices for hill sheep, such as the Scottish Blackface, include both lamb and maternal traits and have led to substantial improvements in economic returns (Conington *et al.,* 2006). However, when offspring are expected to perform in very different environments to where their sires were evaluated, it is often assumed that they will respond the same, regardless of their location. Inevitably this is not always the case, and the variation in genotype performance across different environments can be caused by genotype by environment interactions (GxE). The presence of GxE can reduce the efficiency of index selection and economic performance. The purpose of this study was therefore to identify possible GxE using records from ewes reared in two contrasting environments.

Material and methods The presence of GxE in ewe traits, between two hill sheep flocks, was investigated using performance data from Scottish Blackface ewes collected between 1993 and 2011. Pedigree information was available for 27548 animals and 25 of the sires used during this time period were used on both farms. Farm A is located on the East Coast of Scotland, whereas Farm B is on the West Coast. The farms differ in a number of aspects including annual rainfall, topography, vegetation, temperature and altitude ranges, with Farm B representing a harsher environment overall. Traits studied were 2 of the 6 ewe traits currently included in the breeding index for hill sheep, pre-mating live weight (PMWT; kg;) and the weight of lamb reared by the ewe to weaning, also known as the maternal weaning weight (MATWWT; kg). An additional trait, representing the efficiency of ewes that reared lambs, was calculated by dividing the total weight of lambs weaned by the PMWT of the ewes (EEFF; kg). To determine the level of GxE present for these traits, genetic correlations (r_g) were estimated for each trait between the two farms using bivariate analyses in ASREML (Gilmour *et al.* 2002), based on a model that fitted relevant fixed effects and direct genetic and permanent environmental (pe) random effects. The fixed effects included ewe age (in years), year, and grazing location (at pre-mating for PMWT and EEFF, at weaning for MATWWT). Lamb breed (to account for a small proportion of cross-bred lambs) and weaning category (the nature of the lambs reared: single male, single female, twin males, twin females, mixed twin, and multiple) were also fitted for MATWWT and EEFF. Likelihood ratio (LR) tests were used to determine if the r_g between farms, for each trait, were significantly different from 1. The log-likelihood for the original bivariate analysis ($logL_0$) was taken from an analysis where all parameters were estimated. The r_g between farms was then fixed close to unity (0.999) and the log-likelihood recalculated ($logL_1$). Using the test statistic, $LR = 2(logL_0 - logL_1)$, any significant GxE could be identified. The differences between sire estimated breeding values (EBVs) at each farm, for each trait, were tested using Pearson's and Spearman's rank correlations.

Results Estimates for h^2 and pe, for each trait, at each farm, are shown in Table 1. The r_g and LR estimates are shown in Table 2. A significant deviation from 1 was observed only for PMWT ($P<0.05$), suggesting the presence of GxE for this trait. Re-ranking and scaling of sires were observed, particularly for PMWT. Pearson's and Spearman's rank correlations were all 1 apart from PMWT (0.65 and 0.60 respectively).

Table 1 Univariate heritabilities (h^2), permanent environmental effects (*pe*)and phenotypic variances (σ^2_p) between farms for each trait (s.e. in parenthesis)

Trait	Farm	h^2	*pe*	σ^2_p
PMWT	Farm A	0.42 (0.03)	0.20 (0.02)	30.56 (0.67)
	Farm B	0.36 (0.03)	0.24 (0.03)	26.30 (0.64)
MATWWT	Farm A	0.14 (0.02)	0.03 (0.02)	27.76 (0.44)
	Farm B	0.18 (0.02)	0.04 (0.02)	24.39 (0.49)
EEFF	Farm A	0.13 (0.02)	0.13 (0.02)	0.01 (0.0002)
	Farm B	0.09 (0.02)	0.16 (0.02)	0.01 (0.0003)

Table 2 Genetic correlations (r_g) and likelihood ratios (LR) between farms for each trait

	PMWT	MATWWT	EEFF
r_g	0.453	0.928	0.987
s.e.	0.322	†	†
LR	7.96	1.06	0.40
P-value	0.005	0.30	0.53

† Standard error not estimable

Conclusions The lack of significant GxE observed for MATWWT and EEFF suggests that the offspring of common sires have performed similarly across both farms. Although Farm B is generally considered the harsher of the two farms, either the sires selected were suitable for both farms, or the farms did not differ sufficiently for any GxE to be detected. The GxE observed for PMWT could have implications for the future maintenance of the ewes. If ewes have unexpectantly high mature weights, they will require higher levels of inputs (eg. feed, grazing, and health treatments) to maintain their performance. Further analysis will investigate if similar results are found for the remaining ewe traits currently included in the hill sheep breeding index.

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A preliminary investigation into associations between clinical lameness and reproduction in UK dairy herds

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Introduction Poor foot health is often considered to be an important contributory factor in dairy herd fertility, but there has been little recent UK work in this field. One of the major limitations in wide-scale retrospective studies in this area is the variable quality of recording of clinical lameness cases on farm. The current study aimed to evaluate relationships between farmer-diagnosed clinical lameness and reproductive outcome in a small number of commercial UK dairy herds, with a view to establishing the feasibility of a larger, more detailed investigation.

Material and methods Routinely collected herd management and performance data from 468 herds across England and Wales was collated as part of a larger study. Data quality auditing was performed to remove datasets where there was no year between 2000 and 2008 where fertility, milk recording and clinical lameness data was sufficiently robust. From the remaining 62 herd datasets, 9 were randomly selected for a pilot investigation. In order to facilitate construction of a discrete time survival model, data were restructured into a format whereby each unit of data represented a 2-day risk period at between 20 and 220 days in milk. For each risk period, a binary outcome variable representing whether or not the cow became pregnant during the risk period was calculated (using subsequent calving events). In addition to this, a number of potential explanatory variables were also available. At risk period level, these included binary indicators of clinical lameness events at a variety of timeframes relative to the risk period and stage of lactation. Further potential explanatory variables at lactation level (including lactation 305d milk yield and parity) were also included.
Discrete time survival modelling was then carried out using the explanatory and outcome variables mentioned above. Model construction was by backward selection, with a quadratic term for the natural logarithm of days in milk used to represent stage of lactation and a categorical fixed effect used to represent the effect of herd. A two-level hierarchical framework was used, with risk periods nested within cows. Parameter estimation was performed using penalised quasi-likelihood methods in MLwiN (Rasbash, 2009). Terms were retained in the model where the estimate of their coefficient was more than twice the standard error associated with the estimate (equivalent to $P<0.05$).

Results The mean probability of a cow becoming pregnant in a risk period was 0.0146: this would equate to around 15% of eligible cows becoming pregnant every 21 days. Table 1 shows estimated odds ratios for the explanatory variables related to lameness: a variety of other (potentially confounding) explanatory variables were included in the model but are not presented in Table 1 for brevity.

Table 1 Odds ratios representing the associations between different timings of clinical lameness and the odds of a cow becoming pregnant during a 2-day risk period

Lameness recorded in timeframe relative to risk period?		n	Odds ratio	95% confidence interval	P-value
57-70d before	No	221684		-	
	Yes	4803	0.75	0.58 – 0.97	0.03
Within 7d	No	225485		-	
	Yes	1002	0.78	0.63 – 0.96	0.02
29-42d later	No	219110		-	
	Yes	7377	0.78	0.63 – 0.97	0.03

Clinical lameness cases recorded at 57-70 days before, within 7 days of, and 29-42 days after a risk period were all associated with a reduction of approximately 20-25% in the odds of a pregnancy becoming established during the risk period. A number of other timings of clinical lameness showed trends towards negative associations, but these effects were not significant at the chosen level (equivalent to $P<0.05$). Increasing parity, increasing milk yield, and time of year between April and September were all also associated with a decrease in reproductive performance: these were included in the model but not described in Table 1 for brevity.

Conclusions The results of this work suggest that significant associations between reproductive performance and clinical lameness events exist, at least in some herds. The magnitude of association revealed raises the possibility that a clinically significant association exists with fertility performance at cow level. This justifies a larger-scale investigation to improve understanding of these associations, including whether they are mediated predominantly by decreased submission or pregnancy rates and their potential impact on herd-level reproductive performance.

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Amylase addition increases starch ruminal digestion in dairy cows

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Introduction Exogenous feed enzyme additives are used to improve feed utilisation and animal performance. Addition of an amylase preparation has been shown to increase total tract digestibility of diets and boost milk production in dairy cows (Klingerman *et al*. 2009; Gencoglu *et al*., 2010). However, the mechanisms involved are not fully known. In this work we studied the effect of an exogenous amylase on rumen and total tract nutrient digestion parameters.

Material and methods Four lactating Holstein primiparous cows (avg. 545 kg, 90 DIM) fitted with rumen and proximal duodenum cannulae, were used in 4×4 Latin square design. Dietary treatments differed in starch level (300 vs. 200 g/kg DM) and amylase supplementation (Rumistar, DSM Nutritional Products, Basel, Switzerland). Diets (59% corn silage, 8% hay and 33% concentrate) were iso-energetic (1640 kCal NEL/kg DM) and iso-nitrogenous (160 g CP and 105 g digestible protein par kg DM) according to INRA (2007). High and low starch level was adjusted by changing the proportion of corn grain (44.4 vs 1.3%) and citrus pulp (6.3 vs 33.8%) in the concentrate. Periods lasted 4 weeks and heifers were fed at 95% of *ad libitum* intake during measurements. Total tract digestibility was determined by total faeces collection for 6 days. Duodenal digesta flow was determined using $YbCl_3$ as marker. Microbial N duodenal flow was determined using nucleic bases as markers, from a mixed rumen bacteria sample. In ruminal liquid, kinetics of ammonia and volatile fatty acids were determined before feeding and 1, 2.5, 5 and 8 h after feeding, and protozoa and microbial parameters were counted before and 2.5 h after feeding. Statistical analysis was performed using the Mixed procedure of SAS, with fixed effects for starch level, amylase supplementation, period and time (repeated for kinetics) and random for animal.

Results The DM intake and milk yield were not affected by treatments. Amylase supplementation increased the ruminal digestibility of starch and the true ruminal digestibility of OM at both starch levels. The exogenous enzyme in the high starch diet also increased the proportion of propionate. Other effects observed were a higher amylase activity detected in the solid-associated microbial community and a tendency for lower protozoal numbers. In contrast, the enzyme did not affect rumen pH and total VFA, total tract digestibility of starch and OM, rumen and total tract digestibility of NDF, rumen N balance and intestinal N digestibility, microbial N flow at duodenum, and N partition between faeces, urine, and milk. There were no changes on the microbial community as assessed by DGGE and no or small changes on selected fibrolytic and amylolytic bacteria numbers monitored by qPCR.

Table 1 Performances and digestion in dairy cows receiving diets differing in starch level with and without amylase supplementation

	High starch		Low starch			Starch and Amylase
	Control	Amylase	Control	Amylase	SE	effects
Dry matter intake (kg/d)	18.4	18.9	19.2	19.1	0.61	NS
Milk yield (kg/d)	24.7	25.3	24.0	23.7	0.82	NS
OM total tract digestibility (%)	71.4	71.5	72.1	70.8	0.81	NS
NDF total tract digestibility (%)	50.7	50.0	56.0	52.9	1.80	S*
Starch total tract digestibility (%)	98.4	99.2	97.6	97.6	0.34	S**
OM true rumen digestibility (%)	64.6	67.3	62.6	67.9	1.52	A*
NDF ruminal digestibility (%)	44.6	47.5	48.8	49.9	3.27	NS
Starch ruminal digestibility (%)	73.9	81.1	76.2	82.0	2.98	A*
Microbial N duodenum (g/d)	361	378	365	375	24	NS
Rumen pH	6.32	6.26	6.30	6.31	0.08	NS
Rumen N-NH3 mg/l	257	280	270	235	81	NS
Rumen total VFA mM	114	123	121	120	5.4	NS
Rumen acetate mol/100 mol	64.7	60.6	64.9	65.3	1.2	S** A* SxA*
Rumen propionate mol/100 mol	16.3	21.7	17.5	17.3	1.6	SxA†
Rumen butyrate mol/100 mol	15.2	13.4	14.6	14.1	1.0	NS

S : effect of starch level; A: effect of amylase (Rumistar) addition; SxA : starch x amylase interaction
NS: not significant; † P<0.10; * P<0.05; ** P<0.01

Conclusions The amylase additive increased starch digestion in the rumen of heifers fed high and low starch diets and modified VFA profiles with the high starch level. In the present work heifers had a moderate intake and production, with ruminal parameters remaining within optimal ranges for feed fermentation and digestibility. In high producing animals, where starch digestibility is compromised, exogenous amylase may be an alternative to improve performance.

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Evaluation of the efficiency of utilisation of nitrogen for Holstein heifers and steers at age of six months

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Introduction The efficiency of utilisation of nitrogen (N) is a key component for ruminant nutrition. Excretion of N in faeces and urine has two important impacts on environment, namely pollution of ground and surface water and stimulation of production of N_2O (an important greenhouse gas). However, there is little information available regarding to the efficiency of N utilisation in young cattle. The objective was to investigate N utilisation from young Holstein cattle managed under a typical UK feeding condition.

Material and methods The data used were obtained from a single period study (28 days) with 20 Holstein cattle (10 male steers vs. 10 heifers) at age of 6 months. These animals were selected from the dairy herd at the Agri-Food and Biosciences Institute when they reached 5 month age, and blocked into 10 pairs (steers vs. heifers) according to birth date, birth and weaning weights, growth rate and body condition score (BCS). Nitrogen intake and output data were measured for 4 days at the end of the study when they were housed in calorimeter chambers for measurements of gaseous exchange (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 diet typical of that used in UK production systems (grass silage/concentrates = 0.45/0.55, DM basis). The grass silage was prepared from the first harvest of perennial ryegrass sward and ensiled without application of silage additives. The concentrates were based on barley, maize, sugar beet pulp and soybean meal. All data were analysed using one-way ANOVA and some data were also analysed using linear regression techniques. The statistical programme used was Genstat 6.1 (6th edition; Lawes Agricultural Trust, Rothamsted, UK).

Results There was no significant difference between heifers and steers in DM intake (3.9 vs. 3.8 kg/d), BCS (2.60 vs. 2.63), live weight (175 vs. 176 kg) or live weight gain (0.71 vs. 0.72 kg/d). Data on N intake and outputs and the efficiency of N utilisation are presented in Table 1. The gender had no significant effects on N intake, N outputs in faeces and urine and N retention (g/d). There was no significant difference in urine N/N intake, manure N/N intake or N retention/N intake between the two groups, although heifers had a higher faecal N/N intake ($P < 0.05$). Three linear regression equations were developed using all data from heifers and steers. The relationship between urine N output and N intake was relatively poor with a R^2 of 0.25. The R^2 value was increased to 0.48 for the relationship between faecal N output and N intake. The R^2 value was further increased to 0.57 when using manure N output (y, g/d) against N intake (x, g/d) ($y = 0.940x - 33.0$, Figure 1).

Table 1. N utilisation data for Holstein heifers and steers of 6 month old

		Heifers	Steers	s.e.d.	Sig.
N intake and outputs (g/d)					
	Intake	97.2	93.4	4.53	NS
	Faeces	36.7	30.1	3.16	NS
	Urine	24.2	22.1	3.79	NS
	Manure	60.9	52.2	5.36	NS
	Retention	36.4	41.2	3.58	NS
Nitrogen utilisation					
	Faecal N/N intake	0.376	0.321	0.0247	P<0.05
	Urine N/N intake	0.249	0.232	0.0369	NS
	Manure N/N intake	0.625	0.554	0.0396	NS
	N retention/N intake	0.375	0.446	0.0396	NS

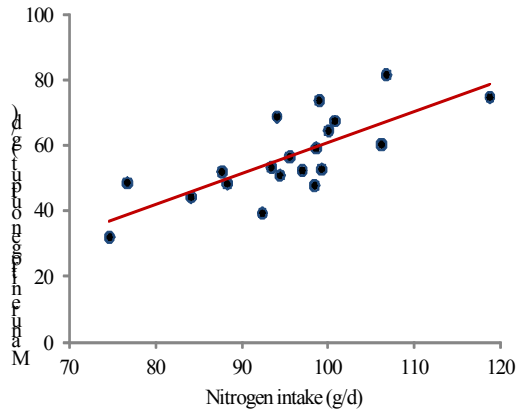

Fig 1. The relationship between manure N output and N intake

Conclusions There was no difference in N intake and outputs between Holstein heifers and steers of 6 month age. A linear relationship was developed to predict manure N output using N intake data for young Holstein cattle.

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Measurements of enteric methane emissions from Holstein heifers and steers at age of six months

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Introduction Methane (CH_4) emission from ruminant animals is a significant source of greenhouse gases which are responsible for global warming. Although CH_4 emission data for adult cattle have been reported extensively, there is little information available regarding CH_4 emissions from young cattle. The lack of data for young stock can impact the accuracy for development of CH_4 emission inventories for the dairy and beef industries. The objective of this study was to investigate CH_4 emissions from young Holstein cattle managed under a typical UK feeding condition.

Material and methods Twenty Holstein cattle (10 male steers vs. 10 heifers) at age of 5 months were selected from the dairy herd of the Agri-Food and Biosciences Institute for a single period study (28 days). The animals were blocked into 10 pairs (steers vs. heifers) according to birth date, birth and weaning weights, growth rate and body condition score (BCS). They were housed in a cubicle accommodation for the first 20 days, and then transferred to metabolism units where they stayed for further 3 days. Afterwards, they were housed in indirect open-circuit respiration calorimeters chamber for 5 days with measurement of gaseous exchange (CH_4, CO_2 and O_2) over the final 96-h period. All cattle were allowed free access to water and offered a diet typical of that used in UK systems (grass silage/concentrates = 0.45/0.55, DM basis). The grass silage was prepared from the first harvest of perennial ryegrass sward and ensiled without application of silage additives. 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 feed intake and CH_4 emission as the gender had no significant effects on these results. The statistical programme used was Genstat 6.1 (6th edition; Lawes Agricultural Trust, Rothamsted, UK).

Results There was no significant difference between heifers and steers in DM intake (4.0 vs. 3.9 kg/d), BCS (2.60 vs. 2.63), live weight (175 vs. 176 kg) or live weight gain (0.71 vs. 0.72 kg/d). Gender had no significant effects either on total CH_4 emission (g/d), CH_4 emission as a proportion of live weight or feed intake (DM, OM, Digestible DM or digestible OM), or CH_4 energy output (CH_4-E) as a proportion of energy intake (GE, DE or ME) (Table 1). The average CH_4-E/GE intake obtained in the present study was 0.075 for cattle at age of 6 months, which is higher than 0.070 of lactating dairy cows (Yan *et al*., 2010), but lower than 0.080 of growing-finishing beef cattle (Yan *et al*., 2009). The statistical analysis revealed that there were strong relationships between CH_4 (y, g/d) and DM intake (x, kg/d) (y = 29.0x - 13.2, R^2 = 0.70) and between CH_4-E (y, MJ/d) and GE intake (x, MJ/d) (Figure 1).

Table 1. Methane data for Holstein heifers and steers of 6 month old

	Heifers	Steers	s.e.d.	Sig.
CH_4 (g/d)	105.4	98.9	6.26	NS
CH_4/live weight (g/kg$^{0.75}$)	2.2	2.1	0.11	NS
CH_4/DM intake (g/kg)	26.0	26.3	0.86	NS
CH_4/OM intake (g/kg)	28.0	27.2	0.91	NS
CH_4/Digestible DM intake (g/kg)	33.1	31.6	1.24	NS
CH_4/Digestible OM intake (g/kg)	34.8	33.4	1.27	NS
CH_4-E/GE intake	0.076	0.074	0.0024	NS
CH_4-E/DE intake	0.097	0.093	0.0035	NS
CH_4-E/ME intake	0.112	0.106	0.0047	NS

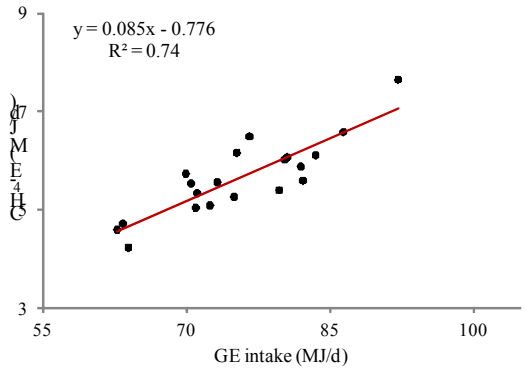

Figure 1. The relationship between CH_4 energy and GE intake

Conclusions There was no difference in CH_4 emissions between Holstein heifers and steers at 6 month of age. However, there were strong relationships between CH_4 emissions and GE intake. These relationships can be used to predict CH_4 production from young cattle for development of CH_4 emission inventories for dairy and beef cattle industries.

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 the breeding program at Terling dairy farm Essex on the resistance and susceptibility to disease in Holstein dairy cows

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Introduction Maximising dairy production was achieved through selective breeding cattle based on phenotypic traits classified as 'dairy type' (Shook, 2006). Continuous selection of cattle based on these traits required utilisation of superior sires and dams worldwide, incorporating intentional inbreeding to maximise their genetic contribution. However, over time this has increased relationships within dairy breeds, successively narrowing the gene pool (Powell and Norman, 2006). Inbreeding has been linked to decreased disease resistance (Sørensen *et al.,* 2006), thus compromising cattle welfare and productivity. Additionally, the Major Histocompatibilty Complex (MHC) genes (BoLA-DRB) are associated with genetic resistance and susceptibility to disease (Tizard, 2009). The aim of this study was to assess the effect of inbreeding on the heritability of disease resistance and susceptibility in cows from Terling dairy farm in Essex.

Material and methods Identification of two groups was established based on individual presentation of disease (mastitis, endometritis, peritonitis, dirty septic metritis, infectious bovine rhinotracheitis). The number of cows identified with high incidence of disease was n=23, and low incidence of disease n=22. An index was created based on the inverse relationships of incidence of disease and the number of lactations. Cows were classified as high incidence if they were >1 and low if they were <1. Extended pedigrees were obtained for analysis to ascertain the percentage of unique bloodlines in the two groups. 106 recurring individuals were identified, and the frequency of occurrence in the pedigree was analysed statistically using the chi-square test (χ^2). The frequency of recurring bloodlines between the two groups was analysed statistically using the χ^2. Hair samples from each cow were collected for DNA extraction and amplification of BoLA-DRB alleles of the MHC by polymerase chain reaction (PCR), to assess expression of alleles in relation to incidence of disease.

Results A three-generation pedigree for one cow consisted of 14 individual animals. A total of 308 animals make up the extended pedigrees of the cows from the low incidence group (n=22 x 14), and 322 individual animals made up the extended pedigrees from the high group (n=23 x 14). 106 recurring individuals were identified from the extended pedigrees for either the high group, low group or both. The cows sampled from Terling dairy farm did not have a significant difference in the unique bloodlines in relation to the incidence of disease (χ^2=0.26, p>0.05). Figure 1 illustrates the percentage of unique bloodlines from both low and high groups, where error bars represent the standard error of the mean. Additionally, the cow groups did not have a significant difference in the frequency of recurring bloodlines in relation to incidence of disease (χ^2=3.6, p>0.05), as shown in Figure 2. The success rates of DNA extractions and PCR amplifications were 14/45 and 1/45 respectively.

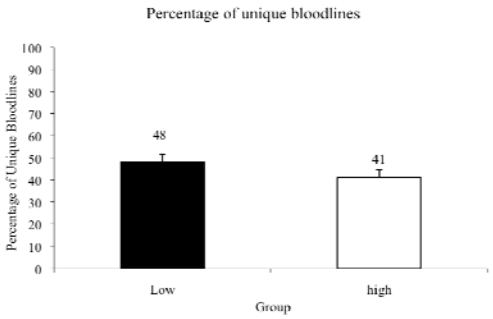

Figure 2 The percentage of unique bloodlines occurring in the groups.

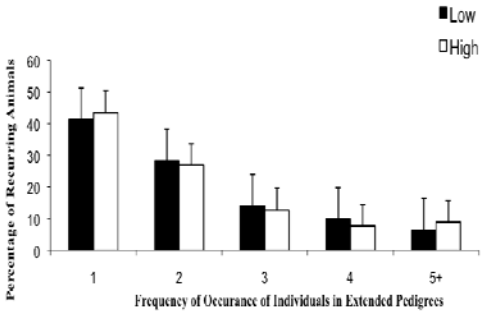

Figure 1 Frequencies of recurring bloodlines, (e.g. in the low group an individual animal appears 39 times out of 92; 41%).

Conclusions Although no statistical significance was found when comparing the pedigrees with incidence of disease, the low group did have an increased percentage of unique bloodlines, suggesting that inbreeding may negatively affect disease resistance in some animals from this herd. DNA analysis was unable to determine heritability of disease resistance in this study possibly due to a low quality template. Further research is needed to ascertain the heritability of disease resistance, and susceptibility in the Terling dairy herd.

Acknowledgements I gratefully acknowledge funding from BBSRC.

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Comparison of nitrogen-use efficiency and energy conversion efficiency as measures of feed conversion efficiency in Holstein-Friesian cows over an entire lactation cycle

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Introduction Over the last decade there has been an increased focus on feed conversion efficiency (FCE) in ruminant livestock, however direct selection has been limited by our inability to measure it for animals, particularly Dry Matter Intake (DMI). FCE can be difficult to interpret in dairy cows because of the mobilisation and replenishment of body reserves. In this work, an energy based measure of FCE, Energy Conversion Efficiency (ECE: milk energy/ME intake, MJ/MJ); and a nitrogen based measure, N-use Efficiency (NUE: Milk N/N intake, g/g) were compared over a 12-month period to investigate repeatability over time, as well as within- and between- lactations. We hypothesised that larger fluctuations in body fat may make NUE more consistent than ECE.

Material and methods Data was available from a previous study of the effects of concentrate level in the second half of lactation on production and body reserves of 43 primiparous Holstein-Friesians (Dewhurst *et al*., 2002). There was a 2 x 2 arrangement of treatments with cows having calved at either 2- or 3-years of age, and receiving either 2 or 7 kg/day of concentrates in the second half of the first lactation. All diets were based on *ad libitum* access to grass silage and all cows received the same allocation of concentrates (8 kg/day for 120 days, then 5 kg/day) in the second lactation. Individual recording lasted for one year, from the middle of the first- to the middle of the second-lactation. Efficiency, intake and milk production information were calculated as weekly means. All statistical analysis was conducted using Genstat for Windows (13th Edition). Linear regression analysis investigated the relationships between ECE (MJ/MJ), NUE (g/g), milk energy (MJ/day), and milk N (g/day). An initial analysis of variance identified a trend ($P < 0.1$) for a concentrate level x age interaction on both ECE and NUE, so the analysis of variance for repeated measures (Genstat PROCEDURE 'Repmeas') included effects and interactions of age, concentrate level and time.

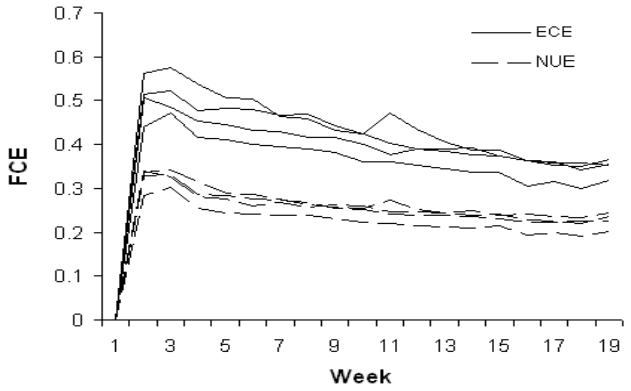
Results There were highly significant relationships between NUE and ECE for the average of both lactation periods (P<0.001), as well as for the lactation 1 (P<0.001) and lactation 2 periods separately (Figure 1; P<0.001). Milk N yield was a better predictor of NUE ($r^2 = 0.82$) than milk energy output ($r^2 = 0.76$), whilst milk energy output was a better predictor of ECE ($r^2 = 0.84$) than milk N yield ($r^2 = 0.79$). Results of the repeated measures analysis of variance are presented in Table 1. The SEM and CV% were lower for models using NUE rather than ECE, for both the lactation 1 and lactation 2 periods.

Table 1 *P*-values for treatment effects on ECE and NUE, with associated standard errors and coefficients of variation

	Measurement[1]			
	ECE(1)	ECE(2)	NUE(1)	NUE(2)
P-values				
age.conc	0.759	0.145	0.376	0.040
time	< 0.001	< 0.001	< 0.001	< 0.001
time.age.conc	0.007	0.379	< 0.001	0.360
SEM				
time	0.004	0.008	0.002	0.004
age.conc	0.018	0.026	0.010	0.012
time.age.conc	0.021	0.031	0.011	0.012
subject	0.048	0.069	0.026	0.032
subject.time	0.026	0.046	0.014	0.024
CV				
subject CV%	14.30	16.90	12.40	12.80
subject.time CV%	7.90	11.30	6.90	9.40

[1]Measurements: (1) = second half of the first lactation; (2) = first half of the second lactation

Figure 1 Average NUE and FCE for treatment groups over time in lactation period 2



NUE = Nitrogen-use efficiency (g/g); ECE = Energy Conversion Efficiency (MJ/MJ)

Conclusions There were highly significant correlations between NUE and ECE as estimates of feed conversion efficiency. Mobilisation of body fat in the first part of the second lactation led to an increase in ECE relative to NUE and this explains the greater consistency over time and better model fit for NUE measurements. It appears that NUE might provide more insight into inherent effects on feed conversion efficiency, independently of those involving mobilisation of body reserves.

Acknowledgement This work was supported by funding from a Teagasc Walsh Fellowship.

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Pastures fertilized with melamine contaminated fertilizers results in the deposition of melamine in cow's milk

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Introduction Melamine, an industrial chemical used in the production of various household items, contains 667 g/kg N. This makes it an attractive protein adulterant, as it has the ability to inflate the crude protein content of feed- and foodstuffs artificially. Our research confirmed for the first time that a pathway exists for the transmission of melamine from feed to milk (Cruywagen *et al*., 2009). Melamine has been used in fertilizers in the past as a source of slow release N. The aim of the present study was to determine whether melamine, when applied to pastures via fertilizers, would be absorbed by grass and deposited in milk by grazing dairy cows.

Material and methods In a pilot pot plant study, four pots with kikuyu grass (*Pennisetum clandestinum)* were used, of which two were fertilized at a rate equivalent to 8.8 kg of melamine/ha. Seven days after fertilization, the mean concentration of melamine in the grass was 228 mg/kg. It was thus decided to do the current trial with grazing dairy cows. Three pastures, 0.3 ha each, were used. One pasture served as control and received N fertilization in the form of limestone ammonium nitrate (LAN) at a rate of 40 kg N/ha. The other two pastures also received LAN, but with 10% (Treatment 1) and 20% (Treatment 2) of the LAN-N substituted with melamine-N. The respective melamine application was thus 5.97 and 11.94 g/ha. Pastures were also fertilized with P and K according to current recommendations. Pastures were cut to a height of 70 mm before fertilization. Pasture samples were taken once a week for 10 weeks. Eighteen lactating Holstein cows were stratified according to milk production and randomly allocated to three pasture groups. Cows were turned to pasture 28 days after fertilization to ensure sufficient regrowth. They were kept on the melamine fertilized pastures for 9 days and were allowed to graze for approximately 10 hours/day. After the 9 day period, melamine was withdrawn by placing the cows on the control pasture for another 7 days. Milk was collected twice daily for the duration of the trial and analysed for melamine (LC/MS-MS) and milk components. From Day 3 to Day 9, faecal samples were taken once daily from cows on the melamine contaminated pastures and analysed for melamine content. A one-way ANOVA was done on data pertaining to milk composition, using Statistica version 9 (2010). Means were separated with a Bonferonni test and significance was declared at $P<0.05$. For milk melamine concentrations, SE values were determined for inclusion in the bar chart in Figure 1.

Results Seven days after fertilization, the melamine content of the pasture grass was 10 and 32 mg/kg for Treatments 1 and 2, respectively. It declined rapidly and during the 7 days grazing period the pasture melamine content was 2 and 7 mg/kg for Treatments 1 and 2, respectively. In the same period, the faecal melamine content was 0.51 and 1.02 mg/kg for Treatments 1 and 2, respectively. Treatment had no effect on milk production or milk composition. Milk production and milk melamine contents are shown in Figure 1.

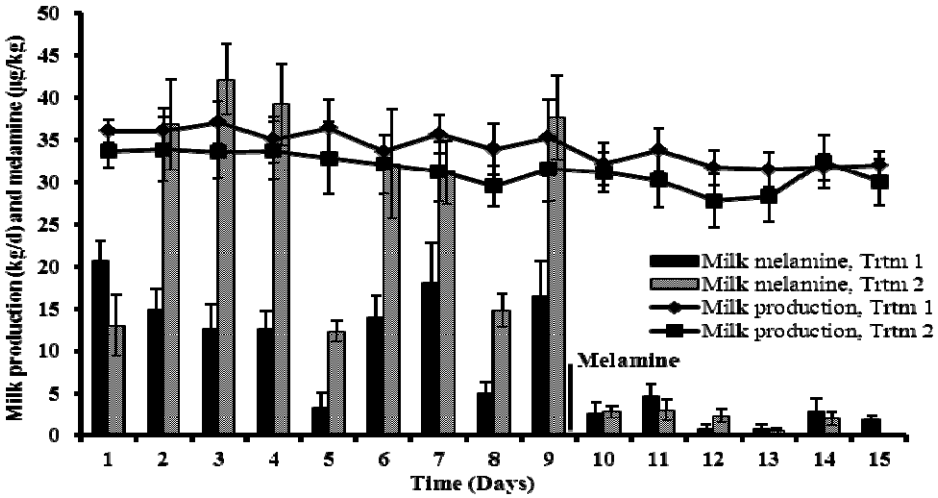

Figure 1 Milk production and milk melamine concentration of cows that grazed on melamine fertilized pastures

Conclusion It was concluded that melamine in fertilizers was absorbed by pasture grass and deposited in milk. Melamine excreted via faeces may recontaminate pastures.

Acknowledgements Funding from the National Research Foundation and the H. Steenberg Trust Fund is gratefully acknowledged.

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Estimation of correlated response selection for 305 day milk yield using monthly test day milk records in Iranian primiparous Holsteins

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Introduction For dairy cattle, selection for milk yield focuses on the use of 305 day lactation records. However, recently, test days have been used to enable earlier selection decision. The use of test day records to analyse milk production data has several advantages over utilising 305 day records such as better estimation of environmental factors that could affect cows differently during the lactation, allow for different shapes of lactation curve for each cow, account for different genetic and residual variances of yields during the course of the lactation, allow a cow to be evaluated based on only one TD record or any number of TD records during a lactation and decrease in generation interval (Jamrozik and Schaeffer, 1997). So, an alternative way to overcome the problems of 305 day records is to utilize TD yields instead of 305-d records. Genetic improvement of production traits could be achieved either by direct selection or by indirect selection (Olivier *et al*, 2001). For the traits such as daily milk yields, for which the definition varies continuously with time from calving, correlated response to selection for 305 day milk yield using monthly test day milk records seems to be important. The objectives of the present study was to estimate correlated response selection for 305 day milk yield records using monthly test day milk records in Iranian primiparous Holsteins.

Material and methods A total of 95,510 daily milk records collected from 11,054 Iranian Holstein cows first calved between 1994 and 2007 in 76 industrial dairy farms in Khorasan province was utilized. Predicted breeding values of individual cows at each month of lactation were initially obtained by a random regression test day model. In the random regression test day model, fixed effects of lactation stage, contemporary group of herd-year-season-sperm origin, as well as linear covariables of age of cow at recording, Holstein gene percentage, pregnancy and daily temperature were included. Additive genetic and permanent environment random effects were taken into account by orthogonal Legendre polynomials by order 4. The model was fit to the data by DXMRR software (Meyer, 1998). Predicted breeding value for 305 day milk yield was subsequently obtained from the predicted breeding values of individual month of the lactation. At the next stage, simple linear regression was applied to estimate regression coefficient of predicted breeding value for 305 day milk yield based on the predicted breeding value at individual months of the lactation. The estimated regression coefficient is the correlated response for 305 day milk yield as the selection is practiced on milk yield of individual months of the lactation course.

Results The results indicated that all regression coefficients were statistically significant ($P<0.01$). The highest and lowest regression coefficients were found for the second and first monthly test days, respectively. The estimated regression coefficients along with coefficients of determination are given in Table 1.

Table1 Estimated regression coefficients (b) for predicted breeding value of 305 day milk yield based on predicted breeding value at individual months of the lactation

Test day	1	2	3	4	5	6	7	8	9	10
b*	100.57	326.83	273.34	248.89	246.25	249.71	250.63	245.83	236.98	227.08
SE (b)	3.225	1.699	0.907	0.661	0.449	0.280	0.356	0.609	0.882	1.174
R^2	0.08	0.77	0.89	0.93	0.96	0.99	0.98	0.94	0.87	0.77

* All correlation coefficients were significant at $P<0.01$

Conclusion The result of this study revealed that correlated response for 305 day milk yield is maximised when the genetic selection is based upon milk yield of cows at month two of the lactation curve resulting in decreasing generation interval and increasing annual genetic gain. However, if the coefficient of determination is to be taken into account, predicted breeding value for the months six or seven of the lactation could be an alternative to the second month.

Acknowledgements The authors are grateful to the Centre of Animal Breeding, Iran for supplying the data used in this study.

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Evaluation of milk urea nitrogen of dairy cows reared under different feed bases in the different seasons

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Introduction Milk urea nitrogen, a fraction of milk protein that is derived from blood urea nitrogen, may be one of the useful tools that may help monitoring of any change required in the feeding and management of a herd. Milk urea nitrogen concentration for individual cow ranges from 8 to 25 mg/dl while optimum concentration for a herd ranges from 12 to 17 mg/dl (Hwang *et al.*, 2000). When blood urea nitrogen and milk urea nitrogen concentrations are lower than normal values, then more rumen degradable protein may be needed to meet the microbial N requirement for protein synthesis. Evaluation of milk urea nitrogen content during collection time of milk may give a good indication on the protein availability of cows from the plane of nutrition that varies on seasons and cropping systems. It is necessary to determine the concentration of milk urea nitrogen in dairy cows of Bangladesh. Thus, the present work was undertaken to determine the on-farm plane of nutrition and the existing status of milk urea nitrogen in relation to region, season, and genotype.

Material and methods Ten cows, each of native (local cow) and crossbred (local × Holstein-Friesian) origins differing in lactation yield were used to evaluate existing milk urea nitrogen of cows considering regions (good & poor feed base) and seasons (dry: Nov. – Feb. 2009 & wet: Jun. – Oct. 2009). A "good and/or poor feed base" region was classified based on the availability of quantity and quality roughages throughout the year. The roughage and concentrate feeds were supplied 2/2.5 times daily by the respective farmers to the selected cows, in the morning and evening. The concentrate feeds were given before and roughage feeds after each milking. Data on feeds and refusals were recorded and analyzed for nutrient composition according to AOAC (2004). The daily milk yield of individual cow was recorded and preserved at -20°C and analyzed for protein using a Milk Analyzer. Milk and blood serum were analyzed according to Baset *et al.* (2009) for milk and blood urea nitrogen. Data were analyzed in a 2×2×2 factorial experiment and subjected to ANOVA following the principles of RCBD (Randomized completely block design) using computer package GENSTAT and SED or LSD differentiated treatment means.

Results Effect of regions, seasons and genotypes on milk urea nitrogen of dairy cows is presented in Table 1. MUN depends on genotype and plane nutrition of cows, more specifically on dietary crude protein and rumen degradable protein. Dietary intake of nutrients was not fulfilled the requirement of cows, still then, level of MUN was found higher compared to other studies. In this study milk urea nitrogen concentration was found 28.55 to 38.86 mg/dl. Milk urea nitrogen concentration was significantly ($P<0.05$) influenced by the interaction of feed base regions and genotypes.

Table 1 Effect of regions, seasons and genotypes on milk urea nitrogen of dairy cows

Parameter	Regions		Seasons		Genotypes		SED/(LSD) and level of significance		
	Good Feedbase	Poor Feedbase	Dry season	Wet season	Local cow	Crossbred cow			
Liveweight,kg	350.9	215.5	284.0	282.3	247.5	318.8	$(14.0)^{**}$	7.0^{NS}	$(14.0)^{**}$
BCS	3.13	2.07	2.74	2.46	2.34	2.85	$(0.24)^{**}$	$(0.24)^{*}$	$(0.24)^{**}$
DM, kg/d	8.53	5.40	7.00	6.93	5.75	8.17	$(0.45)^{**}$	0.23^{NS}	$(0.45)^{**}$
ME, MJ/d	70.2	40.2	54.6	55.7	45.6	64.8	$(3.8)^{**}$	1.9^{NS}	(3.8)**
CP, g/d	839	386	664	561	517	708	$(50.0)^{**}$	$(50.0)^{**}$	$(50.0)^{**}$
RDP, g/d	488	166	358	296	276	378	$(32.9)^{**}$	$(32.9)^{**}$	$(32.9)^{**}$
4%FCM, kg	6.49	3.31	4.90	4.90	3.28	6.52	$(0.73)^{**}$	0.37^{NS}	$(0.73)^{**}$
Milk Protein,%	3.79	3.63	3.71	3.72	3.73	3.70	$(0.08)^{**}$	0.04^{NS}	$(0.08)^{**}$
MUN, mg/dl	38.86	28.55	36.79	36.87	31.97	35.44	$(2.39)^{**}$	1.31^{NS}	$(2.39)^{**}$
BUN, mg/dl	40.98	33.13	37.35	36.75	34.94	39.17	$(2.65)^{**}$	1.34^{NS}	$(2.65)^{**}$

**. Significant at the 0.01 level, *. Significant at the 0.05 level, NS. Not significant, BCS. Body condition score, FCM. Fat content of milk

Conclusions It may be concluded that the milk urea nitrogen depends on plane of nutrition of cows, seasons and their genotypes.

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Evaluation of nitrogen utilisation efficiency of dairy cows offered diets containing two levels of concentrates with or without yeast supplementation

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Introduction Yeast cultures based on *Saccharomyces Cerevisiae* are increasingly used in ruminant diets to improve rumen fermentation, feed intakes and animal performance. The objective of this study was to evaluate the effects of dietary supplementation of a live yeast culture on the efficiency of utilisation of nitrogen (N) of lactating dairy cows offered diets containing two levels of concentrates.

Material and methods The data used were obtained from a 2 (concentrate level) x 2 (yeast supplement) factorial design study with 4 periods (6 wk/period). Twenty early lactation dairy cows (56 ± 25 d *post partum*) were used, which were of 3 genotypes (4 Norwegian, 4 Norwegian x Holstein-Friesian and 12 Holstein-Friesian) and had various parities (4 primiparous and 16 multiparous cows). Animals were offered grass silage-based diets consisting of 2 levels of concentrates (300 and 600 g/kg DM), without or with supplement of 0.5 g/d of a concentrated live yeast. The grass silage had pH of 3.9, and contained CP, NDF, ADF and ash of 132, 464, 301 and 86 g/kg DM, respectively. The concentrates were based on soyabean meal, rape meal barley maize, soya hulls and citrus pulp. During each period, animals were housed as a single group in a cubicle accommodation and then transferred to individual stalls in digestibility units for measurements of N intake and outputs in faeces, urine and milk for 6 days at the end of each period. There was a 3-wk interval between each period. All data were analysed using two-way ANOVA with periods as block, and linear regression technique was also used to analyse relationships between N intake and excretions. The statistical programme used was Genstat 6.1 (6th edition; Lawes Agricultural Trust, Rothamsted, UK).

Results Increasing dietary concentrate level significantly increased feed intake, milk yield and milk protein concentration ($P < 0.001$), while yeast supplementation had no significant effects on live weight, feed intake, milk yield or milk composition. The effects of concentrate level and yeast supplementation on N intake and outputs and N utilisation efficiency are presented in Table 1. Dietary supplementation of yeast had no significant effect on any variable on N intake, output or N utilisation. Dietary concentrate levels also had no significant effect on any source of N output as a proportion of N intake, although cows offered the high concentrate diet had higher N intake and outputs in faeces, urine and milk ($P < 0.001$). The statistical analysis of all data demonstrated that there were good relationships between N intake (x, g/d) and N outputs (y, g/d) in faeces ($y = 0.248x + 39$, $R^2 = 0.59$), urine ($y = 0.385x - 11$, $R^2 = 0.49$), manure ($y = 0.633x + 29$, $R^2 = 0.70$), and milk ($y = 0.202x + 20$, $R^2 = 0.50$). When omitting the constant, manure N output was found to be 0.69 of N intake. This value is slightly lower than that of 0.72 reported by Yan *et al*. (2006) using a meta-analysis of lactating dairy cow data (n = 564) obtained at this Institute.

Table 1. The effects of concentrate levels and yeast supplementation on the efficiency of N utilisation of lactating dairy cows

	30% conc. diets		60% conc. diets		s.e.d.	Significance		
	Control	Yeast	Control	Yeast		Conc.	Yeast	Interaction
N intake (g/d)	407	403	576	574	22.7	***	NS	NS
Faecal N (g/d)	134	144	181	184	8.6	***	NS	NS
Urine N (g/d)	153	136	205	215	14.5	***	NS	NS
Milk N (g/d)	101	104	140	138	7.7	***	NS	NS
Retained N (g/d)	23	21	50	36	16.5	NS	NS	NS
Faecal N/N intake	0.33	0.35	0.32	0.33	0.014	NS	NS	NS
Urine N/N intake	0.38	0.34	0.36	0.37	0.027	NS	NS	NS
Manure N/N intake	0.71	0.70	0.67	0.70	0.032	NS	NS	NS
Milk N/N intake	0.25	0.26	0.25	0.24	0.011	NS	NS	NS
Retained N/N intake	0.05	0.05	0.08	0.06	0.031	NS	NS	NS

Conclusions Dietary supplementation of yeast culture had no significant effects on the efficiency of N utilisation in lactating dairy cows offered diets containing two levels of concentrates. An equation was developed to predict manure N output using N intake data.

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Regional and seasonal variation in the fatty acid profile of bulk milk, predicted using infra-red spectroscopy, from farms across the United Kingdom

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Introduction Milk and dairy products are a major source of saturated fatty acids (SFA) in UK diets, contributing to between 30 and 40 % total SFA intake (Hulshof *et al*., 1999). Most published information on milk fatty acids involves small numbers of cows and individual cow samples, and there are few studies where milk available to the UK consumer has been analysed and how its composition is affected by season and geographical area. Also, until relatively recently, accumulating large amounts of data has been hampered by traditional means of analysis which are complex and slow. Analysis of milk by mid-infrared (MIR) spectroscopy, traditionally used to predict milk fat and protein concentration, can now be applied for predicting key families of fatty acids conveniently and quickly. The objective of this study was to identify any regional and seasonal differences in average milk fatty acid profile from farms supplying one milk purchaser.

Material and methods Farms supplying the milk purchaser were located around one of seven regional depots throughout the UK (Denbighshire, Dorset, Dumfriesshire, Essex, Lancashire, Leicestershire, north Devon). Bulk tank milk obtained at regular intervals dependent on depot was analysed for milk fat and protein content, as well as total SFA, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) by Fourier Transform MIR spectroscopy prediction (National Milk Laboratories, NML, Wolverhampton, UK). Data were compiled on a monthly basis over the course of one year (June 2010 – May 2011), with monthly means for each farm. Fatty acid results as g/100 g milk were converted to g/100 g fatty acids using total fat content and a conversion factor of 0.95 (IDF, 2010). Effect of month, region and month by region interaction were analysed using a general linear model ANOVA (Minitab), with region as a fixed effect and month as a random effect. Pairwise comparisons were conducted to distinguish differences between individual regions. Least square means are presented, and all effects were deemed significant when $P < 0.05$.

Results There was an effect of month ($P<0.001$) for all parameters measured. Mean milk fat content was highest and lowest across all regions during December and June, respectively (4.57 vs 3.97 g/100 g milk), whereas mean milk protein content was highest and lowest during December and July/April respectively (3.47 vs 3.29 g/100 g milk). Milk fat SFA concentration peaked during October (70.6 g/100 g fatty acids) and fell to 64.0 g/100 g fatty acids in May, and unsaturated fatty acids were highest and lowest during summer and winter months, respectively (MUFA, August vs December, 30.0 vs 24.0 g/100 g fatty acids; PUFA, May vs January, 4.35 vs 2.68 g/100 g fatty acids). Region affected milk composition, with Essex having the highest ($P<0.05$) fat and protein content (Table 1). Essex and north Devon also had higher ($P<0.05$) SFA concentrations than the other regions, and Lancashire the lowest. Inversely, milk fat from the Lancashire (and Dumfriesshire) region had higher ($P<0.05$) proportions of MUFA, whereas Essex had the lowest. Milk fat PUFA concentration was greatest ($P<0.05$) in milk from Dumfriesshire followed by Lancashire.

Table 1 Effect of region on milk composition (least square means and s.e.m.).

	Denbighshire	Dorset	Dumfriesshire	Essex	Lancashire	Leicestershire	North Devon	s.e.m.	P (region)
Composition (g/100 g milk)									
Milk fat	4.29^{b}	4.13^{d}	4.18^{cd}	4.50^{a}	4.19^{c}	4.19^{bcd}	4.18^{cd}	0.101	<0.001
Milk protein	3.40^{b}	3.34^{c}	3.32^{cd}	3.48^{a}	3.30^{d}	3.34^{c}	3.34^{c}	0.048	<0.001
Fatty acid profile (g/100 g fatty acids)									
∑SFA[1]	68.8^{b}	69.1^{b}	69.2^{b}	69.8^{a}	67.6^{c}	69.0^{b}	69.4^{ab}	0.91	<0.001
∑MUFA[1]	27.2^{b}	26.8^{bc}	28.3^{a}	27.0^{d}	28.3^{a}	27.0^{b}	26.5^{c}	0.57	<0.001
∑PUFA[1]	3.35^{c}	3.36^{c}	3.53^{a}	3.32^{c}	3.42^{b}	3.36^{c}	3.33^{c}	0.100	0.002

[1] SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.
Means within row with different superscripts are significantly different ($P<0.05$).

Conclusions Monthly variations in milk composition and fatty acid profile are consistent with seasonal variation in cow feeding and management systems typical to the United Kingdom. Similar seasonal variation has been observed previously (e.g. Stergiadis *et al*., 2009). Regional averages suggest those regions associated with more intensive dairy production (i.e. south east of the UK) produce milk with higher fat, protein and SFA content than those associated with less intensive systems. These variable results highlight the need to reconsider the approach to retail labelling of milk and dairy products, which at present uses fixed reference values.

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Effects of dietary rumen-protected conjugated linoleic acids on fatty acid composition of lipids of milk, liver, muscle and adipose tissue of dairy German Holstein heifers in early lactation

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Introduction Among the many different ruminal derived isomers of conjugated linoleic acids (CLA), *cis*-9,*trans*-11 C18:2 (*c*9,*t*11-CLA) has quantitatively the highest percentage in dairy products whereas *trans*-10,*cis*-12 C18:2 (*t*10,*c*12-CLA) is beside *c*9,*t*11-CLA mainly found in synthetic CLA products. During the transition and early lactation periods of dairy cattle, the need for nutrients and energy increases rapidly and might lead to a negative energy balance (Bell *et al*. 1995). Therefore, the milk fat-reducing effect of *t*10,*c*12-CLA became of interest in reducing the energy output *via* milk lipids. The objective of the present study was to compare the effect of a rumen-protected CLA supplement on the fatty acid distribution of milk lipids and lipids of different tissues with focus on selected fatty acid parameters.

Material and methods Twenty-five primiparous lactating German Holstein cows were randomly assigned to 5 groups. All animals received a prepartum diet consisting of a partial mixed ration (PMR; 60% corn silage, 40% grass silage) *ad libitum* and additionally 2 kg concentrate/d. An initial group of 5 cows was slaughtered one day after parturition. Starting at 1 DIM, the other four groups were fed a PMR (25% grass silage, 38% corn silage, 37% concentrate on DM basis) *ad libitum* and additionally 3.5 kg concentrate/d which contained either 100 g of the CLA supplement or 100 g of a control fat preparation. The CLA-supplemented diet provided 6.0 g/d of the *t*10,*c*12-CLA and 5.7 g/d of the *c*9,*t*11-CLA isomer. After 42 DIM (period 1), 5 cows of a control group (42/CON) and 5 cows of the supplemented group (42/CLA) were slaughtered. After 105 DIM (period 2), 5 cows of the control (105/CON) and of the supplemented group (105/CLA) of the remaining animals were slaughtered. Each week, milk samples were taken. Furthermore, tissue samples from liver, mammary gland, muscle *longissimus dorsi* and retroperitoneal adipose tissue were sampled immediately after slaughter. Lipids of the collected milk samples and of the different tissues were extracted and transesterified into fatty acid methyl esters (FAME). Separation and detection of the resulting FAME were carried out *via* gas chromatography and flame ionization detector in two steps. In the first step, FAME with a chain length of 4 to 26 carbon atoms were separated with a medium polar column (DB-225MS, 60 m) following a temperature program. In the second step, the separation of *cis* and *trans* isomers of octadecenoic FAME was performed with a high-polarity column (Select™ FAME, 200 m) under isothermal conditions. Statistical analysis was performed using SPSS Statistics Version 19.0 (IBM, Armonk, USA). For the statistical analysis of fatty acids of milk samples the linear mixed model of SPSS was applied. Statistical analysis of fatty acids of different tissues (liver tissue, retroperitoneal adipose tissue, muscle tissue and tissue of the mammary gland) was performed with the independent samples *t*-test (mean ± SD). For all statistical analyses significant differences were defined as $P < 0.05$.

Results The supplementation led to a significant increase of both CLA isomers in milk lipids after 42 and 105 days (Table 1). Saturated fatty acids (SFA) declined whereas monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and total *trans* C18:1 increased, with a more pronounced effect after 42 days. Preformed fatty acids (>C16) were increased after 42 and 105 days of supplementation. Significant changes of the supplemented isomers in the other tissues were not observed with the exception of the mammary gland adipose tissue. Different fatty acid ratios remained unaffected in all tissues (data not shown).

Table 1 Fatty acids of milk lipids (period 1 and 2) and of mammary gland (42 DIM) [% of selected FAME]

	Milk (period 1)				Milk (period 2)				Mammary gland (42 DIM)		
	CON	CLA	SEM	P	CON	CLA	SEM	P	CON	CLA	P
*c*9,*t*11-CLA	0.36[b]	0.52[a]	0.025	<0.001	0.33	0.5	0.024	<0.001	0.37 ± 0.03	0.49 ± 0.11	0.076
*t*10,*c*12-CLA	<0.01[b]	0.05[a]	0.002	<0.001	<0.01	0.04	0.005	<0.001	n.d.	0.05 ± 0.01[*]	0.001
SFA	73.4[a]	68.2[b]	1.052	0.002	77.8	71.9	1.067	0.003	69.0 ± 3.20	64.8 ± 3.74	0.093
MUFA	23.9[b]	28.6[a]	1.004	0.004	19.7	24.9	1.028	0.006	26.3 ± 2.89	29.0 ± 2.90	0.180
PUFA+CLA	2.64[b]	3.24[a]	0.087	<0.001	2.41	3.20	0.134	0.006	4.64 ± 0.69	6.14 ± 0.86[*]	0.016
C18:1 *trans*	2.56[b]	3.65[a]	0.237	0.005	1.95	2.90	0.13	0.001	1.81 ± 0.34	2.37 ± 0.20[*]	0.018
>C16	36.1[b]	42.4[a]	1.293	0.003	29.4	37.6	1.547	0.004	-	-	-

Period 1: 1 to 42 DIM; period 2: > 42 to 105 DIM; [ab] means of one sample per week; * *t*-test

Conclusions The supplementation of rumen-protected CLA mainly affected fatty acid parameters of milk lipids as well as lipids of the mammary gland. Changes in fatty acid distribution of other tissues - if at all - were marginal. Thus, the given amount of CLA seems insufficiently to act as a potent medium for influencing the fatty acid distribution of different tissues except milk lipids and lipids of the mammary gland.

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The effect of persistent volcanic activity on the composition and nutritive value of the grass *Hyparrhenia rufa*

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Introduction Hazards related to volcanoes are eruptions, earthquakes and landslides. These hazards can cause wide spread, even global devastation depending on the vulnerability of the local environment and the magnitude of the effects. Less well known are the ongoing or chronic effects associated with persistently active volcanoes that can be just as damaging in many ways as they restrict biodiversity and impede economic development and poverty alleviation over long periods. Masaya Volcano in Nicaragua persistently produces a low altitude plume of gas which has produced a devastated 'kill zone' apparently devoid of plant life, but outside of this there is evidence of other effects on vegetation and public health in areas exposed to the plume (Delmelle *et al*., 2002). The plume contains mostly water, but also CO_2, SO_2, HCl, H_2S and other compounds (Langmann *et al*., 2009). The objective of this experiment was to determine the impact of this continuous volcanic plume on the chemical composition and nutritive value of the grass *Hyparrhenia rufa* which is found in many sites around the volcano, and which is grazed by cattle within the Masaya Volcano National Park boundaries.

Material and methods Samples of *H. rufa* were collected in the dry season. In Experiment 1, samples (n=3 per treatment) were taken from sites either directly under the plume (PLUME: samples taken 700, 1300 and 1800 m from the crater) or diametrically opposite the plume (CLEAR: samples taken 1900 m from crater in an area where there were active fumaroles) were analysed for organic matter (OM), acid detergent fibre (ADF), neutral detergent fibre (NDF) and ADF/NDF ratio. In Experiment 2, the three PLUME samples, one of the CLEAR samples and an additional five samples (taken randomly from different sites around the park, with varying distance from the plume) were analysed for *in vitro* organic matter digestibility (IVOMD, Tilley and Terry, 1963) using rumen fluid taken from two lactating cows. Differences between PLUME and CLEAR in Experiment 1 were determined by analysis of variance. In Experiment 2, the distance of samples from the plume was measured with reference to their elevation and from aerial photographs of the volcano. The relationship between IVOMD and distance from the plume was analysed by regression (Minitab 15, Minitab Inc., Pennsylvania, USA).

Results Grass was greenest and most abundant in areas under the plume, or near the fumaroles. The composition of PLUME and CLEAR grasses is summarised in Table 1. Both grasses had high fibre contents, but grass growing away from the plume (CLEAR) had a slightly lower fibre content compared with that which was growing under the plume. The IVOMD values of the grasses taken from different areas around the Park are summarised in Table 2. Again, all grasses were characterised as being of low nutritive value, but there was a significant relationship between IVOMD and the distance from the plume (R^2=0.551, P=0.022); IVOMD = 210 + 0.00735x where x is the distance (m) from the plume.

Table 1 The effect of plume proximity on the chemical composition of H.rufa

	Composition, g/kg DM			
	OM	ADF	NDF	ADF/NDF
PLUME	901	535	794	0.673
CLEAR	912	502	756	0.664
SEM	6.6	12.0	9.2	0.0105
P	0.172	0.042	0.005	0.474

Table 2 IVOMD of different samples of H. rufa

Distance from plume (m)	IVOMD (g/kgDM)
190	208
30	210
140	212
1667	233
2667	223
50	207
83	211
917	211
583	223

Conclusions This dry season forage was of low quality (as evidenced by the low values and small variation in IVOMD). However, it was greener and more abundant in areas beneath the plume suggesting the plume's water encouraged grass growth. However, forage quality was lower in areas subject to the plume. The selection of grass species that could maintain nutritive value in this environment would enhance the potential of livestock production in this area.

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The effect of different C_{32} alkane dosing regimens on forage intake predictions for grazing beef cattle of either dairy or suckler origin

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Introduction The n-alkane technique estimates herbage intake of grazing livestock by using the ratio, in their faeces, of a C_{32} alkane marker dosed intra-ruminally in known amounts, to a C_{33} alkane naturally present, in known concentration, in the herbage consumed (Mayes *et al* 1986). When applying the technique to grazing cattle, a daily dose of 1g of C_{32} alkanes is administered as two separate 500mg amounts (am and pm) to ensure that the dosed marker passes through the alimentary tract at an acceptably constant rate (Mayes *et al*. 1986). However, twice-daily dosing at approximately 12 hour intervals may be impractical in extensive grazing situations. The objective of the current study was to compare the effect on intake estimations of dosing once daily, or twice daily at either a 12 hour interval or at a shorter, more practical interval of 9 hours. An additional objective was to compare the herbage intake of dairy-origin beef cattle with suckler-origin beef cattle.

Material and methods Forty eight spring-born beef cattle (12-15 months old), were allocated to three treatments (T1, T2 and T3) each comprising 3 Charolais heifers (mean initial live weight 386 kg (s.d. 45)), 5 Charolais steers (mean initial live weight 432 kg (s.d. 35)) and 8 Holstein-Friesian steers (mean initial live weight 365 kg (s.d. 30)). Treatments were balanced according to animal live weight, age-within-breed and gender. The study was undertaken in the late spring (April 2011). Treatments were as follows: T1, dosing once daily (1030h) with two 500mg boluses of C_{32}alkanes; T2, dosing twice daily (0900h and 1600h) with one 500mg bolus each time and T3, dosing twice daily (0700h and 1900h) with one 500mg bolus each time. Each treatment group grazed a perennial ryegrass sward divided into three 0.9 ha paddocks. Herbage samples (200g), representative of herbage consumed, were taken daily from each paddock between 16th and 20th April. Faeces samples (at least 50g) were taken *per rectum* from all animals between 18th and 22nd April at 1030h (T1), 0900 and 1600h (T2) and 0700 and 1900h (T3). An additional faeces sample was taken from T1 cattle at 1500h so that all animals were subjected to a twice-daily faeces sampling regime. This additional sample was not analysed in the current study. Faeces samples were bulked for the 5 days of collection, on an individual animal basis, with samples collected twice per day contributing approximately equally by volume to the sample for that day. Herbage and faeces samples were freeze dried and oven dried, respectively, and then hammer-milled to pass a 0.8 mm screen. The concentration of each alkane in the dried and milled herbage and faeces samples was determined by capillary gas-liquid chromatography after saponification, extraction into heptane and passage through a silica gel mini-column. Data were analysed using REML procedures.

Results There were no significant (P>0.05) interactions so only the main effects of dosing interval and animal origin are presented in Table 1. Dosing interval had no significant (P>0.05) effect on estimated herbage intake expressed either as dry matter intake (kg per day) or dry matter intake per kg animal live weight. However, dairy-origin cattle had significantly higher (P<0.01) estimated dry matter herbage intake per kg live weight than their suckler origin counterparts.

Table 1 The effect of dosing interval and origin on dry matter intake

	Dosing interval					Origin			
	T 1	T 2	T 3	sed	Sig	Dairy	Suckler	sed	Sig
Dry matter intake									
kg per day	7.27	6.72	7.07	0.290	NS	7.13	6.91	0.237	NS
kg per day / kg live weight	0.019	0.017	0.018	0.0010	NS	0.020	0.017	0.0008	**

Conclusion The three C_{32} alkane dosing regimens evaluated in the current study predicted the grazing beef cattle to have a similar forage intake, consequently dosing beef cattle with two 500 mg of C_{32} n-alkane boluses administered simultaneously (i.e. once daily) is a more practical regimen for estimating the forage intake of free grazing beef cattle relative to the traditional twice daily dosing regime. The origin of beef cattle (dairy vs suckler) affected herbage intake in the current study with dairy-origin animals having a higher estimated dry matter intake per kg live weight relative to suckler-origin animals.

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Roughage intake by captive browsing ruminants: the case of the greater kudu (*Tragelaphus strepsiceros*)

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Introduction Diet-related disorders are a major problem in the husbandry of captive browsing ruminants. The problem is generally attributed to the difficulty in providing suitable roughages, which have led to the relative oversupply of highly digestible feeds, such as commercial fruits and cereal-based pellets. Feeding diets high in sugars and starch and cause ruminal acidosis and a number of other diet-related disorders, which can have serious implications on captive animal health, welfare and longevity, and, consequently, conservation strategies for endangered species. Comparing the diet of captive individuals to their free-ranging counterparts could highlight existing problems in captive feeding practices to help reduce the putative incidence of diet-related disorders. Therefore, the aim of this study was to assess the current feeding practices for captive greater kudus (*Tragelaphus strepsiceros*) by comparing the dietary and fecal nutrient composition between captive and free-ranging greater kudus and the current feeding recommendations for captive browsing ruminants.

Material and methods Fifteen greater kudus from three zoological collections were assessed during November 2010. Each individual animal was assigned a body condition score (BCS) and faecal consistency score (FS) and the body mass was estimated. Food intake was measured over a consecutive 3-day period by weighing individual food items offered and subtracting the mass of the food not consumed on a daily basis. Both feed and faecal samples were taken and analysed for dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and crude protein (CP) content. Cellulose content was then calculated as ADF-ADL. Lucerne hay quality was assessed using the NDF, ADF and CP content of the lucerne hay (Baylor and Rohweder, 1979). Spearman's rank correlation coefficient was used to test for a correlation between BCS or FS and the dietary and/or faecal nutrients. Finally, the results were compared to the dietary nutrient composition of free-ranging greater kudus, which were calculated from the dataset of Owen-Smith and Cooper (1989).

Results Only four of fifteen captive individuals had a normal BCS and FS. Dry matter intake (DMI) varied considerably in captivity, from 49.2 to 75.7g DM kg $BM^{-0.75}$ d^{-1}. Roughage intake determined DMI, with lucerne hay intake increasing as hay quality increased, and a low DMI of roughage was associated with low BCS. In addition, FSs were positively correlated with dietary CP and faecal nitrogen, and negatively correlated with faecal ADF, indicating that a higher proportion of highly digestible feeds resulted in softer FSs. The level of ADF in the zoo diets was comparable to that in the diet of free-ranging greater kudus (24.7-29.4%), but the latter consumed diets with less cellulose and more ADL.

Conclusions These results suggest that diets fed to captive browsing ruminants are too digestible, even in terms of fibre composition, leading to low BCSs and softer faeces. Hence, rather than trying to meet energy and nutrient requirements by offering a nutrient dense ration and roughage, the findings of this study indicate that feeding high-quality roughage is a better strategy, because it is ingested disproportionally more and thus increases overall intake and, in particular, fibre intake. Additionally, pelleted feeds used to supplement roughage should contain a high proportion of fibre, a moderate proportion of protein, and be limited in the proportion of easily digestible carbohydrates such as starch or sugars.

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Investigation of the feedstuff composition by Fournier Transform Infrared Spectroscopy (FTIR)

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Introduction Good knowledge of the nutritional value of feeds is vital to optimize animal nutrition. However feedstuffs used to feed ruminants are very variable in their physical, chemical and nutritional characteristics, therefore the development of new methods to evaluate them is critical. Relative to conventional laboratory procedures, prediction of compositional parameters by chemometric analysis of feedstuff spectra would offer the advantages of simplicity, speed, reduced chemical waste, and provide more cost-effective predictions. The aim of this study was to investigate the potential of FTIR technique to discern between the spectra of a wide set of feedstuffs samples differing in their nutritional value.

Material and methods A total of 786 feedstuff samples were investigated and classified into 16 groups: 38 samples of barley/wheat forage (silage, whole crop and straw), 111 of grass/clover forage (silage and hay), 39 legume forages (peas silage, galega, lupin, field beans, lucerne and lucerne pellets), 32 maize forages, 22 maize silages, 19 total mixed ration samples, 9 tropical feeds, 18 cereal mill by products (wheat bran, maize/wheat feed-meal and gluten-feed), 127 concentrate mixtures, 35 distillers by-products (corn, wheat and barley), 63 cereal grains (barley, oat, wheat, triticale, maize, ryegrass and corn cob), 16 soybean hull samples, 200 oil by-products (soybean, sunflower, rapeseed, cotton, coconut, sesame and palm kernel cake), 22 beet by-products (fodder beets and dry sugar beet pulp), 17 protein products (>30% CP, guar meal, brewers grains, malt spouts and potato protein), 18 legume seeds (field beans, lupin, peas, repeseed, soybeans and roasted soybeans). All samples were dried and ground to pass through a 1 mm diameter sieve, duplicate infrared spectra were collected by attenuated total reflectance (ATR) from 4000-600 cm^{-1} at a resolution of 2 cm^{-1} using an Equinox 55 FTIR spectrometer (Bruker Optik GmbH, Germany) fitted with a Golden Gate ATR accessory (Specac, UK). Spectra were averaged, derivatised to the 1st Savitsky-Golay derivative using a 13 point window and centre normalised using MatLab (version 2008a, The MathWorks Inc., UK). Spectral differences between forage and non-forage feedstuffs were investigated by principal component analysis (PCA) and subsequently a multivariate analysis of variance (MANOVA) was performed to identify differences between groups. Finally, a canonical variate analysis (CVA) showing the 95% confidence interval was achieved by using GenStat.

Results Principal component analysis of the spectra showed that 81% of the total variance was captured in the first 5 components and forage and non-forage spectra were separated along PC axis 2. Analysis of the first 5 PC scores by MANOVA detected significant differences between forage and non-forage samples (P<0.001, Wilk's lambda: 0.345) and also between the 16 feedstuff groups considered (MANOVA P<0.001, Wilk's lambda: 0.008). Canonical analysis of variance (Figure 2) showed a clear separation between spectra from most of the 16 feedstuff groups considered, canonical axis 1 allowed separation between forage (left) and non-forage samples (right), while canonical axis 2 allowed for separation between feedstuffs with high protein (bottom) and high non-structural carbohydrate contents (top).

Figure 1 PCA of feedstuffs analyzed by FTIR.

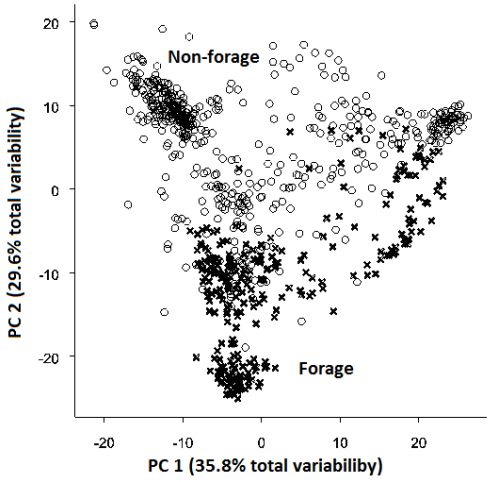
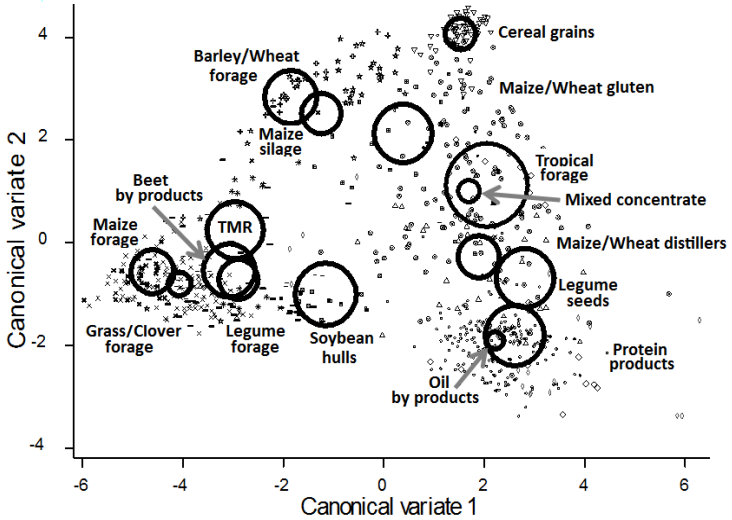


Figure 2 CVA and 95% confidence interval of feedstuff samples



Conclusions This work shows that analysis of mid-infrared spectra can indentify clear differences between feedstuffs of different nutritional value. More research needs to be done to determine whether this approach can provide a reliable, and potentially cheap high through-put technique to predict the composition and nutritional value of feedstuffs.

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Prediction of feedstuff composition by Fourier Transform Infrared Spectroscopy (FTIR)

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Introduction Crude protein (CP) and neutral detergent fibre content (NDF) are the two parameters most commonly used to describe the nutritional value of ruminant feedstuffs. Both components have traditionally been determined using laborious and time consuming procedures which limit the number of samples analyzed. In comparison, prediction of CP and NDF from infrared spectra offers advantages including simplicity of analysis, improved throughput and more cost-effective predictions. Near infrared reflectance spectroscopy (NIRS) has successfully been used during the last two decades to predict several parameters of nutritional interest. However, the use of FTIR has been relatively unexplored, requires less sample material, can now be performed on high performance portable instruments, and has the potential for better resolution of important vibrational features than can be achieved using NIRS. The aim of this study was investigate the potential of FTIR to predict the CP and NDF content in a range of feedstuffs. We hypothesized that it is possible to develop a global calibration to predict CP and NDF in most feedstuffs used for ruminants.

Material and methods A total of 750 samples of feedstuff commonly used to feed ruminants were used in the present experiment (Table 1). All samples were dried and ground through a 1 mm diameter sieve; CP concentration was determined in 664 samples as total Kjeldahl N × 6.25, and NDF concentrations was determined in 120 samples following the AOAC method using amylase and correcting for ash. Infrared spectra were collected by attenuated total reflectance (ATR) from 4000-600 cm^{-1} at a resolution of 2 cm^{-1} using an Equinox 55 FTIR spectrometer (Bruker Optik GmbH, Germany) fitted with a Golden Gate ATR accessory (Specac, UK). Duplicate spectra were averaged and data were modelled using partial least squares (PLS) regression on SIMPLS algorithms using the PLS Toolbox of MatLab (ver. 6.5, Mathworks Inc., Eigenvector Research Inc., USA). Models were trained on a randomly selected subset of samples (573 for CP and 97 for NDF) using a 'Venetian blind' cross validation protocol of 10 data splits, to a number of latent variables giving a minimum root mean square error of cross validation (RMSECV). The fit of the model to the data was given by the determination coefficient (R^2) and the root mean square error of calibration (RMSEC). Predictive accuracy was assessed by root mean square error of prediction (RMSEP) using an independent subset of samples (90 for CP and 23 for NDF). Prior to calibration, spectra were pre-processed using the multiplicative scarlet correction and centre normalization for the CP model, or by Savitzy-Golay derivitisation (2^{nd} derivative fitted to a 2^{nd} order polynomial with a window of 13 wave numbers) followed by detrending and mean centring for the NDF model.

Results Feedstuffs used in this experiment varied in CP (from 5.6 to 54.7 % in DM) and NDF content (from 17.5 to 85.6%), therefore these global models covered the range in which most ruminant feedstuffs are placed. Cross validated PLS regression models based on a training set of data allowed CP and NDF concentrations to be predicted in a test set of data with an acceptable degree of accuracy, with CP being slightly better predicted than NDF (RMSEP = 4.70 and 5.39 %, respectively).

Table 1 Chemical composition of feedstuffs (in % DM).

	n	CP (±SD)	*n*	NDF (±SD)
Straw and hay			*7*	74±7.4
Wheat/barley forage	*12*	10±2.2	*24*	42±5.4
Grass/clover forage	*76*	19±4.8	*51*	38±7.7
Legume forage	*26*	18±3.4	*12*	39±6.7
Maize silage	*12*	10±0.6	*15*	43±4.2
Distillers by products	*34*	31±3.8	*4*	32±4.3
Soybean hulls	*16*	13±1.2	*2*	71±1.6
Oil by products	*200*	40±7.4	*3*	25±6.2
Beet by products	*22*	9±2.5	*2*	21±0.4
Total mixed ration	*19*	17±4.7		
Tropical feeds	*9*	18±5.9		
Cereal mill by products	*17*	19±5.5		
Concentrates	*127*	25±8.0		
Cereal grains	*62*	12±2.0		
Protein products	*16*	45±18.3		
Legume seeds	*16*	31±8.2		

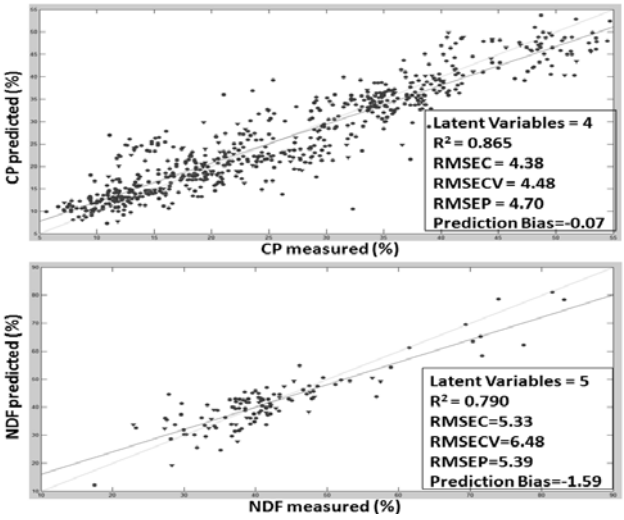

Figure 1 FTIR models to predict the CP and NDF content in feedstuffs

Conclusions This work shows that FTIR can be used to develop a general equation to predict the CP and NDF content valid for most feedstuffs used for ruminants. More research is required to predict feedstuff digestibility using this technique.

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Simultaneous HPLC analysis of alkaloid and phenolic compounds in green and black teas *(Camellia sinensis* var. Assamica*)*

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Introduction Tea is rich in antioxidants such as phenolics and alkaloids. As these compounds may affect microbial metabolism, tea has the potential as a natural feed supplement for ruminants to replace growth-promoters or antibiotics banned in the EU to manipulate rumen fermentation and mitigate methane production. However, chemical characterization of specific tea samples is important before testing their nutritive value by *in vitro* and *in vivo* studies. The existing methods of analysis have been successfully used to chemically analyse either green or black tea in separate studies (Chen *et al.*, 2008; Turkmen and Velioooglu, 2007). This study used an HPLC method for the simultaneous analysis of alkaloids and phenolics in both green and black teas.

Material and methods Green and black teas were obtained from a tea processing company, located in Bandung, West Java, Indonesia. The black tea was factory graded as 'Broken Orange Pekoe Fanning' and green tea was of a common grade. Both teas were plucked from *Camellia sinensis* var. Assamica tea plants at the same farm. About 200 mg of each sample was extracted with 10 ml of 80% methanol by continuous mixing overnight, centrifuged (4^{o}C, 3000 rpm, 10 min.) and stored in a screw-capped brown vial at -20^{0}C. Each extract was then analysed by HPLC (Shimadzu, Kyoto, Japan) with a C_{18} reverse phase column, 250 x 4.6 mm and i.d. 5 µm (Phenomenex, Cheshire, UK) with guard column (Waters spherisorb ODS2, 5 µm 4.6 x 10 mm,UK). The temperature of the column was set at 40^{o}C and the eluate UV spectra were recorded from 227-550 nm. Here, 270 nm was chosen as the optimum wavelength to identify all peaks. Two mobile phases, (A) orthophosphoric acid (0.1%, w/v) and (B) acetonitrile (≥99.9%), were utilized for gradient elution at 1 ml/minute using the gradient profile described by Turkmen and Velioooglu (2007) as follows: 8% B for 10 minutes increasing to18% B at 57 minutes; 24% B at 78 minutes; 26% B at 80 minutes; 28% B at 92 minutes; 80% B at 98 minutes; 8% B at 108 minutes. Column equilibration was 20 minutes and an automatic batch run started and operated by Shimadzu LC solution software integrated to a computer. The injection volume was 20 µl. Each compound was identified and quantified according to the retention time and spectrum view of the corresponding standard purchased from Sigma Aldrich UK. Each calibration standard was analysed in duplicate at the following concentrations (mg/ml): Theobromine:0.01, (-)-gallocatechin (GC): 0.05, (-)-epigallocatechin (EGC): 0.1, (+)-catechin (C): 0.01, caffeine: 0.1, (-)-epicatechin (EC): 0.01, (-)-epigallocatechin gallate (EGCG): 0.5, (-)-gallocatechin gallate (GCG): 0.025, (-)-epicatechin gallate (ECG): 0.05, (-)-catechin gallate (CG): 0.01, Rutin: 0.01, and black tea extract (theaflavin, theaflavins gallate and digallate basic): 0.1. Each sample of either green or black tea was analyzed in triplicate.

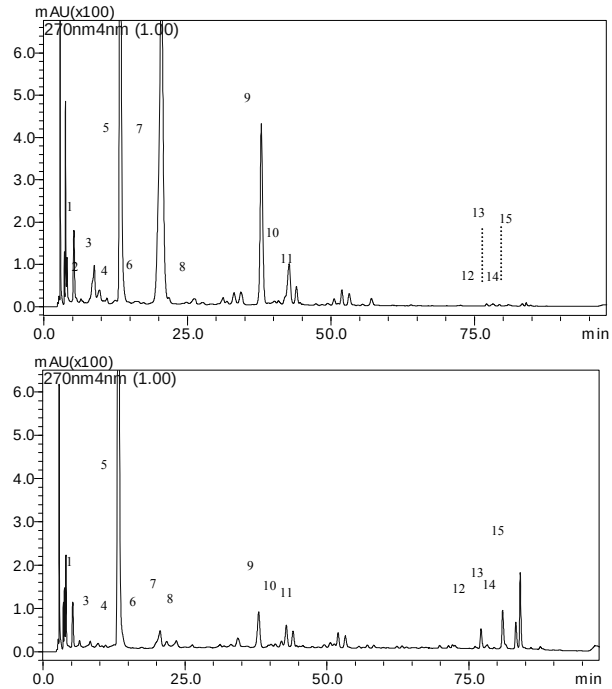

Figure 1 Example chromatograms of green tea (above) and black tea (below) samples

Result Fifteen compounds were identified: (1) theobromine, (2) GC, (3) EGC, (4) C, (5) caffeine, (6) EC, (7) EGCG, (8) GCG, (9) ECG, (10) CG, (11) rutin, (12) theaflavin, (13) theaflavin-3-gallate, (14) theaflavin-3'-gallate and (15) theaflavin-3,3'-gallate (Figure 1). As expected, caffeine was the most abundant alkaloid in both green and black teas. According to their peak areas, green tea had higher concentrations of catechins but lower concentrations of theaflavins than black tea and all theaflavins peaks appeared at longer retention times indicating that they had a more complex structure than catechins. Rutin (a phenolic quercetin glycoside) was identified in both teas in almost comparable peak areas.

Conclusion The method worked well to analyse alkaloids and phenolics such as catechins, rutin and theaflavins in both green and black teas. If only green tea is to be analysed, adjusting the HPLC gradient profile to reduce the run time to no longer than 50 minutes is preferable for time efficiency as only minor theaflavins were found in green tea. Further study will assess the potential of these compounds to manipulate rumen fermentation and methane mitigation in ruminant by a series of *in vitro* and *in vivo* studies.

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Effect of beam electron irradiation on tannin and total polyphenolic compounds content of pomegranate seed pulp

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Introduction Pomegranate seed pulp (PSP) which is a by-product in the industrial decoction of pomegranate, contains large amounts of oil (with some varieties having total lipid contents on a dry matter basis ranging from 66 to 193 g/kg DM of the fruit) and other nutrients which are valuable in meeting the nutritional requirements of ruminants, but it also contain some tannins. Anti-nutritional factors such as tannins reduce the nutrient utilization and intake of some feeds like PSP in animal nutrition. They also bind dietary protein and digestive enzymes to form complexes that are not readily digestible. Tannins are usually subdivided into two groups: hydrolyzable tannins (HT) and condensed tannins (CT). HT are made up of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids (mainly gallic and hexahydroxydiphenic acid). CT or proanthocyanidins are non-branched polymers of flavonoid units and usually have a higher molecular weight than the HT (1000-20000 Da compared to 500-3000 Da) (frutos *et al*, 2004). Different methods are applied to neutralize the anti-nutritional properties of these compounds and irradiation is one of the newest of these methods.

Material and methods Samples of pomegranate seed pulp (about 1 kg) in plastic packets treated with electron beam at three doses of 10 (PSP10), 15 (PSP15) and 20 (PSP20) KGy. Irradiated and non-irradiated (PSP0) samples analyzed for total phenolic compounds (TP) and total tannins (TT) by Folin-Ciocalteu's reagent, and CT were determined by the butanol–HCl assay and expressed at leucocyanidin equivalents according to Porter *et al*. (1986). Data were statistically analyzed as a completely randomized design (with 4 treatments and 3 replicates per treatment) using the GLM procedure of SAS software.

Results Effects of different doses of irradiation on phenolic compounds content of PSPs are presented in Table 1. Irradiation significantly decreased TP and TT compounds in PSP but CT content of samples was not affected. The radiation at 20 KGy was more effective than 10 and 15 KGy doses in reducing the phenolic compounds of pomegranate seed pulp.

Table 1 Phenolic compounds content of pomegranate seed pulp irradiated at different doses (mg tannic acid/g DM)

Phenolic compounds[1]	Treatments[2]				S.E.M	P
	PSP0	PSP10	PSP15	PSP20		
TP	39.2[a]	25.2[b]	23.9[b]	18.6[c]	1.26	<0.0001
TT	26.3[a]	10.7[b]	9.9[b]	6.5[c]	0.16	<0.001
CT	1.11	1.09	1.06	1.02	0.06	0.30

[1]TP, TT and CT are total phenolic compounds, total tannins and condensed tannins, respectively.
[2]PSP0: non-irradiated PSP, PSP10: irradiated with 10KGy dose, PSP15: pomegranate seed pulp irradiated with 15KGy dose, PSP20: pomegranate seed pulp irradiated with 20KGy dose.

Conclusions Dubravka *et al*. (2007) showed the total phenolic and tannin contents of soybean seeds were increased with y-irradiation at 10KGy dose but Hamza and Abu-tarbush (1998) reported that doses of 7 and 10 KGy y-irradiation significantly reduced the tannin content of Shahla sorghum seed. So it seems that irradiation effects on phenolic compounds content of different feedstuffs depend on their physical and chemical properties, kind of applied irradiation beam and the dose of irradiation. The authors are not aware of any report on the effects of any other kind of irradiation on phenolic compounds content of PSP but the electron irradiation at doses which were applied in present study effectively reduced the TP and TT content of this by-product and the effect was amplified at dose of 20 KGy compared with 10 and 15 KGy irradiation.

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Chemical composition, *in situ* degradation and *in vitro* gas production of saffron (Corcus sativus) residues

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Introduction Crop residues are of importance in provision of livestock nutrient requirements specially in developing countries and feeding these feedstuffs usually cause to lower the feeding costs, but the nutritive value of many of these feeds are not known well and determination of nutritional characteristics of them is imperative. Saffron residues which are consisted of stamen, leaves, sepal and petal are produced in large-scale in Iran, the largest saffron producer in the world, as the total production of them is about 87000 tons annually. Therefore, the objective of this study was to elucidate the different features of the nutritive value of the saffron residues.

Material and methods Saffron dry residues were harvested at the maturity stage of plant (approximately 6 months after saffron flowering, at the end of April). Proximal analysis of samples was done based on AOAC (2000) methods, and NDF and ADF content were measured using Van Soest (1994) method. Total phenolic compounds and total and condensed tannin content of residues were determined using procedure of makkar (2003). *In situ* DM degradability parameters were estimated using Orskov and Mcdonald (1979) equation and *in vitro* gas production parameters were determined using the method described by Menke & Steingass (1988).

Results The chemical composition of saffron residues are shown in Table 1. *In situ* DM degradability parameters and *In vitro* gas production parameters are also shown in Tables 2 and 3, respectively.

Table 1 The chemical composition of saffron residues (g/kgDM)

DM	CP	NDF	ADF	EE	ASH	TP*	TT	CT
940.0	67.0	459.0	380.0	47.0	52.0	44.0	32.0	3.1

*TP, TT and CT are total phenolic, total tannin and condensed tannin, respectively.

Table 2 *In situ* DM degradability parameters of saffron residues

Estimated parameters			effective degradability (g/kgDM)		
			passage rate (/h)		
a*	b	c (/h)	0.02	0.05	0.08
0.32	0.39	0.043	585.0	499.0	455.0

*a, b and c are quickly degradable fraction, slowly degradable fraction and degradability rate constant, respectively.

Table 3 *In vitro* DM gas production parameters of saffron residues

Estimated parameters			
b*	c	OMD (g/kgDM)	ME (Mj/kgDM)
0.49	0.091	539.0	8.0

*b and c are slowly degradable fraction and gas production rate constant, respectively; OMD and ME are organic matter digestibility and metabolisable energy, respectively.

Conclusion The results showed saffron residues have a high potential as a roughage source in ruminant nutrition. More *in vivo* researches are needed to elucidate the impact of feeding this feed on ruminant performance.

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Effect of N underfeeding on digestibility, N balance and rumen fermentation in faunated, defaunated and *Isotricha*-monofaunated sheep

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Introduction The absence of rumen protozoa often results in an improvement on N use due to a better efficiency of ruminal N metabolism (Hristov and Jouany, 2005). N environmental pollution by ruminants could be reduced in animals without protozoa due to a reduction of N losses in urine and/or faeces. The effect of protozoa on N metabolism has been seldom studied with diets containing low N. In this study the effect of a low N diet on N balance, digestibility and rumen fermentation was tested on sheep differing in their protozoal communities (faunated, defaunated and monofaunated). *Isotricha prostoma* was chosen as *Isotrichidae* might maintain a positive 'protozoal effect' on rumen fermentation without affecting rumen N catabolism (Ivan, 2009).

Material and methods Eighteen adult Texel wethers fitted with ruminal cannulae were used. They were split into 3 groups of 6 animals: conventional, defaunated or monofaunated. Within each group, animals received a diet high (H) or low (L) in N in a crossover design with two 5-week periods. On a DM basis, diets contained 70% low-quality hay, 15% maize grain and either 15% beet pulp (L) or 15% soybean meal (H). Diet H contained 15.0% CP in DM and was calculated to meet requirements of rumen degradable N, diet L contained 8.3% CP in DM and was deficient in rumen degradable N. Diets were given twice daily at 0800 and 1600 h. Total tract digestibility and N balance were determined by total faeces and urine collection. In rumen liquor, concentrations of non-protein N and ammonia N were determined before feeding and at 1, 2.5, 5 and 8 h after feeding, and volatile fatty acids (VFA) were determined before and 2.5 h after feeding. Statistical analysis was performed using the MIXED procedure of SAS.

Results Organic matter digestibility was lower in L but was unaffected by faunation status. Although N intake was very different between H and L diets, faecal N remained unchanged. In contrast, urinary N decreased markedly in the N-deficient diet and was particularly lower in defaunated and monofaunated treatments ($P > 0.05$). As expected the L diet decreased non-protein and ammonia N contents in the rumen. Concentration of rumen ammonia was lower for defaunated and monofaunated animals in both diets. Total ruminal VFA concentration did not vary among treatments before and after feeding but the L diet increased the proportion of acetate at the expense of butyrate. In defaunated and monofaunated animals propionate increased while butyrate decreased. For all variables measured no interaction was detected between N level and protozoal presence in the rumen.

Table 1 Organic matter (OM) digestibility, N partitioning and ruminal fermentation characteristics in conventional, defaunated or monofaunated (*Isotricha prostoma*) sheep receiving a diet high (H) or low (L) in N

	Conventional		Defaunated		Monofaunated		SEM	Statistical analysis
	H	L	H	L	H	L		
OM intake (kg/d)	759	765	766	760	765	765	2.7	Ns
OM total tract digestibility (g/kg)	64.0	61.8	63.5	60.7	64.8	64.3	1.17	N*
N intake (g/d)	19.8	11.0	19.9	10.9	19.8	11.0	0.03	N**
N in faeces (g/d)	5.6	5.5	5.7	5.7	5.7	5.7	0.22	Ns
N in urine (g/d)	11.3	5.3	10.9	4.0	10.2	3.7	0.46	N** P*
Rumen acetate after feeding (mol/100 mol)	70.1	71.0	68.9	72.1	70.1	71.7	0.62	N**
Rumen propionate after feeding (mol/100 mol)	18.2	18.4	20.2	19.5	20.2	20.1	0.65	P*
Rumen butyrate after feeding (mol/100 mol)	9.2	8.8	8.3	6.9	6.6	6.1	0.36	N* P**
Non protein N, average (mg/l)	429	288	449	304	436	311	18.1	N**
Ammonia N, average (mg/l)	208	116	163	62	165	87	20.3	N** P**

N: effect of N level; P: effect of protozoa presence; ns: non significant; *: $P < 0.05$ - **: $P < 0.01$

Conclusions The effect of a low N diet on digestibility and N balance in sheep was as expected. Defaunation reduced the proportion of butyrate in the ruminal VFA and the concentration of rumen ammonia N but did not affect rumen non-protein nonammonia N concentrations. This data suggest a better use of N by rumen microbes resulting in lower urinary N excretion. The absence of difference between defaunated and monofaunated animals indicates that, under the conditions of the trial, *Isotricha prostoma* did not modify rumen fermentation. The absence of interaction between N level and protozoa presence shows that the effects of defaunation on digestibility, N partitioning between urine and faeces, and ruminal N metabolism do not depend on N dietary level.

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Attributes of fresh meat suitable as healthy food from healthy organic animals, (cattle and camel)

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Introduction Healthy animals help to ensure a safe food supply. They also grow properly, make best use of the food (grasses) they eat and produce good quality foodstuffs, such as meat, dairy products and eggs, at affordable prices (IFAH, 2005). One of easiest ways to produce a healthy animal in Nigeria is through organic farming systems. This is the combination of scientific knowledge of ecology and modern technology with traditionally farming practices, based on naturally occurring biological processes (FIBL, 2006). Raising livestock and poultry, for meat and eggs is a traditional, farming activity that complements the growing number of, organic farms in an attempt to provide animals with natural living conditions and feed. In such practice, free-ranging outdoor with access to grazing and exercise, avoids crowding. Feed is also organically grown and drugs including antibodies are not ordinarily used because they are prohibited under organic regulatory regime. It is therefore the aim of this study, to evaluate the qualities attributes of fresh meat suitable for food from healthy organic animals.

Material and methods Animals used for this experiment were organic animals, fed only on grasses, salt-lick and water. 3 kg of semimenbranous muscles from 2-3years old animal from both Camelus dromedarous and White Fulani from the Teaching and Research farm of Osun State University, Osun State Nigeria were used. Muscles were kept overnight at 4 ^{0}C for 24 hours, and evaluation was carried out on parameters like; cooking loss, shear force determination, water holding capacity, cold shortening, thermal shortening (during cooking), according to methods used by Fakolade, (2008). Proximate evaluation (moisture, protein, ether extract and ash content) were measured according to A. O. A. C, (2000). Histological samples were fixed in buffered formalin for 24 hours and processed by routine histological techniques which involves dehydration, clearing, embedding, blocking, sectioning and staining, muscle sections were cut and stained using Masson's Trichrome method, according to Luna,1968. Statistical analysis conducted, data collected were subjected to analysis of variance (ANOVA) and significant differences between means were separated using Duncan's Multiple range text (SPSS). The SAS (1999), software was used for all statistical analysis.

Results There appeared to be distinct differences between beef and camel meat. The physio-chemical properties showed that camel meat had higher significant values ($P<0.05$) for thermal shortening, cooking loss, shear force, and water holding capacity. For proximate composition, camel had the highest significant moisture and protein value while beef was high in ether extract than in camel meat. Meat from organic animals had lower ether extract, enough protein and having high percentage of ash content than meat from inorganic animals which could probably be due to high percentage of lignin in their diet. Histological study of fresh bovine semimembranous muscle shows intact muscle bundle with little or no muscle fibre disintegration, but there appears to crimped think collagen fibers were more pronounced, while that of camel muscle showed that the muscle fibers, which were also intact with little or no muscle fibre disintegration, the perrimysial connective tissues are more pronounced in the muscle fibres. Shear force values in the table denote that the meats appear to be tough according to Miller *et al*., (2001).

Table 1 Physico-chemical properties of organic animals

Parameters	Camel	White Fulani	SEM
Cold shortening (%)	2.4^{b}	2.9^{a}	0.19
Thermal shortening (%)	23.0^{a}	15.8^{b}	3.10
Shear forces (%)	9.0^{a}	7.0^{b}	1.90
Water holding capacity (kg/cm^3)	68.1^{a}	66.9^{b}	1.12
Cooking loss (%)	27.8^{a}	33.2^{b}	0.94

^{ab}: means in the same row with different superscript are ($P<0,05$) significantly different.

Conclusion Organic meat produced lean meat, having less fat and more nutritive values than typical non-organic meat. Cattle meat had more fat than camel but was generally of superior eating quality. Lean meat from such animals are suitable for adults or people running away from fat consumption in food, it therefore become choice meat and demand high price.

Acknowledgement I acknowledge all the Departmental and Laboratory staff of Animal Science, Ejigbo campus, Osun State University.

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The effects of season of shearing and terminal sire breed on the performance of a yearling flock

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Introduction Ewe replacements are a significant cost in mid-season lamb systems. Mating ewe lambs represents an opportunity to offset this cost through an increase in flock output. In a review of the literature on breeding from ewe lambs Hanrahan (2011) concluded that lambing at 1 year of age can increase ewe lifetime productivity by 10 to 20%, depending on prolificacy and longevity. Previous studies have shown that the season of shearing influences subsequent lamb birth weight and lamb performance (Keady and Hanrahan 2009). However, there is limited information on the potential effects of season of shearing on the performance of ewes joined to lamb at 1 year and on that of their progeny. The objective of this study was to evaluate the effect of season of shearing on the performance of ewe lambs and that of their progeny, which were by two terminal sire breeds.

Material and methods The study was undertaken on a commercial farm in Co. Wicklow. Crossbred (lowland breeds) ewe lambs (n= 316), sourced from 7 flocks, were assembled in early August and were assigned, at random within farm of origin, to one of the following treatments, autumn shorn (25 August) or winter shorn (3 January). All lambs were managed as one flock. The ewe lambs present were weighed, and condition score was assessed, at joining and weaning. Ewe lambs (n=229) that were over 40 kg were joined (single mob) with mature rams (3 Suffolk & 3 Charollais) on 7 October for a 47 day period. Ewe lambs were housed in mid December and offered grass silage *ad libitum* (DM 316 g/kg, DMD 740 g/kg). From 1 January the lambs received 300 g/day of concentrate (12.7 MJ/kg DM, CP 147 g/kg DM) per head daily. For the final 6 weeks of pregnancy single and twin bearing ewes received (total) 12.6 kg and 20.3 kg, respectively, of a concentrate formulated to have 12.8 MJ/kg DM and CP 190 g/kg DM. Lambing events were logged as either assisted or none. All lambs were tagged and weighed at birth and breed of sire was recorded based on visual criteria. Lamb mortality, either at birth or subsequently, was also recorded. Following turnout to pasture ewes rearing singles were managed separately from those rearing twins and received no supplementation. Ewes rearing twins were supplemented with 0.5 kg/day concentrate daily for 5 weeks post lambing and their lambs had access to concentrate until weaning (maximum 300g/day). Lambs were weighed at 12 weeks of age (weaning). Data were analysed using linear model procedures of SAS: Proc GENMOD for pregnancy rate and mortality; Proc GLM for other ewe performance traits; Proc MIXED, with dam as random effect was used for lamb growth traits. Orthogonal contrasts were used to evaluate differences among treatment groups.

Results The effect of shearing on ewe weight and reproductive performance was not significant (Table 1). Shearing (autumn or winter) increased lamb birth weight (P<0.01) and weaning weight (P=0.09). Total lamb mortality was 19.6% (13.6% dead at birth) and was unaffected by treatment. Lambs sired by Suffolk were heavier at birth (P < 0.07) and had higher growth rate (P<0.05) than those sired by Charollais. Pregnancy rate was positively related to condition score at joining (P<0.05) but was not influenced by weight at joining. Litter size was positively related joining weight (P<0.05) but was not influenced by condition score at joining. The overall incidence of assistance at lambing was 60% and was not associated with either shearing treatment or breed of sire.

Table 1 Effect of season of shearing on ewe and lamb performance

	Season of shearing				
	Unshorn	Autumn	Winter	s.e.	Significance
Ewe weight at - joining	43.7	42.8	44.1	0.48	P = 0.08
- weaning	51.7	54.2	51.6	0.60	P = 0.10
Pregnancy rate (%)	73	78	85	-	NS
Litter size	1.21	1.31	1.26	0.07	NS
Lamb weight (kg) - birth	4.3	4.9	4.9	0.16	**
- 12 weeks	27.0	28.3	28.4	0.87	P =0.09
Growth rate to 12 weeks (g/day)	270	281	281	9.26	NS

Table 2 Effect of Terminal sire breed on lamb birth weight and subsequent performance

	Sire breed			
	Suffolk	Charollais	s.e.	Significance
Lamb weight (kg) - birth	4.9	4.5	0.12	P = 0.07
- 12 weeks	29.0	26.8	0.86	*
Growth rate birth to 12 weeks (g/day)	290	266	9.2	*

Conclusion Shearing ewe lambs prior to joining or during the winter increases lamb birth weight and weaning weight. Lambs sired by Suffolk have higher growth rate than those sired by Charollais.

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The refractory UK sheep production system

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Introduction The sheep industry in the UK has the reputation of being resistant to change. But change is needed to meet the increasingly complex demands of healthy food from healthy animals, while remaining economically sustainable and benefiting the environment. Methane emissions from ruminants have gained increasing profile in the context of climate change. A range of different animal breeding and other technologies have been suggested as providing ways of reducing these emissions. However, sheep producers have historically been slow at adopting breeding technologies to improve productivity for reasons that are not well understood. The aim of this project was to understand why the adoption of breeding technologies has been slow and uneven and how the context of methane emissions might influence this situation.

Material and methods The research undertaken was qualitative in nature and consisted of 42 individual, semi-structured interviews with a range of livestock farmers and relevant associated stakeholders (e.g. vets, meat processors etc.) around England and Scotland. Farmers interviewed included hill and lowland producers, organic and conventional, selling direct, deadweight and live weight. In the sample, 17 holdings kept sheep. The aim was to sample a diversity of stakeholders systematically but these were not viewed as statistically valid samples. However, the approach does provide rich insights into farmer behaviour. Interviews lasted approximately one hour and were recorded and transcribed (with permission). Subsequent analysis proceeded on an inductive basis with specific themes identified from the interview transcripts.

Results Sheep farming is embedded in a range of different 'systems of production' which have co-evolved with the geographical location and the opportunities afforded. More broadly, some of the critical issues identified include:
Scientific: Estimated Breeding Values (EBVs) are not trusted and are associated with intensive rearing. There is a lot of disillusionment with 'experts' and their advice.
Environment: the need for genotypes that thrive in harsh hill environments was stressed.
Socio-cultural: breeding was seen as providing a particularly enjoyable and interesting aspect to farming and one that involved considerable craft knowledge. It is not necessarily viewed as a primarily economic activity.
Uncertainty of data: growth performance is often judged by eye rather than weighing, group mating means pedigrees are uncertain.
Supply: while EBVs are used in selecting terminal sires, such evaluations are missing in the dam (cross-bred) side.
Resource: labour is a limited resource and often drives towards a less labour-intensive approach.
Economic: economic benefits from faster growth rates or improved feed efficiencies are not immediately obvious to farmers.
Market for meat: the EUROP grading scheme gives emphasis to weight, conformation and fat cover and may not be linked to efficiency.
Market for breeding stock: the complexities of the traditional sheep pyramid make change difficult due to many interactions.
Value chain: the sheep value chain is extremely complex and lacks consistent feedback messages. Different aspects are particularly valued at different stages in the chain.
Structural: systems of production may preclude using other technologies to reduce methane emissions. Environments often preclude changing grass varieties, limited concentrate feeding preclude use of feed additives

In the public sphere, methane production from cattle and sheep may be presented as an important cause of climate change but to livestock farmers, methane is a natural product that has always existed and they see few ways in which methane production can be reduced. The context of methane emission alone is unlikely to facilitate adoption of breeding and other techniques.

Conclusions There is no single one barrier to adoption of breeding techniques by sheep farmers but rather a whole host of barriers to adoption, many of them inherent in production systems. The development of alternative systems of production or more joining up of the current production chain may prove more effective in changing practices than focusing purely on knowledge transfer. The term 'refractory' can refer to lack of willingness to change but it can also refer to retaining strength under exposure to extreme environments. Evidence from these interviews suggests that sheep farmers are continually adjusting their production systems but are rarely making whole-scale systemic changes. The question remains whether this approach provides greater resilience to the sheep production system than one that is more fluid.

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Effects of plane of nutrition during the rearing phase and pregnancy on the performance of ewes lambing at 2 years of age

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Introduction The cost of rearing ewe replacements is influenced by the plane of nutrition offered during the first winter and the second grazing season, both of which impact on body size at joining (18 months) and subsequent lambing. The aim of the current study was to evaluate the effects of plane of nutrition during the first winter, the subsequent grazing season and during first pregnancy, and potential interactions, on ewe productivity at first lambing (2 years old), and on subsequent lamb performance using breed types with contrasting prolificacy.

Materials and methods 292 ewe lambs (60 Charmoise x S Blackface, 150 Belclare x S Blackface, 82 Belclare) were allocated, over 3 consecutive years, to 2 herbage DM allowances [0.75 (L) and 1.75 kg (H) per head] on winter grazing (winter-1) from late November to 1 April. From 2 April to 30 August the animals from each winter treatment were divided equally between two planes of summer nutrition, achieved by set stocking to maintain sward heights of 4 cm (L) or 6 cm (H) (summer). From 30 August to early December all ewes were grazed as one flock and were synchronised (progestogen sponges) and joined with a panel of Suffolk rams to lamb in mid March. The ewes from each of the four rearing treatments were allocated to either a high (H) or low (L) feed-value grass silage at housing (winter-2). Thus, there were 8 treatments in a 2 x 2 x 2 factorial design. During the last 6 weeks of pregnancy the ewes carrying 1, 2, 3 or 4 lambs were offered 13, 22, 28 and 31 kg concentrate per head, respectively. Post lambing, ewes rearing singles or twins and their lambs received no concentrate supplement at pasture. Ewes rearing triplets were offered concentrate (0.5 kg/day each) for 5 weeks post lambing while their lambs were offered up to 300 g/day each until weaning at 14 weeks. The data were analysed as a 2 x 2 x 2 factorial using Proc GLM and Proc MIXED of SAS for ewe and lamb traits, as appropriate; all models included terms for ewe breed and year, models for lamb traits had terms for sex and birth type (fixed), and dam (random).

Results The DM and metabolisable energy concentrations for the L and H feed value silages were 222 and 271 g/kg; and 10.8 and 11.6 MJ/kg DM respectively. The effects of plane of nutrition at each stage up to lambing at 2 years on ewe and subsequent lamb performance are presented in Table 1. Increasing winter-1 and summer planes of nutrition increased ewe weight pre mating and post lambing and ewe condition score pre mating. Increasing winter-1 plane of nutrition tended (P=0.06) to increase lamb birth weight. There was a significant interaction (P<0.05) between winter-1 and summer nutrition levels for ewe weight post lambing. Increasing winter-2 plane of nutrition increased live weight and condition score post lambing, and lamb birth and weaning weights. The effects of ewe genotype on ewe and subsequent lamb performance are in Table 2. The Charmoise-X were lightest at lambing, had the lowest litter size, and their lambs were lightest at birth and weaning. Interactions involving ewe breed were not significant. Relative to the Belclare-X, the Belclare ewes were heavier at lambing and their lambs tended to be heavier at birth and weaning.

Table 1 Effect of plane of nutrition during the rearing phase on ewe and subsequent lamb performance

		Winter-1 (W1) x Summer (S) nutrition level					Winter-2 (W2)			Significance			
		Low		High									
		Low	High	Low	High	s.e.	Low	High	s.e.	W1	S	W2	W1xS
Pre-mating	- condition	3.24	3.44	3.48	3.69	0.039	-	-	-	***	***	-	NS
	- weight (kg)	53.3	56.8	56.8	61.7	0.77	-	-	-	***	***	-	NS
Post lambing	- condition	3.13	3.05	3.11	3.23	0.063	2.73	3.54	0.046	NS	NS	***	NS
	- weight (kg)	50.6	51.7	51.3	55.9	0.79	49.0	55.7	0.58	**	***	***	*
Litter size		1.95	1.98	2.05	1.92	0.085	1.96	1.99	0.062	NS	NS	NS	NS
Lamb weight (kg)	- birth	3.53	3.58	3.64	3.77	0.144	3.48	3.78	0.132	P=0.06	NS	***	NS
	- weaning	27.4	28.0	28.4	27.8	0.91	27.4	28.5	0.86	NS	NS	**	NS

Table 2 Effect of ewe genotype on ewe and lamb performance

		Ewe genotype			s.e.	Significance
		Charmoise-X	Belclare-X	Belclare		
Pre-mating	- condition	3.50^{b}	3.64^{c}	3.25^{a}	0.043	***
	- weight (kg)	49.1^{a}	57.0^{b}	65.5^{c}	0.84	***
Post lambing	- condition	3.39^{b}	2.98^{a}	3.02^{a}	0.068	***
	- weight (kg)	45.2^{a}	51.0^{b}	60.8^{c}	0.87	***
Litter size		1.7^{a}	2.21^{b}	2.02^{b}	0.093	***
Lamb weight (kg)	- birth	3.18^{a}	3.67^{b}	4.03^{b}	0.152	***
	- weaning	25.9^{a}	28.2^{b}	29.6^{b}	0.95	***

Conclusions Whilst altering the plane of nutrition during different stages of the rearing phase altered pre-mating weight by up to 8.4 kg there was no effect on litter size. Increasing plane of nutrition during the first winter tended to increase lamb birth weight. Plane of nutrition offered during pregnancy had the greatest effect on ewe and lamb performance. Relative to the Charmoise-X the Belclare and Belclare-X increased the weight of lambs weaned due to higher litter size and higher weaning weight of progeny.

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Effect of different levels and frequency of concentrate feeding on growth performance and carcass characteristics of Texel cross lambs

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Introduction The plane of nutrition affects the growth performance and carcass characteristics of lambs (Mustafa *et al*., 2008). It is known that increased level and frequency of concentrate feeding can help ruminant livestock to better utilize their diets perhaps through altered the activity of rumen microbes that can influence animal growth and production. Therefore, this study tested the effect of two levels and frequencies of concentrate (CON) feeding on daily dry matter intake (DDMI), live-weight gain (DLWG) and carcass characteristics of similarly managed Texel cross lambs consuming ryegrass hay *ad libitum* from weaning to slaughter.

Material and methods Twenty four Texel cross weaned lambs comprising 12 wethers and 12 ewes of similar initial live-weight (LW=32 ± 3.5 kg) were used in a factorial experiment by using 2 CON levels each with 2 feeding frequencies and 2 genders with three lambs as replicates per gender. The lambs were individually housed on a sawdust covered concrete floor. Each lambs was offered a CON diet containing (kg^{-1} DM) 260g ground barley, 260g sugarbeet pulp, 230g soybeen meal, 150g maize distillers grains, 70g molasses and 30g mineral premix. Each kg CON DM had 11 MJ ME and 214g CP and each lamb was fed a fixed amount of either 500g or 250 g CON /day as a single meal in the morning only or two equal meals in the morning and afternoon. Also, each lamb had *ad libitum* access to a chopped ryegrass hay containing (kg^{-1} DM) 10 MJ ME and 87g CP. The experiment lasted for 35 days after an adaptation period of 10 days. The lambs were monitored regularly for their health and weekly feed intake, LW and condition scores (CS). The lambs were slaughtered at about 40 kg of LW to assess their carcass and killing out (KO %). The data were analysed by using the Minitab software to test the statistical effects of gender (G), the level (L) and frequency (F) of CON feeding and their respective two way interactions on production and carcass profiles of these lambs for their significance at $P<0.05$.

Results Table 1 presents the mean values for different production profiles of wether and ewe lambs of different treatments. The levels and frequency of CON feeding had no significant effect on DLWG, FCR, CCW, KO%, SFE, CONF and kidney weight ($P>0.05$). While the level of CON feeding had a significant effect on DDMI ($P<0.05$), the effects of feeding frequency and gender on DDMI were not significant ($P>0.05$). The wether lambs had significantly heavier kidneys than the ewe lambs ($P<0.05$). The level of CON feeding x gender interactions were significant ($P<0.05$) for DDMI and KO% suggesting that the wether lambs consumed more dry matter per day than the ewe lambs on higher CON level but the ewe lambs consumed more dry matter on lower CON level. The wether lambs had higher KO% on lower CON level but the ewe lambs had higher KO% on higher CON level ($P<0.05$).

Table1 Effect of level (L) and frequency (F) of concentrate (CON) feeding and gender on feed intake, growth performance and carcass characteristics

	CON (L)		CON (F)		Gender (G)		SEM	P values for main effects		
	500 g	250 g	Once	Twice	Wether	Ewe		L	F	G
Items										
DDMI (kg)	0.84	0.77	0.79	0.82	0.80	0.81	0.017	0.025	0.317	0.764
DLWG (kg)	0.15	0.17	0.16	0.16	0.15	0.17	0.015	0.382	0.913	0.430
FCR	5.77	4.96	5.31	5.42	5.54	5.19	0.477	0.251	0.875	0.613
CCW (kg)	17.3	16.7	17.2	16.8	17.1	16.9	0.304	0.192	0.346	0.703
KO (%)	46.9	45.2	46.6	45.6	46.2	45.9	0.802	0.139	0.395	0.826
SFE (%)	12.0	10.7	11.3	11.4	11.6	11.1	0.651	0.179	0.894	0.625
CONF	3.9	3.9	3.8	4.0	4.0	3.83	0.081	0.792	0.163	0.163
Kidneys (g)	98.8	93.4	95.3	96.9	101.2	91.0	2.653	0.186	0.694	0.023

FCR= Feed conversion ratio as kg DDMI /kg LWG; CCW= Cold carcass weight, SFE= Subcutaneous fat estimate, CONF= conformation score

Conclusions The level and frequency of concentrate feeding had no appreciable effect on any of the growth parameters or carcass characteristics of weaned lambs. The lack of response may be partly due the relatively lower daily DM intake of hay which was not of high quality. However, the tendency of greater daily DM intake and KO % of weather lambs than the corresponding ewe lambs suggest that the wether lambs tended to respond more to changes in the frequency and level of concentrate feeding in this study. It appears that low number of lambs and short feeding duration were perhaps the main reasons for the lack of significant differences between the treatments of this study.

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Utilization of wheat offal-carried pineapple waste by West African Dwarf goats

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Introduction The major problem confronting small ruminant livestock production is the non-availability of feed all year round to meet their maintenance and production requirements of. The search for alternative feed ingredients for livestock feeding has led to investigations into the use of pineapple waste for small ruminant feeding (Babatunde, 1998). This study evaluated the utilization of wheat offal-carried pineapple waste by West African Dwarf goats fed graded levels of the ration with a view of ascertaining the level of optimum performance.

Material and methods In a 16-week feeding trial, 20 West African Dwarf goats of both sexes, aged between 5 and 7 months, were randomly allotted to four treatments of inclusion level (0, 20, 30 and 40%) of wheat offal-carried pineapple waste (WCPW) as supplement to a guinea grass basal diet in a completely randomized design. Chemical component of diets and faeces were determined using the method of AOAC, (1990). Data obtained were analyzed with the General Linear Model of SAS (2008).

Results The crude protein content of diets containing wheat-offal carried pineapple waste was higher than the control diet (Table 1). The performance characteristics of the experimental goats (Table 2) were significantly different ($p<0.05$). The apparent digestibility coefficient of dry matter and nutrient of the experimental goats was significantly different ($p<0.05$) in all the diets (Table 3). There was significant difference ($p<0.05$) between the mean nitrogen balance of the experimental goats (Table 4).

Table 1 Chemical composition of experimental diets

Parameters	Control diet	20%WCPW	30% WCPW	40%WCPW
Dry matter	92.78	92.95	92.78	93.30
Organic matter	92.99	92.73	92.53	92.03
Crude protein	16.38	18.20	17.75	17.75
Crude fibre	10.77	9.12	9.62	11.44
Ether extract	11.87	12.59	10.05	9.88
Ash	7.01	7.27	7.47	7.97
Nitrogen free extract	46.75	45.77	47.89	46.19

Table 2 Performance characteristics of the experimental goats

Parameters	Control diet	20% WCPW	30% WCPW	40% WCPW	SEM	P
Total dry matter intake (g/d)	421.55^{b}	462.43^{a}	402.49^{c}	399.90^{d}	0.66	<0.0001
Feed efficiency (%)	9.66^{b}	9.90^{a}	8.60^{d}	8.82^{c}	0.05	<0.0001
Initial weight gain (kg)	6.68^{a}	6.61^{b}	6.43^{c}	6.18^{d}	0.02	<0.0001
Final weight gain (kg)	10.51^{b}	11.06^{a}	9.85^{c}	9.63^{d}	0.02	<0.0001
Live weight gain (kg)	8.59^{b}	8.83^{a}	8.15^{c}	7.92^{d}	0.08	<0.0001
Average daily weight gain (g/d)	39.21^{b}	45.54^{a}	35.00^{c}	35.24^{c}	0.10	<0.0001

Table 3 Apparent digestibility coefficient of the dry matter and nutrient by experimental goats

Parameters	Control diet	20%WCPW	30%WCPW	40%WCPW	SEM	P
Dry matter	61.77^{a}	60.74^{b}	57.73^{d}	60.20^{c}	0.38	<0.0001
Crude protein	51.27^{c}	60.74^{a}	52.70^{b}	50.23^{d}	0.28	<0.0001
Crude fibre	60.97^{c}	65.13^{a}	63.21^{b}	52.52^{d}	0.30	<0.0001
Ether extract	64.81^{d}	68.32^{c}	70.11^{b}	72.36^{a}	0.46	<0.0001
Ash	44.50^{d}	56.36^{a}	49.22^{b}	43.15^{c}	0.27	<0.0001
Nitrogen free extract	83.64^{d}	90.41^{b}	91.42^{a}	86.06^{c}	0.68	<0.0001

Table 4 Mean nitrogen balance (g/animal/day) of the experimental goats

Parameters	Control diet	20% WCPW	30% WCPW	40% WCPW	SEM	P
Nitrogen intake	4.64^{c}	4.93^{a}	4.85^{b}	4.85^{b}	0.01	<0.0001
Feacal nitrogen	1.33^{c}	1.41^{b}	1.41^{b}	1.47^{a}	0.03	<0.0001
Urinary nitrogen	0.93^{b}	0.89^{c}	1.02^{a}	0.96^{b}	0.01	<0.0001
Retained nitrogen	2.37^{c}	2.63^{a}	2.44^{b}	2.42^{b}	0.01	<0.0001
Nitrogen retention (%)	51.19^{b}	53.46^{a}	50.17^{c}	49.88^{d}	0.08	<0.0001

Conclusion It was concluded that wheat offal carried-pineapple waste can be included in the diet of West African Dwarf Goats up to 40% without any adverse effect. However, 20%WCPW level of inclusion gave the best performance.

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Potentiated glycerol: a functional feed ingredient with antimicrobial and mycotoxin inactivating properties

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Introduction Glycerol, as a co-product from the biodiesel industry, is now regularly used as a feed ingredient. Glycerol may also improve feed hygiene by inhibiting mould growth (Südekum *et al*., 2008). Potentiated glycerol (PG) is a patented calcium/glycerol complex which is registered as a feed ingredient in the EU. Additional antimicrobial and mycotoxin inactivating functions of PG have now been investigated.

Material and methods PG in liquid and powder versions were tested in various *in vitro* trials. For antibacterial effect against *Salmonella enterica abony* and *Campylobacter jejuni. a* 0.1mL bacterial suspension was inoculated into 9.9 mL of test substance solution to give *ca*. 2 x 10^6 cfu/mL, incubated for 5 min at room temperature and pour plates prepared for counting. Biofilms of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*s were produced over 24 hours in microtitre plates. Aliquots of 100 µl of the products were added to the wells, and incubated 5 min. Resazurin viability staining was applied and fluorescence of the biofilms measured. For mycotoxin inactivation, an aqueous solution containing aflatoxin B1 (AFB1), ochratoxin A (OTA), zearalenone (ZEA), fumonisin B1 (FB1), deoxynivalenol (DON), and HT-2/T-2 toxins (HT-2/T-2) was treated with PG at 10% (w/v). The mycotoxins remaining were assayed after 2 hrs and 1day.

Results The PG liquid tested against two important bacterial pathogens gave complete kill at a dilution factor of 1/16. Crude glycerol from the biodiesel industry, the raw material for the production of PG, had no effect against *S. enterica abony* but had a modest effect upon *C. jejuni* (Table 1).

Table 1 Antibacterial activity of Potentiated Glycerol liquid

Treatment	Product dilution	Micro-organism	
		S. enterica abony	C. jejeuni
None (initial count)	0	3.55 x 10^6	1.14 x 10^6
Potentiated Glycerol	1/16	0	0
	1/32	3.6 x 10^5	0
Crude Glycerol	1/1	2.9 x 10^6	5.35 x 10^3
(Bio-diesel)	1/2	4.35 x 10^6	1.36 x 10^4

Biofilms are important problems in both feed and food production. There was a good control of three micro-organisms implicated in biofilm formation (Table 2). Glycerol on its own actually supported the growth of the micro-organisms.

Table 2 Activity of Potentiated Glycerol liquid against biofilms. Data are fluorescence/well (average ±S.D.) obtained after resazurin-based viability staining. A lower signal means less surviving cells.

Micro-organism	Treatment		
	Control (none)	Potentiated Glycerol (w/v)	Glycerol (w/v)
S. aureus	160,000^{a*} ± 40,000	60,000^b ± 60,000 (3.1%)	180,500^a ± 30,000 (10.0%)
P. aeruginosa	25,000^a ± 8,500	0^b (6.25%)	29,000^a ± 30,000 (10.0%)
C. albicans	130,000^a ± 12,000	30,000^b ± 7,500 (12.5%)	150,000^a ± 17,800 (10.0%)

*Means with different superscripts in the same row are different P<0.00001

PG inactivated all mycotoxins tested within 2 hrs and after 1 day only 16% of AFB1 remained (Table 3).

Table 3 Recovery of mycotoxins from a multi-toxin aqueous solution (2 µg/mL) with potentiated glycerol (10% w/v)

Treatment	Mycotoxin Recoveries (%, Mean ±S. D., n=3))					
Time	AFB1	ZEA	OTA	DON	FB1	HT-2/T-2
2 hours	64 ± 1	38 ± 1	0 ± 0	43 ± 0	2 ± 0	0 ± 0
1 day	16 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Conclusion Potentiated Glycerol has useful antimicrobial properties against a wide range of important pathogens. It can also inactivate mycotoxins. It is a multifunctional feed ingredient as a source of energy and calcium that will also improve feed hygiene and safety.

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Effect of different levels of urea and molasses on organic matter digestibility and estimation of metabolizable energy with *in vitro* methods (gas test, Tilley and Terry) in ensiled pistachio epicarp

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Introduction Given the increasing demand for animal products and lack of food in the recent years, nutritionists and farmers have focused on using crop residues and byproducts in feeding domestic animals. One problem in storing epicarps arises from the fact that this byproduct is harvested and available only once in a year. Ensiling is one method for storing epicarps in long term. If the method is used properly, the products can be used over a long period. The present study aims to examine effects of different levels of urea and molasses on improving quality as well as to estimate metabolizable energy in ensiled pistachio epicarp.

Material and methods Urea and molasses were added to ensiled pistachio epicarp at different levels. The study was conducted based on a randomized factorial design. Four levels of urea dry matter (0, 0.5, 1, and 1.5%) and three levels of beet molasses (0, 3, and 6%) were used in 12 groups each consisting of 6 replicates.
After 2.5 months, the silos were opened for sampling. Tilley and Terry [2] and gas test [1] were used to determine digestibility.

Results Adding different levels of urea and molasses increases metabolizable energy. This can be attributed to both the energy contained in molasses and improved digestibility of organic matters as a result of adding urea and molasses. A significant difference was observed between the estimated level of energy in the urea treatment groups (particularly those with higher levels of urea) found using the abovementioned methods. As far as molasses treatment with lower levels of urea is concerned, the gas test provides a better estimation of metabolizable energy.

Table1 Determination of OMD, DOMD, ME, CP, Gas production and SCFA in experimental silage

Molasses	Urea	OMD	DOMD	ME[1]	CP	Gas_{24}(mlit)	ME[2]	SCFA[3]
	0	41.04	37.12	1.49	9.65	26.25	1.51	0.579
	0.5	41.86	37.24	1.50	11.08	23.25	1.43	0.512
0	1	42.32	40.36	1.62	11.39	26.00	1.53	0.573
	1.5	48.64	43.14	1.73	13.18	26.50	1.57	0.584
	0	46.65	41.11	1.65	10.28	29.25	1.62	0.645
	0.5	48.50	42.66	1.71	11.49	30.00	1.66	0.662
3	1	45.11	42.84	1.72	12.59	28.75	1.63	0.634
	1.5	51.11	45.12	1.81	13.00	29.50	1.66	0.651
	0	48.67	43.91	1.76	10.05	32.75	1.73	0.723
	0.5	50.94	45.41	1.82	11.46	34.00	1.79	0.751
6	1	52.91	47.21	1.90	12.60	34.25	1.81	0.756
	1.5	51.85	46.04	1.93	13.51	33.50	1.80	0.739

1.MCal/Kg DM from Tilley and Terry experiment
2.Mcal/Kg DM from Gas Test experiment and related equation
ME (MCal/Kg DM)= $2.2+0.136Gas_{24}+0.075$ CP
3.Short Chain Fatty Acids (mmol/100 mg DM)
SCFA= $0.0222Gas_{24}-0.00425$
4.from Tilley and Terry experiments: OMD=organic matter digestibility, DOMD=dry organic matter in dry matter(%)

Conclusion As result indicate SCFA, OMD, ME and DOMD increase when the level of molasses and urea increase in silage but the higher level of molasses is more effective than adding urea in this increasing. The rule of adding urea in increasing of metabolizable energy is in increasing in protein portion (see the equation 2 below the table) and this is an inexpensive method for improvement of silage quality. Adding urea and molasses improves quality of ensiled pistachio epicarp through increasing organic matter and digestible organic matter in the dry matter percentage and increasing metabolizable energy.

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To graze together or to graze apart: the epidemiological consequences of host resistance to *Teladorsagia circumcincta* in lambs

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Introduction Selection for increased host resistance to gastro-intestinal parasitism using faecal egg count (FEC, eggs/g) is supported by evidence of heritable variation in FEC (Bishop and Morris, 2007), and by results of selection in practice (Kemper *et al.* 2010). However, predicting the impacts of selection for decreased FEC in grazing ruminants is difficult, due to the complex interactions between parasite epidemiology and host resistance. The mathematical model of Laurenson *et al.* (2012) which includes heritable between lamb variation in host-parasite interactions, the epidemiology of *Teladorsagia circumcincta* and anthelmintic drenching protocols, was used to address the interaction between host resistance genotype and parasite epidemiology. The hypothesis was that host populations differing in resistance will differ in FEC, which in turn will lead to pasture contamination differences. Furthermore, we predict that these differences will be greater when populations are grazed separately compared to when grazed together.

Material and methods A population of 10,000 Scottish Blackface lambs was simulated to be grazing on a medium-quality pasture (CP=140g/kg DM, ME=10MJ/kg DM), at a grazing density of 30 lambs/ha, for a time period of 4 months from weaning to 6 months of age. Lambs were assumed to be initially immunologically naïve and the initial larval contamination of pasture (IL_0) was set to 3000 *T. circumcincta* larvae/kg DM. Further, the lamb population was either given no anthelmintic treatment or drenched at 30 day intervals (days 30, 60 and 90) aimed at achieving suppressive nematode control, representing the two extremes of commercial practice (Sargison *et al.* 2007). In order to explore the impact of host resistance on the model predictions, the individual lambs were assessed according to their FEC as predicted by the model. The 1,000 lambs predicted to have the smallest FEC were deemed resistant (R_t) and the 1,000 lambs with the largest FEC were deemed susceptible (S_t). These groups were then re-simulated to be grazing separate pastures ($\mathbf{R_a}$ and $\mathbf{S_a}$), under the same conditions as the population, to assess the impact of host resistance to nematodes on the epidemiology of *T. circumcincta*. Simulations were run for the population, $\mathbf{R_a}$ and $\mathbf{S_a}$ groups for 3 consecutive grazing seasons to allow time for the predicted differences between pastures and groups to stabilise. Live weight (LW, kg), FEC and pasture contamination (PC, larvae/kg DM) were predicted on a daily basis and the results are given as averages over the whole grazing season.

Results Average PC predictions for year 3 are given in Table 1. Grazing $\mathbf{R_a}$ and $\mathbf{S_a}$ lambs separately dramatically altered predicted average PC. Frequent drenching decreased average PC by *ca*. half, although the relative effect was greater in S lambs. Average FEC predictions for year 3 are given in Table 2. Differences between resistant and susceptible lambs were considerably magnified when grazed apart compared to when they are grazed together as part of a larger population. As expected, drenching substantially reduced FEC. However, the drenching effect was markedly greater, both in real and relative terms, in the susceptible population of lambs. Results for year 2 were similar to those from year 3. However, the differences between the $\mathbf{R_a}$ and $\mathbf{S_a}$ populations, whilst greater than the difference between R_t and S_t, were not so pronounced in year 1, suggesting that it takes more than one grazing season for the epidemiological effects to cumulate.

Table 1 Average PC predictions (larvae/kg DM) for year 3 for the whole lamb population grazed together and for $\mathbf{R_a}$ and $\mathbf{S_a}$ lambs grazed separately, given either no anthelmintic treatment or drenched at 30 day intervals

Anthelmintic treatment	Population	$\mathbf{R_a}$	$\mathbf{S_a}$
Un-drenched	4005	547	10087
Drenched	1951	376	4391

Table 2 Average FEC predictions (eggs/g) for year 3 for R_t and S_t lambs (grazed together) and $\mathbf{R_a}$ and $\mathbf{S_a}$ lambs (grazed separately), and given either no anthelmintic treatment or drenched at 30 day intervals

Anthelmintic treatment	R_t	S_t	$\mathbf{R_a}$	$\mathbf{S_a}$
Un-drenched	98	862	58	1305
Drenched	62	240	35	360

Conclusions Selection for host resistance is predicted to ultimately lead to large differences in the expected average PC and FEC. Further, these differences are predicted to be magnified when susceptible and resistant lambs graze separate pastures. Anthelmintic treatment reduces both average PC and FEC, however the effect is much greater in susceptible lambs, suggesting that resistant lambs have a reduced requirement for treatment. This supports the use of targeted selective treatment of lambs, which has implications in terms of reducing costs and reducing the potential for evolution of anthelmintic resistance.

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Modelling the effects of behavioural economics in animal disease surveillance: impact on BVD eradication in Scotland

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Introduction Effective surveillance of endemic disease prevalence and monitoring for exotic disease incursion can be extremely resource intensive. The financial support for such systems is also often limited, so it is crucial that the resources available be allocated in the most efficient manner possible. This is of particular relevance to coordinating strategies that may be employed as part of a disease eradication policy, such as the BVD eradication currently on-going in Scotland. Modelling of animal disease surveillance systems allows the effectiveness of different surveillance systems to be quantified, and therefore facilitates the optimisation of resource allocation. While heterogeneity between farms, for example in terms of animal numbers, is known to affect prevalence estimates and therefore is frequently incorporated into these models, it is much more difficult to incorporate behavioural effects which modulate the responses of individual farms to the perceived state of other farms around them. One solution is to utilize an agent-based approach, where the agents are further subdivided into actors representing farms of various types, and regulators such as media, government and veterinary interventions that have a feedback effect onto these actors. Each agent class is associated with a set of rules based on behavioural phenomena such as the perceived threat of disease incursion, and impacting on the probability that farmers will look for disease. Epidemiological disease transmission models of arbitrary complexity can also be incorporated into within and between farm disease transmission models. This modelling framework can be used with specific rules for BVD disease characteristics and on-farm behavioural responses to various potential eradication strategies employed at a national level in order to evaluate the influence of these behavioural parameters on the rate of reduction in national BVD prevalence.

Material and methods We present an object-oriented programming framework for an agent-based, stochastic model written in the C++ programming language. At the core of this model are objects representing a population of farms, and one or more objects representing regulators such as government policy and veterinary testing with feedback effects onto their behaviour. At each simulated time step, farms pass information about their observed state to regulators (incorporating the sensitivity and specificity of the diagnostic tests), and receive information about the wider population in return. This framework is modified to incorporate specific BVD rules representing disease transmission, diagnostic test sensitivity/specificity and uptake, vaccination status and efficacy, as well as BVD effects such as abortion and infertility on farm. Farm demography information and cattle movements are taken from real census and cattle movement database information between 2003 and 2010 in order to model disease transmission patterns that conform to the real situation in Scotland. Behavioural mechanisms are included to reflect possible impacts on the propensity to test for disease based on on-farm abortion/infertility as well as local and national trends in observed BVD prevalence, with heterogeneity in behavioural effects allowed for between farms. This simulation was repeated one thousand times, recording the observed proportion of infected farms at each time step, in order to capture the variation in stochastic model output. This exercise was repeated with different parameter values for the magnitude of various behavioural effects in order to evaluate the impact of these parameters on the system as a whole.

Results Incorporating behavioural feedback effects into the model increased the stable prevalence of BVD in Scotland from approximately 1% to approximately 10% towards the end of the simulated time period. Increasing the magnitude of the behavioural feedback effects resulted in a greater disparity in average observed prevalence to the model without behavioural feedbacks, and introduced auto-correlative effects into the observed disease prevalence that tended to increase the discrepancy between observed and true disease prevalence. This resulted in a stable observed disease prevalence of only 5% at the same time period as the true prevalence was 10% for the model incorporating behavioural feedback mechanisms, making the simulated disease eradication appear to be more successful than it actually was. Removing heterogeneity between farms also tended to improve the effectiveness of disease eradication, but to a lesser extent than removing behavioural feedback effects.

Discussion The results presented demonstrate that incorporating behavioural feedback mechanisms into disease surveillance modelling has a profound impact on the observed disease prevalence. Based on these results, it is also apparent that the success of any disease eradication campaign is likely to be strongly influenced by the changing behavioural responses of farmers to the perceived threat of disease. These feedback effects appear to be non-random and often counter-intuitive, and introduce auto-correlative effects into sequential prevalence estimates resulting in a random-walk-like effect in the observed prevalence even when the true prevalence is in fact static. The magnitude of these effects is highly dependent on the parameter estimates used to model the behavioural responses, which is concerning given the current lack of data available on which to base these parameters, but is likely to be at least as influential as epidemiological parameter estimates such as diagnostic test characteristics and disease transmission parameters. Inclusion of behavioural effects into disease surveillance modelling is undoubtedly difficult, but can be achieved using the agent-based approach employed here. We conclude that obtaining better estimates for behavioural parameters should be a priority for future data collection, and that pursuing collaboration with economists and social scientists to improve attempts to model behavioural economics should be regarded as essential for future disease surveillance programmes.

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Modelling the impact of *Teladorsagia circumcincta* larvae contamination of pasture and anthelmintic treatment on genetic parameter estimates for parasite resistance in grazing sheep

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Introduction Breeding programs for host resistance to nematodes require knowledge of genetic parameters for host resistance and production traits, such as heritabilities and genetic correlations between traits. Whilst heritabilities for faecal egg count (FEC, eggs/g) and body weight (BW, kg) are relatively consistent (e.g., 0.2-0.4), estimates of genetic correlations between FEC and BW vary widely, ranging from -0.8 (Bishop *et al.* 1996) to +0.4 (McEwan *et al.* 1995). Variation observed in such correlations may be due to interactions between host genetic resistance and the environment, including parasite epidemiology. The mathematical model of Laurenson *et al.* (2012), which includes heritable between-lamb variation in host-parasite interactions, the epidemiology of *Teladorsagia circumcincta* and anthelmintic drenching protocols, was used to address epidemiological effects (e.g., level of pasture larval contamination) and anthelmintic input on the estimates of genetic parameters for a population of grazing lambs. The hypothesis was that both will strongly influence the direction and magnitude of the correlation between production and resistance traits.

Material and methods A population of 10,000 lambs was simulated (Laurenson *et al*. 2012) to be grazing on a medium-quality pasture (CP=140g/kg DM, ME=10MJ/kg DM), at a grazing density of 30 lambs/ha, for a time period of 4 months from weaning to 6 months of age (N.B. lambs are weaned at 3-4 months in some systems). The lambs modelled were similar to Scottish Blackface lambs, with the mean, the coefficient of variation and the heritability of input parameters matching those of Bishop *et al*. (1996) and Bishop and Stear (1997). Lambs were assumed to be initially immunologically naïve and the initial larval contamination of pasture (IL_0) was set to either control (i.e., 0), 1000, 3000 or 5000 *T. circumcincta* larvae/kg DM, corresponding to a range of challenge levels that normally lead to subclinical *T. circumcincta* infections. Further, the lamb population was either given no anthelmintic treatment or drenched at 30 day intervals (days 30, 60 and 90) aimed at achieving suppressive nematode control, representing the two extremes of commercial practice (Sargison *et al.* 2007). Empty body weight (EBW, kg) and FEC were predicted on a daily basis. FEC was log transformed for the calculation of genetic parameter estimates, and genetic parameter estimates (i.e., heritabilities and genetic correlations) were reported at the end of the simulation on day 121, to reflect values for lambs close to both market and selection age.

Results Heritability estimates for *ln*(FEC+1) was 0.23 for un-drenched sheep, and 0.22 for drenched sheep across all levels of IL_0, falling within the range of published values. Heritability estimates for EBW (Table 1) decreased with increasing levels of IL_0, however the impact of IL_0 was much less on drenched sheep in comparison to un-drenched sheep, where it remained almost constant. Genetic correlations between EBW and *ln*(FEC+1) (Table 2) became increasingly negative with increasing levels of IL_0, and similar to heritability estimates, the impact of IL_0 was much reduced in drenched sheep in comparison to un-drenched sheep.

Table 1 EBW heritability estimates at day 121 post-infection for un-drenched and drenched sheep grazed on pasture with an initial larval challenge (IL_0) of control, 1000 3000 or 5000 larvae/kg DM

L_0	Un-drenched	Drenched
control	0.56	0.56
1000	0.36	0.54
3000	0.29	0.52
5000	0.27	0.52

Table 2 Genetic correlation between EBW and *ln*(FEC+1) at day 121 post-infection for un-drenched and drenched sheep grazed on pasture with an initial larval challenge (IL_0) of 1000, 3000 or 5000 larvae/kg DM

IL_0	Un-drenched	Drenched
1000	-0.43	0.00
3000	-0.65	-0.10
5000	-0.72	-0.18

Conclusions Both level of pasture larval contamination and anthelmintic input had substantial influence on the heritabilities for EBW and the magnitude of the correlation between production and host resistance traits. Increasing levels of IL_0 led to increasingly negative (i.e. favourable) correlations, and anthelmintic drenching resulted in much weaker correlations being predicted. These findings shed light on one possible cause of variability in the genetic parameter estimates reported within literature and suggest that interpretations of results and subsequent breeding decisions should be made in accordance with the environment in which sheep are maintained.

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A mechanistic approach to modelling macro-parasite transmission in a grazing system

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Intro Macro-parasites affect the fitness and survival of their herbivorous hosts and have an adverse impact on the livestock industry. The development of mechanistic models encapsulating key factors driving the parasite transmission process will allow us to understand the potential impacts of global change on future disease risk, and explore long term control strategies. Previous models have looked separately at the dynamics of helminth infection (Roberts and Grenfell, 1991), and the impacts of host grazing behaviour and spatial heterogeneity on pathogen risk (Marion *et al*., 2005). Through building on these approaches and formulating a model that incorporates the pivotal elements of the parasites lifecycle and livestock grazing behaviour, we aim to identify the key drivers of helminth infection in a grazing system.

Methods A spatially explicit individual based stochastic model was formulated to incorporate the fundamental elements of the transmission process including survival and development of larvae both in host and on pasture, host immunological response, and host grazing behaviour. All simulations modelled a herd of cows, in set stocking, over one grazing season. To determine the influence of the main drivers in helminth transmission the model was run with incremented values for key parameters including parasite fecundity, probability of an ingested larvae establishing as an adult parasite in the host, and the development rate of larvae on pasture from a non infectious to an infectious state.

Results The trends in parasite dynamics shown by Roberts and Grenfell (1991) were successfully reproduced (Figure 1), with the introduction of susceptible hosts onto contaminated pasture accounting for the rapid increase in ingested larvae and adult parasites in the host, the subsequent acquisition of immunity accounts for the consequent decline in parasite numbers.

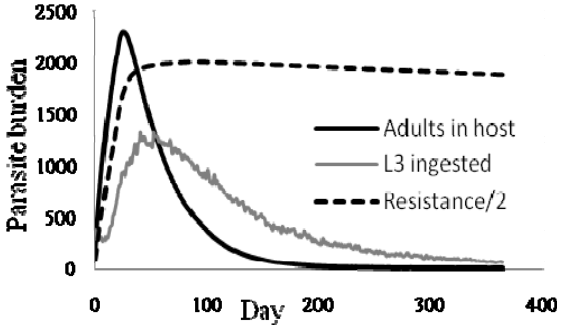

Figure 1 The trend in parasite burden, infectious larvae ingested and host resistance over one grazing season.

Figure 2 shows the influence of importance parameters on peak host parasite burden. There is a non linear relationship, with both establishment rate and larval development rate showing distinct sigmoidal responses. These results indicate that the influence of acquired immunity on parasite establishment and fecundity has a large impact on parasite burden. Further results demonstrate that host grazing behaviour affects both the timing and magnitude of outbreaks.

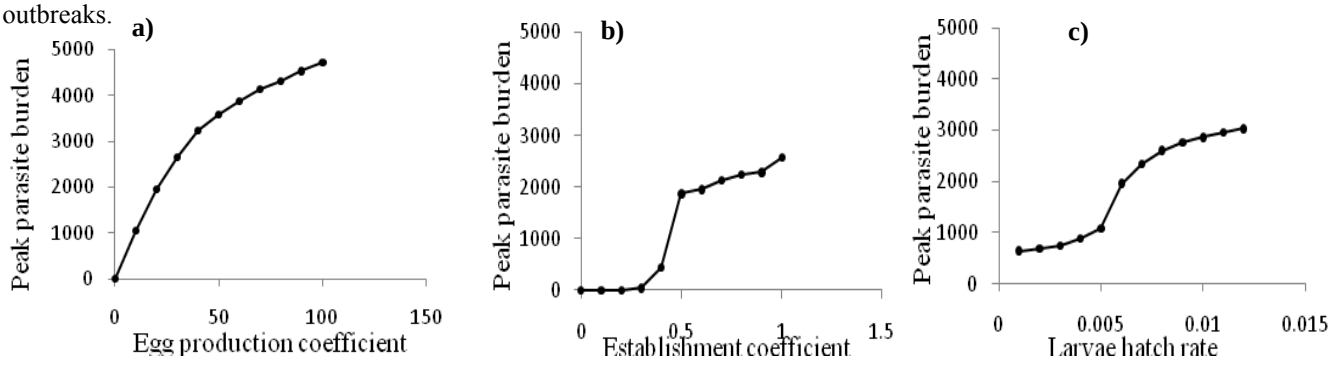

Figure 2 Variation in peak parasite burden in the host under changing levels of a) parasite fecundity, b) proportion of ingested larvae establishing in the host, and c) larvae hatch rate.

Conclusions The timing and intensity of helminth outbreaks is driven by multiple factors including the survival and development of larvae on pasture, parasite establishment and fecundity, host resistance and grazing behaviour. It is important these elements are considered when developing transmission models. This model can ultimately be used to understand the potential impacts of climate change on specific parasite risk and the efficacies of different control strategies in a changing climate.

Acknowledgements SAC and BioSS receive funding from the Scottish Government.

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Detecting emerging and re-emerging zoonotic pathogens in rodent species using microarrays

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Introduction Emerging and re-emerging diseases have the potential to have a significant impact on public health, animal health and the global economy. Increasing urbanisation and climate change leave us facing an unprecedented worldwide impact from animal diseases, particularly zoonoses. The order *Rodentia* is the largest of all the mammal orders, and they can act as reservoirs for many zoonoses. The aim of this project is to validate a number of zoonotic pathogens transmissible by rodents on a microarray platform. Samples from rodents from urban centres across Europe and from Vancouver, Canada will then be screened for pathogens using this new tool. Sequencing of isolates and epidemiological analyses will then be performed.

Material and methods The following pathogens were selected: *Yersinia* species (*Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis*), Hantavirus (Puumala Virus and Seoul Virus), Hepatitis E Virus and *Toxoplasma gondii*. The choice of pathogens was made by the WildTech members as these pathogens have the potential to be carried by *Rattus rattus, R. norvegicus and Mus musculus*. Several genomic regions were identified as targets for probe design. For the viruses: SEOV, PUUV, generic Hantavirus and HEV the entire genome was considered as these have a relatively small genome. For *T. gondii*, a literature search revealed genes that contain specific regions that could be used for detection purposes. Similarly for the *Yersinia* species, various genes have previously been studied in the literature and can be used to identify the different species of *Yersinia*. Two free software packages were used for probe design; Unique Probe Selector (UPS) (Chen 2010) and Oligwiz 2.0(Wernersson 2007). The programmes identified potential regions for oligonucleotides and checked that there would be no complications such as cross-hybridisation with or folding. Oligonucleotides were designed so that they would be 60 nucleotides long. The selected probe sequences were sent to Metabion International for synthesis. Oligonucleotide primers were designed from the target sequences from which probes were designed. Using Primer3 software, amplicon size was set between 250 and 750 bases with an optimum of 500 bases. The synthesised probes were sent directly to Alere Technologies for microarray production. Three arrays were requested: Hanta_HEV_01, Yersinia_01 and Toxoplasma_01. Each probe was duplicated on the array and the remaining spots were used as control probe, orientation markers and biotin markers (an assay control). PCR was then performed on the *Yersinia* and *T. gondii* samples. The samples were initially amplified using sequence specific primers and then by a second round of PCR using random primers. Amplified products were checked for correct length, by electrophoresis on 1% (w/v) agarose gels stained with ethidium bromide. Direct labelling of the randomly-amplified product was carried out by PCR. The samples were then added to the arrays for the hybridisation process to be carried out. The images were recorded using an array reader and the resulting image was analysed using Alere's IconoClust software and analysis script.

Results 453 probes have been designed, and all of these have been analysed *in silico* using BLAST. No significant cross hybridisation was apparent for any of these sequences with any on the NCBI database. The identities of the nucleic acid samples received were confirmed by using pathogen-specific primers. The gel electrophoresis results showed bands at the expected size for the samples of *Yersinia* and *T. gondii*. Hybridisation was performed on the relevant microarray using the labelled PCR products from *Y. pestis, Y. enterocolitica*, *Y. pseudotuberculosis* and *T. gondii*. Figure 1 shows the image of Toxoplasma_01 hybridised with multiple biotin-labelled amplicons of *T. gondii*. Biotin markers are indicated by arrows.

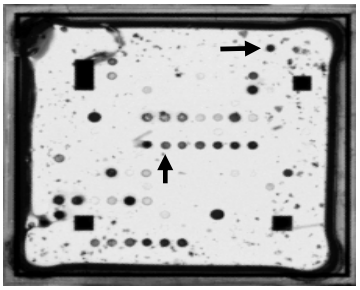

Figure 1

Conclusions This shows that the hybridisation protocol for *T. gondii* and *Yersinia* work as expected when using cultured samples. This will further be tested on samples of experimental infection and known positive samples before screening is performed on rodent species where it has the potential to identify numerous pathogens in rodent species.

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Cellular changes in the mitral valve of Cavalier King Charles Spaniel with late-stage myxomatous mitral valve disease

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Introduction The Cavalier King Charles Spaniel (CKCS) is recognised to be particularly susceptible to myxomatous mitral valve disease (MMVD), with a more rapid development of valve pathology and consequent heart failure compared to other pedigree and mixed breed dogs. Pathological changes in MMVD have been partially described in the dog, but this has been primarily in a heterogeneous breed group. Recent studies suggest that damage to valve endothelium and activation of valvular interstitial cells might be the key to the valve extracellular matrix remodelling seen with MMVD. The aim of this study was to determine if the cellular changes in CKCS with MMVD is similar to that seen with other dogs or does a difference in pathological outcome explain the unique characteristic of CKCS MMVD.

Material and methods Mitral valve leaflets of late-stage MMVD were collected from CKCS (n=6) at the Hospital for Small Animals, Royal (Dick) School of Veterinary Studies, the University of Edinburgh. All dogs were euthanized due to decompensated heart failure and poor quality of life. Following removal and careful examination of mitral valve leaflets, they were immersed in 4% paraformaldehyde overnight. The tissues then were embedded in paraffin wax, and sagittally sectioned and collected onto glass slides. Standard immunostaining (Vector ABC Kit and NovaRed) was performed. Primary antibodies for inflammatory cell markers (CD11c, CD45), interstitial cell markers (vimentin, α-SMA, SMemb), the endothelial cell marker vWF and the proliferation marker Ki-67 were used to identify valve cell phenotype. Leica-DMRB microscope and Leica CCD camera (Wetzlar, Germany) were used to capture images, and images were processed and analysed using ImageJ software (NIH, USA).

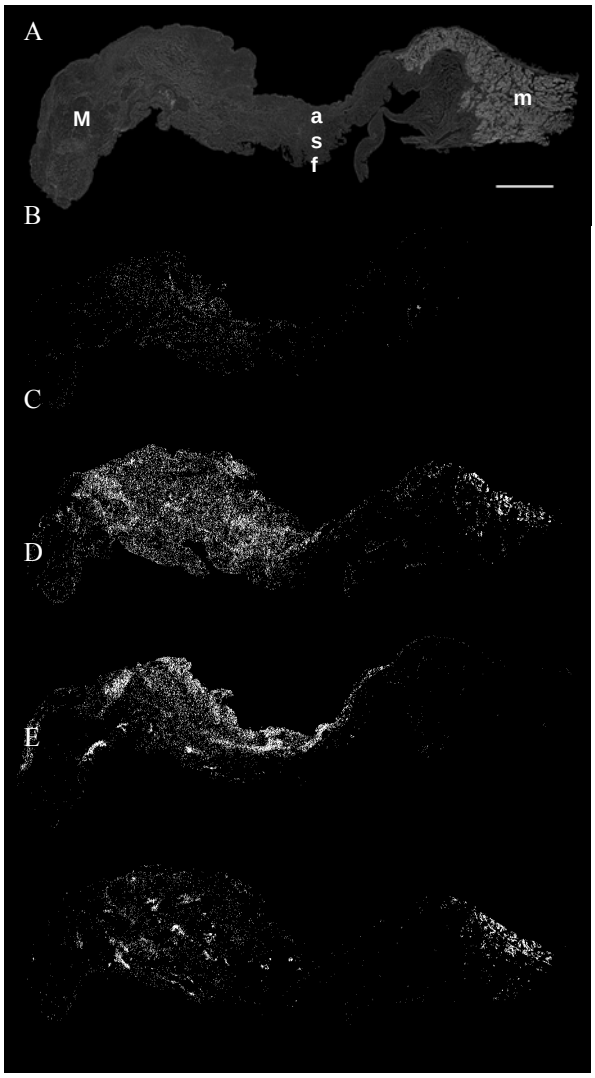

Figure 1 *ImageJ processed immunostaining images of CKCS mitral valves demonstrating staining for (A) H&E (B) α-SMA (C) SMemb (D) Vimentin (E) vWF. Three different layers (a) atrialis, (s) spongiosa, (f) fibrosa in the mid zone of the leaflets and (M) myxomatous area are shown in the top panel. Myocardium (m) is present at the base of the mitral valve. Bars = 1mm.*

Result No inflammatory marker positive cells were observed on any slides. Individual Ki-67 positive cells were found solely distributed at the tip of the leaflets with some cells clustering in the spongiosa layer. Immunohistochemistry staining with SMemb and Vimentin both showed extensive cytoplasmic staining throughout the entire valve leaflet, whereas α-SMA positive cells formed a linear zone immediately beneath the endothelium and also showed sparse aggregation in the mid zone. vWF staining were patchy and localized to the endothelium, but there were some positive cells in the valve stroma.

Conclusions The cellular changes of mitral valves found in CKCS are generally similar to those descriptive studies previously reported for mixed canine breed dogs and for human patients with mitral valve prolapse. This suggests there is no difference in CKCS MMVD pathology compared to other dogs, and that the early onset of CKCS MMVD does not result in a different pathology end-point. Furthermore, there is no evidence for an inflammatory component in late stage MMVD, but that does not exclude a role for inflammation and immune mechanism as initiators of disease in the early stages. Interestingly, the differences in pattern of expression of α-SMA and SMemb (activated myofibroblast markers) do show heterogeneity of mitral valve interstitial cells.

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Variation in resistance and resilience to nematode parasites between selected mice strains

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Introduction Medicinal plants play an important role for parasite control in developing countries, and are of growing significance 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 aim to identify a mouse model to scientifically evaluate the suitability of plant preparations for parasite control. Whilst between-strain variation in nematode resistance is well described (Behnke *et al*., 2006), there is no evidence of between-strain variation in nematode resilience. This needs to be demonstrated, as mice will serve as model organisms for livestock, which experience performance penalties during parasitism. Our hypothesis is that resistant strains of mice may experience greater penalties on resilience to parasitism than their more susceptible counterparts.

Material and methods Three inbred strains of mice were used, i.e. C57BL/6, BALB/c and NIH, characterized as having high, intermediate and low susceptibility to infection with the nematode *Heligmosomoides bakeri* as defined by their phenotype (Behnke *et al*, 2006). Mice (male, 6 weeks of age) were either parasitized with a single dose of 250 L_3 *H. bakeri* (P; n=10) in water or were sham-infected with water (C; n=10), as *per* previously established model (Coltherd *et al*, 2011). Mice were offered a maintenance diet (14% crude protein) *ad libitum* for six weeks. Body weight (BW) and feed intake (FI) were recorded thrice weekly; nematode eggs per gram faeces were determined weekly from wk3 onwards. At wk 6 post infection mice were euthanized and dissected; organ weights, egg counts in colon contents and worm counts were determined. FI and BW gain during infection establishment (wks 1-2) and established infection (wks 3-6), with initial BW as covariate, and dissection parameters were analysed through a 3 x 2 factorial ANOVA (3 strains x 2 treatments). FEC and worm counts were analysed by one-way ANOVA (3 strains). FI and faecal egg counts were analysed in a repeated measure model.

Results Although all infected mice showed lower intake compared to their control counterparts (P<0.001), during the first two weeks post challenge the difference was more pronounced in BALB/c mice and less pronounced in C57BL/6 mice (P=0.08). This interaction on FI was not sustained in weeks 3-6 (P=0.450). Parasitized BALB/c mice grew more and parasitized C57BL/6 grew less than their control counterparts during the first two weeks post challenge (P=0.05); this interaction on BW gain was not sustained in weeks 3-6 (P=0.126). FEC were significantly higher in the C57BL/6 mice (P<0.01); worm counts were also higher in C57BL/6 mice, but the difference between strains was not significant (P=0.600). At dissection (Table 1) the weights of liver, spleen, small intestine and caecum were significantly higher in parasitized animals (P<0.001), whereas the carcass weight was significantly lower in parasitized hosts (P=0.005), particularly in C57BL/6 mice (P=0.08).

Table 1 Weights of internal organs (mg/g final BW) and carcass (g) of parasitized and control mice of the three strains

Strain	Treatment	Small intestine	Large intestine	Caecum	Liver	Spleen	Carcass
BALB/c	P	90.5	12.1	22.9	51.8	4.7	21.21
	C	57.6	11.8	18.7	48.9	3.7	21.12
C57BL/6	P	111.2	9.1	36.8	47.6	3.8	17.37
	C	57.9	8.3	23.5	43.4	2.6	19.60
NIH	P	95.2	9.6	20.8	52.1	6.0	23.33
	C	59.9	9.0	19.3	48.8	3.4	25.11
	s.e.d.	3.7	1.2	2.5	2.1	0.2	0.76

Conclusions The study confirmed that C57BL/6 mice are more susceptible to an *H. bakeri* infection than NIH and BALB/c, which appear to have similar susceptibility to the infection. All parasitized mice displayed reduced intake, which was least pronounced in the most susceptible mice strain, as hypothesised. However, C57BL/6 mice seemed to have experienced the greatest penalties on their growth, the extent of which became evident only by accounting for their increased internal organs weights. C57BL/6 mice showed the greatest increase in small intestine weight, which may be indicative of increased inflammation due to parasite susceptibility. It appears that although C57BL/6 mice did not show the greatest drop in their intake, their final carcass weight was severely penalised, maybe as a consequence of different prioritisation of resources. As a consequence, we recommend C57BL/6 mice as the best model to evaluate the suitability of medicinal plants for parasite control. Our results also support the view that impact of parasitism on animal productivity may be underestimated if this is assessed through live weight gain only.

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Expression of carboxymethyllysine, an advanced glycation end product, in myxomatous mitral valve disease and the mitral valve of healthy dogs

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Introduction Advanced glycation end products (AGEs) are a series of complexes that have been shown to be implicated in degenerative changes of long-living tissue proteins, such as collagen, either by forming intermolecular cross-links or through AGE receptor pathways. AGEs are believed to contribute to diseases where there are collagen abnormalities, and myxomatous mitral valve disease has been shown to include collagen damage. The aim of this study was to evaluate the expression of carboxymethyllysine (CML) modified protein, one of the most common AGEs, in canine myxomatous mitral valve disease (MMVD) and compare to expression in the healthy mitral valve (MVH).

Material and methods Canine mitral valves were collected *post mortem* from MMVD (n=3) and MVH (n=3) dogs. Valves in the diseased dogs were graded according to the Whitney grading scheme. CML modified protein expression was determined by protein immunoblotting (Western) and valve localisation in the MMVD dogs and MVH dogs was confirmed by immunohistochemistry. Serum CML modified protein levels were measured in MMVD (n=17) and MVH (n=9) groups using a commercial CML ELISA kit and the concentrations were calculated by applying the mean values of each sample to the standard assay curve. The ELISA results were analysed using the unpaired t-test.

Results On Western blotting, differential CML expression was observed comparing the MMVD and MVH groups. On immunohistochemistry staining, there was in general more obvious CML staining in MMVD dogs than in the MVH dogs. CML modified proteins were detected in valve stroma, endothelial cells and interstitial cells of the MMVD valves. Very weak CML expression was found in valve stroma of one MVH dog but not in the other two MVH dogs (data not shown). For ELISA results the mean ± SEM of the MMVD group was 1.176 ± 0.11 µg/ml and the mean ± SEM of the control group was 1.159 ± 0.1453 µg/ml. The means of the two groups were not significantly different (p>0.05) and this was found to be the same when comparing variances (p=0.9327).

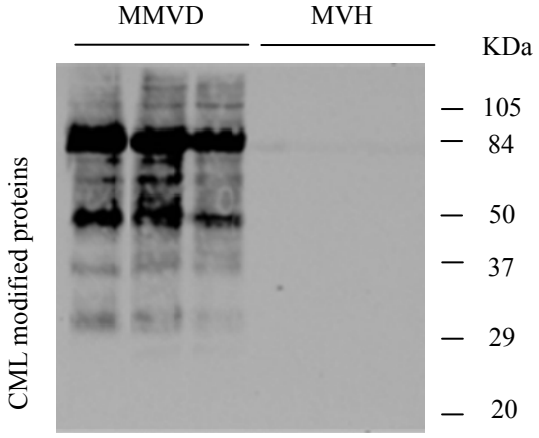

Figure 1 Western blot illustrated marked differential expression of CML modified proteins in the mitral valve of MMVD dogs (n=3) and normal control dogs (n=3). Poncaeu S staining demonstrated equal loading control of proteins (not shown).

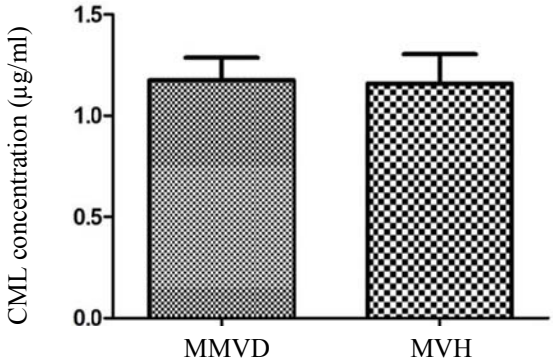

Figure 2 Circulating CML levels were detected in the MMVD group (n=17) and MVH group (n=9) by ELISA. No significant difference was observed between the two groups (P>0.05).

Conclusion This limited study has shown CML modified proteins have higher expression in the mitral valve tissue of MMVD dogs compared to that of MVH dogs. Confirmation and distribution was determined by immunohistochemistry. However, circulating CML concentrations in MMVD and MVH groups were not significantly different. These findings suggest AGEs have a potential role in the pathogenesis of canine MMVD pathology but circulating CML levels cannot be used as a marker for MMVD.

Acknowledgement The authors would like to thank the University of Edinburgh and the Chinese Scholarship Council for funding this project.

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Immunomodulatory potential of salmon oil in domestic cats

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Introduction The potential therapeutic benefits of dietary supplementation with omega-3 fatty acids, which are found primarily in fish oils, including salmon, and establishing the optimal dietary ratio of omega-3 (n-3) to omega-6 (n-6) fatty acids is of great interest in the functional foods arena. The anti-inflammatory properties of n-3 fatty acids have been well documented in several species; however results from work investigating the effect of n-3 fatty acid supplementation on the immune system are often conflicting, possibly due to the different ratios of n-6: n-3 fatty acids used in the trials. Lipid metabolism in cats is different to other mammalian species (cats have low levels of liver Δ-6 desaturase); therefore supplementation with n-3 fatty acids in cats may give different results. To date the limited work in cats indicates that 12 week supplementation at an n-6: n-3 ratio of 5: 1 results in immunosuppression, with decreased lymphocyte proliferation to pokeweed mitogen but not to concanavalin A (ConA) or phytohaemagglutinin (PHA) and a reduction in numbers of B cells and total T and T_h subpopulations (Chew *et al*., 2000, Park *et al*., 2011). In this study we assessed the effects of short term dietary supplementation with salmon oil produced in NZ, to modulate immune parameters in the domestic cat.

Material and methods This study using 16 adult domestic short haired cats, was conducted at the Centre for Feline Nutrition, Massey University, Palmerston North, NZ. Eight animals (4 male, 4 female) were fed the Control diet which comprised a commercial moist diet (protein 41.7%; fat 43.3%; carbohydrate 6.4%; ash 8.6%; DM basis; n-6:n-3 ratio of 4.77: 1), with a second sex-matched group fed the same commercial diet supplemented with 2% (w/w) Salmon oil (New Zealand King Salmon Company Ltd, Nelson, NZ; n-6:n-3 ratio of 0.78: 1), resulting in a test diet with an n-6:n-3 ratio of 2.32: 1. The cats were fed the diets *ad libitum* for 28 days and had access to water at all times. Body weight was monitored weekly throughout the experimental period. Blood samples were taken by jugular venepuncture on days 0, 14, and 28 and collected into heparin tubes. These whole blood samples were used in the assessment of immune cell responses, including lymphocyte proliferation to the T-cell mitogens PHA and Con A by assessing incorporation of ^{3}H-thymidine, expression of the cell surface markers CD 4 (T-helper cell), CD 8 (cytotoxic T-cell), B cell, CD 14 (monocyte) and CD 11b (neutrophil activation marker) and phagocyte function using flow cytometry. Data were analysed by repeat measure with a model including treatment and time as fixed effects and cat within treatment as a random effect (Proc Mixed, SAS). Sex was not included in the model as no difference between male and female were observed.

Results The cats remained healthy for the duration of the trial and maintained constant body weights. As shown in Table 1, dietary supplementation with Salmon oil resulted in a trend for a time dependant enhancement of lymphocyte proliferative responses to the T-cell mitogen PHA after 4 weeks and a significant increase in phagocytic activity after both 2 and 4 weeks. Dietary supplementation did not alter the lymphocyte proliferative responses to ConA. Control group animals showed no change in lymphocyte proliferative responses to Con A, or change in phagocytic activity during the experimental period. Dietary supplementation with salmon oil did not alter the level of expression of cell surface markers.

Table 1 Effect of dietary supplementation on immune parameters over time. ^{a,b,c} supplementation x time values with different superscripts are different to each other ($P<0.05$). ^{x,z} Overall time values with different superscripts are different to each other ($P<0.05$)

	Lymphocyte Proliferation (PHA)				Phagocytic Activity			
	Control		Salmon oil		Control		Salmon oil	
Time (weeks)	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	7.6^{x}	1.42	6.1^{x}	1.33	42.0^{b}	2.23	34.8^{a}	2.09
2	8.1^{x}	1.62	7.3^{x}	1.33	42.1^{b}	2.23	41.4^{b}	2.09
4	9.3^{z}	1.42	10.09^{z}	1.33	41.0^{b}	2.23	53.7^{c}	2.09

Conclusions Dietary supplementation with the salmon oil led to a time dependant enhancement of phagocytic activity and trend for an increase over time for lymphocyte proliferation to PHA. The lymphocyte proliferation results may indicate that specific groups of T-cells were up-regulated by consumption of the supplement, apparently priming them to proliferate in response to an appropriate antigenic challenge, such as a bacterial infection. The phagocyte function assay measures the ability of cells to ingest foreign particles such as bacteria. Since the enhanced phagocyte function occurred without any increase in the level of expression of CD14 and CD 11b cells, the mechanism for the increase was not via increased numbers of monocytic cells or increased neutrophil activation. Taken together these results indicate that short term dietary supplementation with salmon oil can modulate the immune system of cats, and this may translate into having enhanced resistance and a greater ability to fight infection and disease.

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Effect of selenium in cadmium induced renal toxicity in male Sprague-Dawley rats

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Introduction Cadmium, a heavy metal well known to be highly toxic to both human and animals, is distributed widely in the environment due to its use in various industries. Cadmium may induce oxidative damage in different tissues by enhancing peroxidation of membrane lipids in tissues and altering the antioxidant systems of the cells. Conversely, selenium (Se) is generally recognized as an important antioxidant and an antagonist that moderates the toxic effects of many heavy metals in various organisms. Since little information is available on the ameliorating effects of Se against Cd-induced toxicity, this study investigated the ameliorating effect of sodium selenite on the cadmium chloride induced renal toxicity in male Sprague-Dawley rats.

Material and methods A completely randomised study was used to house twenty male Sprague Dawley rats (28 days old) at Quaid-i-Azam University Islamabad, Pakistan as approved by this University's Ethics Committee. . The rats were familiarised to their group housing and feeding for 14 days before their weighing for distribution into four groups of 5 rats each with similar initial body weight per group. These rat groups were randomly housed in metal cages (38x23x10cm) which were kept in a Laboratory at 25±2^{o}C with dark to light cycle of 14 to 10 hours. The same pelleted diet and fresh water was provided *ad libitum* to these rats for the entire study period. The rats were given subcutaneous injections (1mg /kg BW) of normal saline (Control), Cadmium Chloride (Cd group), Sodium selenite (Se group) and Cadmium chloride + Sodium selenite (Cd-Se group) on alternate days for 28 days of this study. All rats were then weighed at 7 day intervals for 28 days before their humane sacrifice on 29th day, to collect their kidneys. The kidneys were weighed and these weights were expressed as g/100g final BW and cleaned in ice cold normal saline. The Cd, Se, Zn and Fe concentrations in kidneys were estimated by using an inductively coupled plasma optical emission spectroscopy (ICP-OES). A known weight of kidney tissue was quickly weighed and then homogenized in 0.1 M Tris–HCl buffer at pH 7.4 by using a Potter-Elvejham homogenizer at 4°C with a diluting factor of 4. The crude tissue homogenate was then centrifuged at 10,000 rpm for 15 min at 4°C to collect the supernatant which was used for the assay of malondialdehyde (MDA), lipid hydroperoxides (LHP), reduced glutathione (GSH) and catalase activities (CAT) according to relevant protocols. The data were statistically analysed as a completely randomised design by using ANOVA in Minitab software to determine the treatment effects on relative weight of kidneys, trace metal levels in kidneys, lipid peroxidation and oxidative stress parameters at P<0.05. Tukey's test was used to compare treatments means at P<0.05.

Results Table 1 shows significant difference in final body weight after 28 days among different treatment groups (P<0.05). The relative weight of kidney differed significantly (P<0.05) in Cd group compared to other treatment groups and control. Table 2 shows significant differences (P<0.05) in trace element levels (mg /kg DM) in kidneys of different treatment groups of rats. Lipid peroxide levels differed significantly (P<0.05) in different treatment groups of rats and more MDA levels were observed in Cd group than the control and se groups, while significant (P<0.05) decrease in MDA level was observed in the Cd-Se compared to the Cd group which showed ameliorating effect of Se on Cd toxicity in rat kidney. Lipid hydroperoxides in the Cd group also differed significantly as compared with the other treatment groups and the control (P<0.05). Significantly lower levels of GSH were recorded in Cd group compared than the other treatments and control (P<0.05), while increased level of GSH was observed in Cd-Se group than the Cd group. Catalae also showed lower levels in the Cd group than the other treatment groups. Oxidative stress enzymes showed reduced levels in the Cd group than the other treatments.

Table 1 Initial and final body weights (BW) and relative kidney weights (g/100g BW) of rats for different treatment groups

	Control group	Cd Control	Se group	Cd-Se group	SEM
Initial BW	87.2	87.6	87.2	84.5	2.71
Final BW	227.6^{b}	200.8^{c}	259.0^{a}	227^{b}	3.73***
Kidney relative weight	0.88^{b}	0.71^{a}	0.81^{b}	0.81^{b}	0.021*

(Means with different superscripts in the same row differed significantly; *=P<0.05; ***=P<0.001)

Table 2 Effect of cadmium and selenium on levels (mean ± SE) of trace elements, lipid peroxides (MDA), Lipid hydroperoxides (LHP), reduced glutathione (GSH) and catalase (CAT) in kidney of different treatment groups of male Sprague-Dawley rats

Treatment Groups	Cd	Se	Fe	Zn	MDA (nM/g)	LHP (mM/g)	GSH (µM/g)	Catalase (U/ml)
Control	1.78±0.26^{c}	10.41±1.45^{c}	258±6^{b}	105±6^{a}	370±10.9^{b}	7±0.25^{b}	2012±80^{a}	2.3±0.21^{a}
Cd	15.47±2.23^{a}	4.14±0.45^{d}	180±7^{d}	80±2^{d}	1230±50^{a}	8.2± 0.22^{a}	1160±21^{b}	1.6±0.02^{b}
Se	1.26±0.24^{d}	17.4±0.21^{a}	300±6.7^{a}	93±1.5^{b}	410±20^{b}	6.8±0.23^{b}	2120±112^{a}	2±0.1^{a}
Cd-Se	12.5±0.21^{b}	15.27±2.3^{b}	211±4.5^{c}	88±1.25^{c}	600±30^{c}	7.1±0.21^{b}	1709±49^{c}	2± 0.1^{a}

(Means with the same superscript within a column did not differ significantly P>0.05)

Conclusions This study showed an ameliorating effect of selenium in cadmium induced renal toxicity in male Sprague-Dawly rats.

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Seasonal effects on activity levels, energy intake and nutrient digestibility in neutered male and female cats in a temperate environment

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Introduction Domestic cats (*Felis catus*) show strong seasonal cycles of reproduction (Goodrowe *et al.* 1996), coat growth (Hendriks *et al*. 1997), and body weight when maintained on *ad libitum* wet diet (Thomas unpublished). Despite these seasonal rhythms, current feeding recommendations and current nutritional adequacy pet food testing protocols (AAFCO, 2011) do not consider these underlying seasonal rhythms. The aim of the present study was to investigate the seasonal fluctuation in activity levels, energy intake and nutrient digestibility in neutered male and female cats.

Material and methods Two groups of adult domestic short-haired cats; neutered females (NF: n=8) and neutered males (MN: n=8), from the Centre of Feline Nutrition, Massey University, Palmerston North, New Zealand (longitude 175° 38' E, latitude 40° 22' S) were used in the study from 20th February until 30th July 2011. Each group was balanced for age (mean±SEM: NM 5.98±0.86, NF 5.44±0.68), and kept in outdoor colony cages (4.5×1.4×2.5 m) according to the Animal Welfare Code of Welfare (Companion Cats, 2007). Environmental conditions (temperature, rainfall and humidity), were recorded using a weather station (La Crosse Technology, La Crosse WI USA). Both groups were fed *ad libitum* with a commercial AAFCO tested wet diet (17.8% DM as is, 8.1%DM ash, 45.9%DM crude protein, 32.5%DM crude fat, 2.0%DM crude fibre and 26.0kj/g DM gross energy). Accelerometers (Actical® Respironics Mini Mitter division, Bend, OR USA) were used to determine the activity rhythms of the cats in autumn (20th February – 23rd May) and winter (5th June – 30th July). Epoch length was set at 0.25 min on each monitor which recorded continually for 6-day blocks (Monday excluded). Data was downloaded once a week and analyzed by Actical® Software (Version 2.0). Apparent nutrient digestibility was assessed in autumn (6th-11th March) and winter (5th-10th June) using a total collection protocol (AAFCO, 2011). Results are presented as mean and standard errors of the mean (SEM), with differences between groups determined using a Fisher-protected least significant difference (LSD). Differences between seasons were determined using paired T-test.

Results The daily activity pattern of domestic cats was inversely related to the temperature pattern. As the season changed from autumn to winter the daily activity level of both groups was lower, with the effect significant (P<0.005) in NF. In both autumn and winter, the activity of NM was significantly (P<0.05) higher than NF.

Table 1 Average daily activity counts per epoch of neutered female (NF) and male (NM) domestic cats during autumn and winter.

Cat group	Autumn	Winter	P-value
Neutered Female	10.79±0.43[a]	8.99±0.31[a]	0.003
Neutered Male	15.17±0.65[b]	13.05±0.84[b]	0.064

Mean within a column with a different superscript differ (P<0.05).

Table 2 Mean±SEM apparent total tract dry matter (DM) digestibility and voluntary metabolisable energy (ME) intake of neutered female (NF), and male (NM) domestic cats during autumn and winter.

Cat group	Autumn	Winter	P-value
	Dry Matter (% as is)	Dry Matter (% as is)	
Neutered Female	72.8±1.1	69.7±1.9	0.027
Neutered Male	74.6±3.0	68.4±5.6	0.043
	Energy (kJ/g DM)	Energy (kJ/g DM)	
Neutered Female	1114.8±56.9	1017.8±42.5	0.016
Neutered Male	1233.3±115.9	1089.0±70.4	0.258

The food intake pattern of the two groups was similar; intake was higher in autumn than winter. The apparent total tract DM digestibility was lower (P<0.05) in autumn compared to winter in both NF and NM, voluntary ME intake was also decreased during winter in both groups with a significant difference found for NF (P<0.05). Gender had no effect on apparent total tract DM or voluntary ME intake during both autumn and winter.

Conclusions Seasonal changes affected activity rhythm, nutrient intake and dry matter digestibility in domestic cats. Gender had a significant effect on activity rhythms but did not affect nutrient intake or dry matter digestibility in domestic cats.

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Risk factors for hock lesions on 76 dairy farms in the Midlands region of the United Kingdom

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Introduction Hock lesions are currently one of the prevalent leg injuries in dairy cattle. The severity of hock lesions vary from hair loss to open wounds and swelling. Although categorical scales have been used to assess the severity of lesions, there is no commonly used, standard scoring system. The impact of hock injures on the welfare of the animal is largely unknown.

Material and methods Seventy seven farms in the Midlands region of the United Kingdom were recruited to the study, which was conducted during the winter housing period of 2007/08. Approximately 50 cows were selected randomly from each herd for assessment. Cow characteristics and farm management factors were measured. Milk records were collected from Cattle Information Service (CIS) or National Milk Records (NMR). The degree of hair loss and swelling were recorded according to the scoring system (score 0-3) previously reported in UK as described by Huxley and Whay (2006c). The area and shape of lesions on the lateral area of the hock (lateral tarsal joint and tuber calcis) was recorded on a hock map. The area of the lesion recorded on the hock maps were analyzed using Matlab and the output was converted to cm^2 using a scaling factor. The area of partial hair loss was used as the outcome variable in a multivariate model. The total area of hair loss and ulceration on each hock were compared with the categorical scores given separately.

Results Seventy six farms had complete information on the recorded variables and 3130 cows (5608 hocks) were selected in this analysis. The majority of the selected cows (92.76%) were Holstein-Friesian. Out of 5608 hocks, 5352 hocks and 976 hocks had area of partial hair loss and ulceration respectively. Out of 5608 areas of hair loss on the hock, 3008(53.64%), 1730(30.85%) and 870 (15.51%) hocks were scored 1, 2 and 3 for hair loss on the categorical scale. For the 1134 areas of ulceration, 592 (52.20%), 397 (35.01%) and 145 (12.79%) were scored 1, 2, 3 for ulceration on the categorical scale. The Mann-Whitney test showed that the median area of hair loss was no different between score 1(median: 45.93cm^2) and score 2 (median: 46.32cm^2) while score 3 (median: 80.89 cm^2) cows had significantly higher median area of hair loss than score 2 (Figure 1). With regard to ulcerated hocks, the median area of ulceration significantly increased with increase in the categorical score (was 0.75cm^2, 3.74cm^2, 11.61cm^2 for scores 1, 2 and 3 respectively) (Figure 2). The risk factors associated with the area of partial hair loss on the lateral area of hock were mobility score, total cleanliness score, mean milk yield (kg), body condition score, days of winter housing and combination base and bedding material in the cubicles. The cows housed in the mattress with straw or rape straw had larger area of partial hair loss compared with the cows housed in concrete with straw or rape straw. On the contrary, the cows housed in concrete with sand and concrete with chopped straw had smaller area of partial hair loss compared with the cows housed in concrete with straw or rape straw.

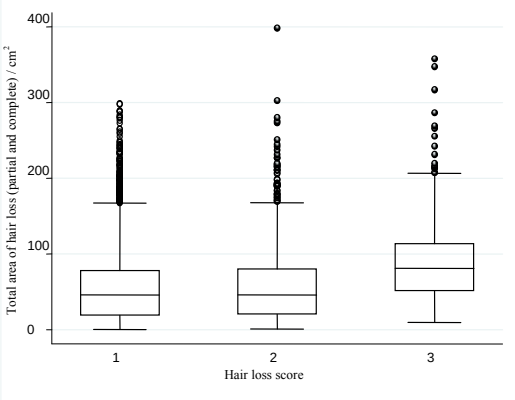

Figure 1 The distribution of total area of hair loss (partial and complete) with hair loss scored on a catagorical scale

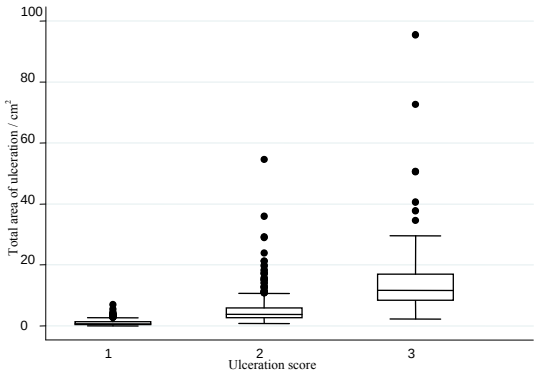

Figure 2 The distribution of total area of ulceration with ulceration scored on a catagorical scale

Conclusions The comparison of total area of hair loss with the categorical scoring system showed that there was no difference in hair loss between score 1 and score 2. This suggests that ordinal categorical scale for hair loss in its current form is not capturing accurately the severity of lesions. Longitudinal studies are required to further our understanding of the development of hock lesions.

Acknowledgements The authors thank to Sarah Potterton to collect the data from the selected farms.

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Production system effects on weekly prevalence of lameness in dairy cows

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Introduction Lameness is a significant problem in many of today's dairy herds and is associated with a decrease in milk production and impaired reproductive performance. Lameness is not only a welfare issue but it also increases the risk of a cow being culled. Locomotion scoring helps to monitor the general lameness situation in the herd (Sherer, 2010). The objective of the current analysis was to determine the effect of the production system on weekly lameness prevalence in dairy cows. The weekly proportion of cows with the locomotion score of 3 and above relative to the whole herd defined the weekly prevalence of lameness.

Material and methods Data were obtained from a research herd of Holstein Friesian cows, which are on a long-term 2x2 factorial, genotype x feeding regime project based at the SAC Dairy Research Centre, Dumfries, Scotland. Two contrasting approaches to dairy herd management systems were practiced. The two management systems were a high forage system (HF) and low forage (LF). In HF system, the cows grazed when sufficient herbage was available and fed a complete diet containing between 70% and 75% forage in the dry matter when grass heights fell below set values and housed in the winter months. In the LF system, the cows were housed throughout the year and were fed a complete diet containing between 45% and 50% forage in the dry matter. Low forage diet contained approximately 1200 kg concentrate while high forage diet contained approximately 3000 kg concentrate per cow per lactation. The forage component of the complete diet consisted of grass silage, maize silage and whole crop wheat (Chagunda *et al*., 2009). Locomotion score was conducted once every week on a 1 to 5 scale with 1 being sound and 5 being obviously lame. In the current analysis, weekly lameness prevalence was calculated as a proportion of the herd that had cows with a locomotion score of 3 and above. Three production systems were defined based on feed type and housing. The 3 systems were, low forage-continuously housed system, high forage-housed (over winter), and high forage-at grass (grazing in summer). In the study 1102 weekly prevalence records from the period 2004 and 2009 were used. To determine the effect of environmental factors and farm management changes on weekly lameness prevalence, analysis of variance using a univariate mixed model was applied. The fixed factors included in the model were the feeding-housing system, genotype, number of milkers, the individual locomotion scorer, and year of production. Feed dry-matter content was included as a covariate. Analysis was done using the GLM procedure of SAS version 9.2 (SAS, 2008).

Results and Discussion The mean weekly lameness prevalence was 0.08 (sd = 0.12) and the median of 0.16. From the analysis of variance, production system ($p<0.001$), year of production ($p<0.001$) and number of milkers ($p<0.01$) had significant effect on weekly lameness prevalence. Least square means are presented in Table 1.

Table 1 Least square means and standard errors for weekly lameness prevalence (proportion of cows with a locomotion score of 3 and above) as affected by number of milkers and production system.

	Weekly lameness prevalence		
Variable	n	Lsmean	Standard error
Number of milkers			
1	646	0.23[a]	0.014
2	454	0.14[b]	0.015
Feeding-Housing group			
Low Forage-Continuously Housed	616	0.21[a]	0.013
High Forage-Housed	296	0.15[b]	0.013
High Forage-At grass	188	0.17[c]	0.014

Non identical superscripts on least square means within the same variable group denote significant difference ($p<0.001$).

In general, the low forage-continuously housed system had higher weekly lameness prevalence than those in the high forage system. Cows that alternated between indoor and outdoor life (high forage) had higher weekly lameness prevalence when they were outdoors than when they were back indoors indicating that whatever benefits on feet health they got whilst outdoors were exhibited when the cows returned indoors and vice versa. When cows were milked by one milker, there was higher weekly lameness prevalence than when milked by 2 milkers. This may be because animals did not have to stand in the holding area waiting to be milked for as much time when they are milked by 2 milkers as they did with one milker, and hence reduced feet stress.

Conclusion The study demonstrated that changes in the production systems significantly affect weekly lameness prevalence in dairy cattle.

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A preliminary investigation into the presence, risk of entry and risk of on-farm spread of Johne's disease in the south west of England

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Introduction Johne's disease is a degenerative infectious disease affecting ruminants (primarily cattle). The disease causes significant reduction in milk yield with likely considerable financial loss through reduction in output and consequential culling. It has been suggested, though not proven or disproven that there may be a link between Johne's disease and possible causes of Crohn's Disease in humans. Any such link would have profound effects for the dairy industry and animal health policy. The study aimed to establish the level of disease as well as examining the level of control measures used to prevent farm entry and farm spread for the disease, within a defined population. This study used data collated by the RDPE-funded Healthy Livestock project led-by Duchy College's Rural Business School, a regional project designed to reduce the level and improve the management of a variety of cattle and sheep diseases. Data was collated using the on-line herd health planning tool 'Myhealthyherd'.

Method Data relating to Johne's Disease was collected from dairy (n=555) and beef (n=348) farms, across Cornwall (n=235), Devon (n=382), Somerset (n=130), Avon (n=30), Dorset (n=87), Gloucestershire (n=72) and Wiltshire (n=33). Data was discriminated by the farm disease status. Disease status was defined as green – no disease recorded, amber – possible disease recorded, red – confirmed recording of disease but with no active observed occurrences, and disease present – confirmed recording of disease with active observed occurrences. Additionally farm disease entry risk status, and on-farm disease spread risk status data was assessed. These two statuses were recorded via a standardised protocol established from a questionnaire and veterinary assessment. Green – effective precautions against entry or on farm spread. Amber –entry or spread protocols in place but not wholly adequate. Red inadequate entry or on farm spread protocols Data was considered by farm type (dairy or beef). Descriptive statistics for the studied population were returned. Further Chi squared analysis was undertaken on the data relating to farm disease status.

Results Data relating to farm disease status was collected from 37% (n=204) of diary and 25% (n=88) of beef farms within the total population. A small number n=23 (11%) of dairy farms were free (green) of Johne's disease, with n=132, 65% either testing positive or having observed incidences (Figure 1). A reverse trend was noted on beef farms. (= 32.319, df= 3 P<0.001). Data relating to farm disease entry risk status, and on-farm disease spread risk status was collated from 64% (n=356) of dairy and 34% (n=118) of beef farms in the total population. Figure 2 shows the percentage distribution for risk of entry and risk of on farm spread.

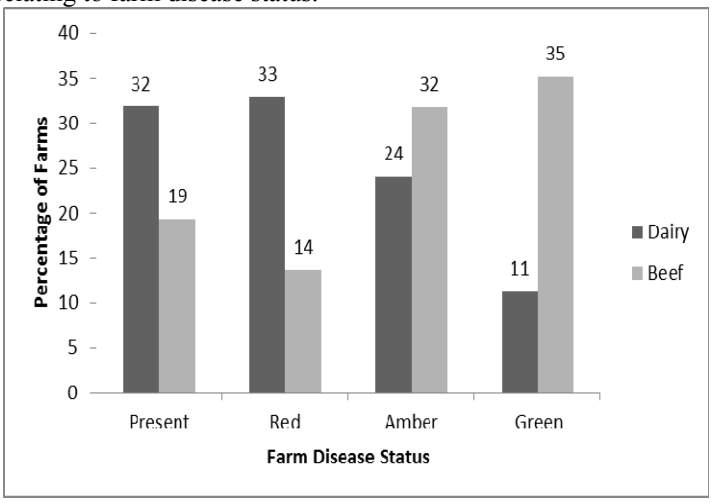

Figure 1 Degree (measure in percentage) of Johne's disease measured on dairy and beef farms in the south west region.

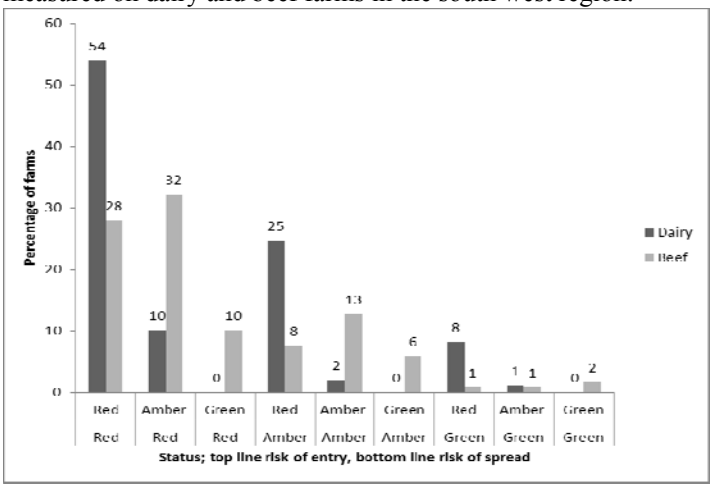

Figure 2 Risk of entry and risk of spread of Johne's disease on dairy and beef farms in the south west region.

Discussion A significant proportion of dairy farms had the presence of Johne's disease. Generally within the population the levels of risk (entry and spread) were found to be lower on beef farms. 54% of dairy farms within the studied population were defined as Red/Red in terms of risk of entry and spread of Johne's disease. When the data is analysed further 87% of dairy farms are defined as having a Red status for disease entry to farms. These figures are of significant concern and appropriate strategies need to be implemented by the farming community and associated agencies to mitigate against the risk Johne's disease possess and the consequential considerable economic loss escalation might cause. Further data collection is required to allow appropriate statistical techniques to be applied to the entry and spread data sets.

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Culture of bone marrow derived haematopoietic stem cells in a pooled colostrum feeding model of bovine neonatal pancytopenia

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Introduction Bovine neonatal pancytopenia (BNP) is a recently emerged disease of young calves, with a documented association with maternal vaccination with an inactivated bovine virus diarrhoea vaccine (Pregsure, Pfizer)(Defra, 2011). The vaccine has been withdrawn but cases continue in calves born to vaccinated animals. The age at presentation is less than 28 days; the clinical signs are associated with thrombocytopenia and involve petechiation and haemorrhage either externally or internally. The underlying lesion is a dramatic bone marrow injury (depletion of haematopoietic cells and precursors). The syndrome can be reproduced by feeding of colostrum from cows which have borne affected calves (BNP-dams) to unrelated calves; following this colostral challenge a high proportion of calves will develop BNP or typical clinicopathological features (Schroter and others, 2011). Antibodies present in serum from BNP-dams have been shown to be capable of binding to peripheral leucocytes of normal calves. Such studies support a working hypothesis is that colostral antibody targeting a bovine antigen causes the bone marrow pathology. While the identity of the antigen is unconfirmed, evidence supporting the induction of anti -bovine MHC Class I antibodies by the implicated vaccine has recently been published (Deutskens and others, 2001; Foucras and others, 2011).

Material and methods Four Holstein Friesian colostrum deprived calves were fed either challenge (n=2) or control (n=2) colostrum pools. A previous study (unpublished observations) had established that the challenge pool could induce clinical signs of early BNP and marked bone marrow lesions consistent with BNP in 5/5 calves and that the control pool did not induce BNP in 5/5 calves. Blood samples were taken before colostrum feeding, at 4,8,12 and 24 hours post-colostrum and daily thereafter to assess haematological parameters. Sternal bone marrow biopsies were taken under sedation and local analgesia at 24 hours and 6 days post challenge using a 0.5cm diameter trephine needle (Rocket Medical, Washington, UK). Bone marrow hematopoietic stem cells (BM-HSCs) were harvested from these biopsies and cultured for 7 days in methylcelluose medium containing EPO (Methocult, StemCell Technologies) and GM-CSF (a gift from Prof. G. Entrican) as previously described (Keller and others, 2004). Microscopic examination was performed at 2, 5 and 7 days post isolation and colonies counted and typed as (1) colony forming unit-granulocytic erythroid, monocyte, macrophage and megakaryocytic (CFU-GEMM), (2) colony forming unit-erythroid (CFU-E) and (3) colony forming unit granulocyte macrophage (CFU-GM). Residual biopsy material was fixed for histopathological examination. The animals were euthanased upon the appearance of haematological abnormalities and/or clinical signs of BNP using a defined clinical scoring system. The animal work was carried out under a home office licence and following consultation with the local ethics committee.

Results Calves which received challenge colostrum developed lymphopenia between 4 and 8 hours post-challenge and marked thrombocytopenia by day 6, consistent with the pre-clinical stages of BNP. Petechiation was observed in the challenged calves from day 7. These clinical and haematological abnormalities persisted until euthanasia was performed in accordance with the clinical scoring system. Post-mortem examination (PME) demonstrated gross lesions consistent with BNP and histopathological examination of bone marrow confirmed extensive depletion of haematopoietic cells and precursors in the challenged calves. The control calves were neither clinically affected, nor did they develop any haematological abnormalities or bone marrow lesions. HSC cultures demonstrated normal colony development in the control animals at all sampling timepoints. In the challenged animals, CFU-GEMM colony growth was compromised as early as the 24 hour biopsy. The BM-HSC collected at 6 days from colostrum challenged calves failed to develop CFU-E colonies and the development of both CFU-GEMM and CFU-GM was markedly reduced as compared to the control animals.

Conclusions This pilot study is the first where the functionality of BM-HSCs has been assessed. We have demonstrated that as early as 24 hours after colostrum intake CFU-GEMM, the pluripotential stem cells, are compromised in their colony forming ability and that by 6 days the functional capacity of all colony types in BM-HSCs is markedly reduced. The methods and observations made here also demonstrate the utility of *in vitro* stem cell culture in the investigation of BNP facilitating further *in vitro* studies to characterise the aetiology, maternal vaccinal responses, colostral antibody titre and specificity in a standardised, non-animal model system.

Acknowledgements The authors gratefully acknowledge funding from the Moredun Foundation and Quality Meat Scotland and the University of Edinburgh Royal (Dick) School of Veterinary Medicine Farm Animal Practice for supplying the colostra.

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Ticks infesting goats in the mountainous area of Jabal Akhdar in Oman

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Introduction In arid and semi-arid regions such as Oman, goats are the major livestock species that are reared by the people. This is because goats can thrive very well under harsh environmental conditions (Alexandre and Mandonnet 2005). In 2008, goats constituted 66.9% of the livestock population of Oman (Ministry of Agriculture, Oman 2011) and are therefore a major source of meat for the human population. There are three major distinct breeds of goats in the country, namely: Dhofari, Batinah and Jabal Akhdar (Zaibet *et al*. 2004). The Jabal Akhdar breed originated from and is found mainly in the Jabal Akhdar area where the goats are reared under an extensive, low input management system. The breed is highly priced and sought after by people in Oman and some surrounding countries. Despite the importance of goats in Oman, very little is known about the health problems of these animals in the country. Ticks and tick-borne diseases are known to be important causes of morbidity in goats (Alexandre and Mandonnet 2005). Hitherto there had been no investigation of ticks infesting livestock in Jabal Akhdar. Therefore, this study was carried out to determine the species of ticks that infested goats in the area. Jabal Akhdar is part of the Hajar mountain range of northern Oman. It is a relatively isolated area of the country and has villages located at elevations between 1,000 and 2,800 metres above sea level. It has a Mediterranean type of climate with an average annual rainfall of about 122 millimetres.

Material and methods In April and June 2009, with the informed consent of the goat owners, ticks were collected from goats at 14 villages in Jabal Akhdar area.The geographic coordinates of each location where ticks were collected were recorded at the time of tick collection and later mapped. At each location, at least 10 goats with visible tick infestation were selected for tick collection. Where there were less than 10 infested animals, all the infested animals were selected. The ticks collected from each animal were preserved in bottles filled with 70% ethanol and each bottle was labelled internally with a strip of paper on which was recorded the animal's details, date of collection and the location. The ticks were later identified morphologically and counted, using a stereoscopic microscope.

Results A total of 1,221 adult, nymph and larvae of ticks were collected off 176 goats whose ages ranged from one month to 7 years. There were 48 males and 128 females. All the ticks were hard ticks (Family Ixodidae) and belonged to five species, namely: *Rhipicephalus (R) camicasi*, *Hyalomma (H) anatolicum*, *H. excavatum, H. marginatum*, and *H. rufipes*. *R. camicasi* was the most prevalent and numerous species being found in all the 14 villages. The sex ratio of the adults of this tick species was close to 1:1 (F: M 409:485). *H. marginatum*, *H. rufipes* and *H. anatolicum* were found in seven, six and three villages respectively. However, the number of *H. anatolicum* collected was greater than that of *H. marginatum*, which in turn had a higher number than *H. rufipes* collected. *H. excavatum* was very rare, only one adult male was found on one goat. The elevations at which the villages were located did not seem to have any distinct effect on the distribution of the ticks. Adults, nymphs and larval stages of *H. anatolicum* were found on goats in one village. The numbers of larvae, nymphs, adult females and adult males were five, 110, 61 and 97 respectively. On the other hand, only the adults of this tick species were found on goats in the other two villages. Majority of the goats were infested with a single species of ticks. The proportions of goats with single species infestation were as follows: 82.9% with *R. camicasi*, 1.7% with *H. marginatum* and approximately 1.1% with *H. anatolicum*. Concurrent infestation with two tick species was found on approximately 12% of the goats; with *R. camicasi* and *H. anatolicum* being found on 5.7%, *R. camicasi* and *H. marginatum* on 4.6%, and *R. camicasi* and *H. rufipes* on 1.7%. Concurrent infestation with three species of ticks was found on 2.3% of the goats.

Conclusions. This is the first record of ticks that infest goats in Jabal Akhdar area and probably the first record of *Rhipicephalus (R.) camicasi* in Oman. The sex ratio of the adults of this tick species in this area, almost 1:1, indicates that the species is well established there. The finding of *Hyalomma* (*H.*) *rufipes* is the first record of the tick infesting livestock in Oman. Thus this study has added another species of ixodid ticks, *Rhipicephalus (R.) camicasi* to the tick fauna of Oman and established a new host record for *H. rufipes*. The finding of larval stages of *H. anatolicum* on goats in one of the villages, though few in number, is probably a first record in the Arabian Peninsula. Also the ratios of nymphs, adult females and adult males indicate that this tick species is well established in the area. Apart from *R. camicasi* whose disease association has not been established, the four other species of ticks identified in this study are known to be vectors of some disease causing pathogens in animals and man. Therefore further investigations are required to determine the effects of these tick species on the livestock, and probably people, of Jabal Akhdar area.

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Genetic diversity of bovine viral diarrhoea isolates in Irish cattle

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Introduction RNA viruses accumulate genetic change at a much higher rate than their DNA counterparts. This leads to the development of specific subtypes, strains and quasi-species frequently right down to animal level. Bovine Viral Diarrhoea (BVD) virus is a RNA virus which infects ruminants and has a widespread distribution in Ireland. The CVRL has had a key diagnostic role in the identification of BVD in Irish cattle and receives field samples for testing based on ill thrive, digestive, respiratory, abortion, and herd screening. The *genetic fingerprint* of specific BVD strains provide a useful opportunity to monitor and map the spread of the virus at national and farm levels.

Material and methods Two hundred and fifty two BVD virus positive samples were selected from the CVRL virus archive to provide maximum coverage of geographical location and date of collection. Samples were chosen to represent all 26 counties in Ireland over the period January 2008 to August 2011. Total nucleic acid was extracted using an automated extraction robot MagnaPur (Roche Diagnostic, Mannheim, Germany). To reduce the risk of cross-contamination, a one-step RT-PCR protocol was carried out using the commercial Quantitect Probe RT-PCR kit (Qiagen) according to the manufacturer's instructions.
To differentiate samples genetically, a 288 bp fragment from the 5'UTR regions of each BVD isolate underwent amplification using primer pair 324 and 326 (Vilcek *et al*. 1994). Amplified products were subjected to automatic dye-terminator cycle sequencing commercially (Source Bioscience PLC, Dublin). Sequence alignment was undertaken using ClustalW 1.6 (Thompson *et al*. 1994) and phylogenetic trees constructed using MEGA5 (Tamura *et al*. 2011) initially by maximum likelihood analysis and repeated using the neighbour-joining method. The reliability of the branches in the tree were tested by bootstrap analysis (1000 replicates) using the same software.
For comparison, pestivirus reference strains NADL (M31182), AF298072, AF298054 were included in the tree analysis representing BVD sub-types 1a, 1b and 1e respectively in addition to some historic Irish BVD isolates.

Results A useful amount of genetic variation was identified across the set of samples tested with intra-herd stability and inter-herd diversity demonstrated. There was no evidence of BVD virus type 2 in any of the samples tested.
The predominant subtype was BVD type 1a (96%) with BVD subtypes 1 e and 1b also identified. Six non-contiguous counties showed BVD subtypes 1e and 1b - both subtypes being found in the same two counties
There was clear evidence that some strains of virus have remained in circulation in Irish cattle for at least 10 years.

Conclusions Although the predominance of BVD subtype 1a and 1b is very similar to that for the UK, the finding of BVD subtypes 1e with the apparent absence of subtype 1i is particular to Ireland.
Both islands show quite different patterns of BVD diversity when compared to other European and American cattle populations.

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A preliminary study investigating factors affecting percentage of lameness in dairy cattle on farms in the south west of England

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Introduction Lameness in dairy cattle is a considerable problem facing farmers and the wider dairy industry. Significant financial loss can be accrued through lameness; this can be observed via loss in production, as well as additional costs in health management. Various farm health planning and management strategies can be implemented to reduce loss. This study is a preliminary investigation reporting a number of descriptive farm parameters and observed levels of lameness within dairy farms across the south west region. The study is the result of initial data collection from the 'South West Healthy Livestock Initiative' a regional project designed to reduce the level and improve the management of a variety of 'on farm' livestock health and disease issues facing farmers.

Method The study population consisted of 102 dairy farms across the south west region. Of these, 90 farms were used within the analysis. Twelve farms returned incomplete data information and were excluded. The dependant variable tested within the analysis was the overall percentage of herd lameness. Overall herd lameness was defined by the number of animals returning lameness assessment score of two or three as a percentage of the total herd size. All lameness assessments were undertaken by veterinary surgeons using a standardised moderated scoring system. Independent variables used within the analysis were farming system, housing system and numbers of full time equivalent (FTE) stockpersons working on farm. These variables were appraised using an analysis of variance. The dependant variable, overall percentage of herd lameness was normally distributed (Pearsons skewness test 0.892) for the population. Therefore it was not deemed necessary to undertake an arcsin transformation. Further analysis was undertaken using Pearson's correlations co-efficiencies for farm size (hectares), herd size and the dependant variable overall percentage herd lameness.

Results The mean farm size was 173.6 (±116.0)ha with a range from 33.2 ha to 606.9ha. Mean herd size was 175.6 (±85.2) with a range from 47 to 466 cows. Herd lameness for the population was returned at 26.5% (±13.5) with a range from 3.3% to 72.9%. 73% (n=66) of the farms studied used a standard farming system, 18% (n=16) extensive and 9% (n=8) organic. 70% (n=63) used a combination of freestalls and cubicles, 27% (n=24) freestalls and yards and 3% (n=3) yards only. 13% (n=12) of farms had one or less stockperson FTE's, 34% (n=31) two FTE's, 34% (n=31) three FTE's and 21% (n=19) four or more FTE's.
A significantly ($df = 89$, $F= 3.3$, $P<0.05$) lower level of lameness was observed on organic farms (15.6%) when compared to intensive (25.2%) and standard (28.1%) farming systems. No differences in lameness levels were observed when housing systems were considered, freestalls and cubicles 25.5%, freestalls and yards 26.7% and yards 25.6%. Higher percentage levels of lameness were observed on farms where one or less FTE's were working (30.2%) when compared with other farms within the studied population; two FTE's24.7%, three FTE's 26.5% and four or more FTE's 27%. This relationship however was not statistically significant. There was no correlatory relationship between herd size and percentage of lameness ($r=-0.025$), or farm size (ha) and percentage lameness ($r=-0.039$)

Conclusion The results from this preliminary study indicate that farming system has a significant effect on levels of recorded lameness within dairy herds in the south west region; organic farming systems having a considerably lower incidence. The reasons for this are likely to be multi-factorial and are worthy of further study. Other factors considered within this study appeared not to be significant although it is noted that those farms with one or less FTE's do see higher (although not significant) levels of lameness. Further study is required on a larger population to investigate these and other factors that might play a role in the level of lameness observed on dairy farms.

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