

## Fatty acid composition of milk produced under systems of different intensity and milking practices in the North East of England

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**Introduction** The relatively high saturated fatty acid (SFA) content in milk fat has raised criticism in the past since it is assumed to be associated with negative effects on human health, especially due to the presence of C12:0, C14:0 and C16:0. On the other hand, monounsaturated fatty acids (MUFA), such as c9 C18:1 (oleic; OA) and t11 C18:1 (vaccenic; VA) and polyunsaturated fatty acids (PUFA), such as n-3 and n-6 groups and c9t11 conjugated linoleic acid (CLA9) found in milk, have been linked to beneficial effects on human health (Haug *et al.* 2007). c9c12c15 C18:3 (ALN) and c9c12 C18:2 (LA) are the main n-3 and n-6 FA in milk respectively. Differences in milk fatty acid composition between different dairy management systems in UK, such as conventional, organic and low input, have been reported in other studies (Butler *et al.* 2008; Ellis *et al.* 2006). Grazing intake, silage and sward composition, forage:concentrate ratio and oilseed supplementation, all factors that widely vary between management systems, can be responsible for milk fat compositional differences (Dewhurst *et al.* 2006). In a previous study, a stronger effect on milk fatty acid composition was found when the differences on grazing intake between management systems were more extreme. The comparison between low input and conventional milk showed higher differences in milk fatty acid composition than when conventional milk was compared with organic (Butler *et al.* 2008). The aim of this study was to investigate and explain possible differences in milk fatty acid composition between farms under different management practices in the North East of England.

**Material and methods** Milk from the bulk tank of 20 farms in the North East of England, representing 4 different management systems, was collected every 8 weeks for 10 month period. Conventional systems are characterized by an average of 41% of their diet as concentrates, with conserved forage fed while animals are fully housed during winter but access to ryegrass pasture when conditions allowed in summer. In organic systems, cows graze in ryegrass/clover swards, usually between April and October, and are housed in winter, with an average 22% concentrate supplementation. On intensive farms, cows are milked 3 times per day, nutrition is consistent throughout the year and is based on silage, which in that case may also contain maize silage, and 49% concentrates. Farms that apply robotic milking were selected to have the same feeding practices as conventional farms in order to investigate the effect of milking procedure. 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 CLA isomer standards. Analysis of variance (ANOVA) using linear mixed effects model (LME) was used to analyze results in R statistical environment using “Management system” (conventional, organic, intensive, robotic) and “sampling month” (7 sampling months over one year) as fixed factors and farm number as random factor.

**Results** Compared to conventional milk, organic milk showed significantly higher concentrations of C14:0, ALN, n-3 and n-3:n-6 ratio while milk from intensive farms showed higher milk n-6 concentrations. When organic milk was compared to milk from intensive farms, significantly higher concentrations of C14:0, VA, CLA9, ALN, n-3, higher n-3:n-6 ratio and significantly lower concentrations of LA were found. Robotic milking farms showed significantly higher milk concentrations of C12:0 and C14:0 and lower concentrations of CLA9 than conventional farms.

**Table 1** Relative proportions (%) of individual fatty acids and fatty acid groups in milk from organic, intensive and robotic farms compared with milk from conventional farms

	C12:0	C14:0	C16:0	Oleic	VA	CLA9	ALN	LA	SFA	MUFA	PUFA	n-3	n-6	n-3:n-6
Organic	+9.7 <sup>ab</sup>	<b>+8.4<sup>a</sup></b>	-6.5	-3.6	+15.2 <sup>a</sup>	+6.7 <sup>a</sup>	<b>+76.3<sup>a</sup></b>	-12.0 <sup>b</sup>	+0.8	-3.1	+7.0 <sup>a</sup>	<b>+69.4<sup>a</sup></b>	-11.0 <sup>b</sup>	<b>+84.0<sup>a</sup></b>
Intensive	-0.9 <sup>b</sup>	-0.8 <sup>b</sup>	+2.2	-3.6	-21.6 <sup>b</sup>	-21.6 <sup>b</sup>	-18.8 <sup>b</sup>	+28.7 <sup>a</sup>	+1.4	-4.9	+9.8 <sup>a</sup>	-18.2 <sup>b</sup>	<b>+28.2<sup>a</sup></b>	-35.1 <sup>c</sup>
Robotic	<b>+16.8<sup>a</sup></b>	<b>+8.1<sup>a</sup></b>	-1.1	-4.9	-20.0 <sup>b</sup>	<b>-25.2<sup>b</sup></b>	-1.7 <sup>b</sup>	-14.2 <sup>b</sup>	+2.7	-5.4	-13.2 <sup>b</sup>	-2.9 <sup>b</sup>	-12.3 <sup>b</sup>	-16.9 <sup>b</sup>
P-values	†	†	ns	ns	†	*	***	*	ns	ns	†	***	*	***

ANOVA P-values refer to the overall effect of management system and bold values indicate significant differences compared with milk from conventional farms. Significances were declared at \*\*\*: P<0.001, \*: P<0.05, †: 0.05<P<0.10, ns: P>0.05. Different superscripts within the same column indicate differences between the reported management systems.

**Conclusions** The high content of ALN in organic milk and LA in milk from intensive farms can be attributed to the increased grazing intake and the use of maize silage and by-products respectively. Many differences can be explained by production intensity and the proportion of grazing in DMI, hence CLA9 and VA concentrations of milk were higher in organic milk only when compared with intensively produced milk. For relatively smaller differences in grazing intake (18% DMI in conventional, 38% DMI in organic), the CLA9 content of milk was similar, regardless the dairy management system. The lower CLA9 content of milk from farms with robotic milking should be further investigated since differences in nutrition, that could possibly affect the CLA9 content of milk between those and conventional farms, were limited.

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## Concentration of $\beta$ -casein variants in UK retail milk

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**Introduction**  $\beta$ -casein makes up 30 to 40 % of the total protein in cows milk. Within  $\beta$ -casein, there are a number of variants which are genetically determined. A1, A2, B and C are the most common versions of the  $\beta$ -casein gene in UK cattle. These genes are co-dominant, so a heterozygous cow will express both genotypic variants in her milk. The Holstein Friesian breed predominates in the UK and, based on known frequencies of  $\beta$ -casein genetic variants within this breed in other countries (Boland, 2003), retail milk is likely to contain approximately equal concentrations of A1 and A2  $\beta$ -casein with substantially less B and C, but this has not been verified to date. It has been suggested that consumption of A1  $\beta$ -casein may exacerbate autistic spectrum disorders in people due to the opioid activity of a peptide known as  $\beta$ CM7 which is released during digestion. The B and C  $\beta$ -caseins also release this peptide, but the A2 form does not. The purpose of this study was to estimate concentrations of different  $\beta$ -casein variants in milk available at retail in the UK.

**Material and methods** Samples of semi-skimmed milk which had been purchased from five supermarkets within a 8 km radius of the laboratory at the same time every month for 11 months as part of a separate study (Kliem and Givens, 2010) were frozen until analysis. Detection and quantification of  $\beta$ -casein variants was conducted using an adaptation of the method described by Bonfatti *et al* (2008) using reverse-phase HPLC and high resolution mass spectrometry. Total protein and casein concentrations were determined by mid-infrared spectrometry. It was assumed that  $\beta$ -casein made up 40 % of total casein. Data were analysed for effects of supermarket using analysis of variance.

**Results** The A2 variant was present in the largest amounts (mean 58.4 % of total  $\beta$ -casein) across all supermarkets, followed by A1 (mean 31.5 %) and small amounts of B and C (Table 1). The concentrations of A1, A2 and C were similar across supermarkets, whilst significant variation in the concentration of B was observed across supermarkets, although this variant contributed only 6 to 7.6 % of the total  $\beta$ -casein.

**Table 1** Concentrations of  $\beta$ -casein variants in retail milk from five supermarkets

Supermarket	mg/g total $\beta$ -casein				mg/g total protein				g/litre milk			
	A1	A2	B	C	A1	A2	B	C	A1	A2	B	C
1	320	593	60	27	98	185	19	8	3.12	5.87	0.59	0.26
2	318	576	73	33	98	179	23	10	3.22	5.87	0.76	0.32
3	316	580	72	32	97	181	22	9	3.21	5.95	0.73	0.31
4	318	580	70	32	98	181	22	9	3.20	5.91	0.72	0.31
5	304	591	76	28	93	182	24	9	3.05	5.96	0.79	0.29
SEM	10.3	9.8	3.3	2.7	3.6	3.4	1.1	0.9	0.126	0.106	0.035	0.028
P <	0.812	0.662	0.017	0.470	0.835	0.835	0.017	0.812	0.870	0.958	0.005	0.669

**Conclusions** This is the first survey of  $\beta$ -casein variant concentrations in UK retail milk that we are aware of. Concentrations of A2  $\beta$ -casein were higher than expected based on the reported frequencies of the genetic variants within Holstein populations in other countries, thus implying that the A2 form of the  $\beta$ -casein gene may be more common within the UK national herd than in overseas populations. This may in part reflect the Friesian ancestry of many UK Holstein cows, but also the contribution of other breeds such as Jersey and Guernsey to the milk pools from which samples analysed in this study were taken. Data from this study were used in conjunction with information from NDNS (2010) on UK food consumption patterns to estimate consumption of  $\beta$ CM7-producing  $\beta$ -caseins (A1, B and C) and A2  $\beta$ -casein (which does not produce  $\beta$ CM7) in different age groups (Table 2).

**Table 2** Consumption of  $\beta$ CM7-producing  $\beta$ -caseins (A1, B and C) and A2  $\beta$ -casein (does not produce  $\beta$ CM7) in different age groups

	1.5 – 3 years	4 – 10 years	11 – 18 years	19 – 64 years
A1 + B + C, g/day	6.3	4.9	4.0	4.2
A2, g/day	8.8	6.9	5.6	5.9

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## Persistency of the effect of dietary milled rapeseeds on the milk fatty acid composition of lactating cows fed maize silage-based diets

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**Introduction** Including oilseeds in the dairy cow diet is an effective strategy to reduce milk fat saturated fatty acid (SFA) concentrations (Givens and Shingfield, 2006). However the rumen ecosystem requires time to adapt to dietary change. Time dependent changes in rumen fermentation and biohydrogenation of unsaturated fatty acids would be expected to alter both the supply of fatty acid precursors for de novo synthesis and long chain fatty acids available at the mammary gland. Most studies examining the impact of rapeseed supplements on milk fat composition have been conducted over relatively short periods. However, maintaining any favourable changes in milk fatty acid profile over an extended period is critical for commercial production of modified milk and milk products. Recent research (Givens *et al.*, 2009) concluded that a mixture of milled rapeseeds and wheat was an effective supplement for lowering milk SFA content whilst minimising adverse effects on intake, rumen function and milk yield. The objective of this study was to assess the persistency of changes in milk fatty acid composition due to milled rapeseeds, over a 10-week period.

**Material and methods** Thirty multiparous, Holstein-Friesian cows (mean yield 40.8 litres/day, mean 79 days into lactation) blocked according to milk yield one week before the start of the study and randomly allocated to one of two dietary treatments according to a repeated measures randomised block design. Treatments fed over 70 days were a control diet (CPO) containing 41 g/kg dry matter (DM) calcium salts of palm oil distillate (Megalac®, Volac International Ltd., Royston, UK), or the same basal diet with Megalac® being replaced by 47 g/kg DM lipid derived from rapeseed milled with wheat. Diets were fed as a total mixed ration (TMR) with a 50:50 forage:concentrate ratio (DM basis) with the forage proportion consisting of 3:1 mixture of maize silage:grass silage. Daily DM intake and milk yield were recorded throughout the experiment. Milk samples collected on days 0, 28, 49 and 70 were submitted for fat, crude protein, lactose and fatty acid composition (Kliem *et al.*, 2008). Data were analysed by ANOVA for repeated measures using the Mixed Models procedure of SAS, with a model that included fixed effects of treatment, day and treatment x day interactions and random effect of block. To assess persistency, changes in response to each treatment between days 28 and 70 were compared.

**Results** There was no effect ( $P>0.05$ ) of treatment on DM intake, milk yield or milk composition. However milk yield decreased ( $P<0.001$ ) over time for both diets, and DM intake and milk composition varied ( $P<0.05$ ) over time. Replacing CPO with a rapeseed supplement enhanced ( $P<0.05$ ) 10:0, 12:0, 14:0 and 18:0 and decreased ( $P<0.01$ ) 4:0 and 16:0 concentrations leading to an overall reduction in total SFA content (Figure 1). The effect of milled rapeseed on total SFA was persistent over 70 days, with no difference ( $P>0.05$ ) between days 28 and 70 for the CPO diet but a further reduction ( $P<0.05$ ) between days 28 and 70 for the rapeseed diet. Rapeseed inclusion also enhanced ( $P<0.001$ ) milk *cis*-MUFA content compared with the CPO diet, mainly due to increases in *cis*-9 18:1 concentrations. The increase in *cis*-MUFA due to rapeseed supplementation was found to persist ( $P<0.05$ ) over the 70-day period. Concentrations of total *trans*-MUFA in

milk fat were higher ( $P<0.01$ ) in milk from the rapeseed-fed cows compared with the CPO diet, mainly due to enhanced concentration of *trans*-11 18:1. Effects of milled rapeseeds in the diet on milk *trans*-MUFA were maintained until the end of the 70 day study for the rapeseed diet. Total conjugated linoleic acid (CLA) concentration in milk fat was also greater ( $P<0.01$ ) following rapeseed supplementation compared with CPO, an increase which persisted for 70 days, although the concentration of non-conjugated 18:2 isomers was lower ( $P<0.001$ ) in milk from cows supplemented with rapeseeds.

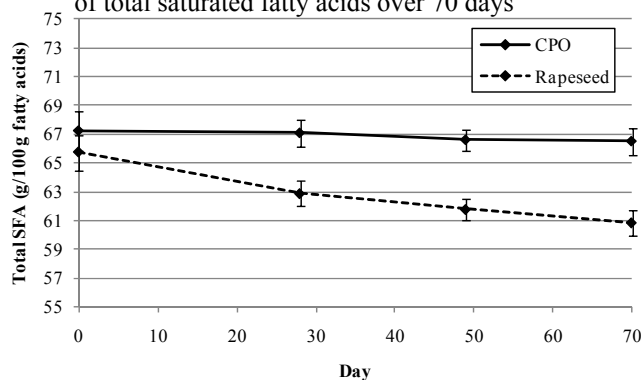
**Conclusions** Supplementing dairy cow diets with milled rapeseed resulted in a significant decrease in milk fat SFA (mainly 16:0) concentration, which was persistent for 70 days. The decrease can be attributed to both partial replacement of the CPO supplement (which has a high 16:0 content) with rapeseed lipids, but also inhibition of mammary de novo synthesis of 16:0 by long chain MUFA and PUFA from rapeseed. The enhanced effect of rapeseed supplementation after 70 days for total SFA and total *trans*-MUFA may reflect time dependent changes in rumen and mammary gland metabolism of fatty acids with advancing lactation.

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**Figure 1.** Mean effect (n=15 per treatment) of rapeseed supplementation on milk fat concentration of total saturated fatty acids over 70 days



# Effect of rumen boluses containing iodine, selenium and cobalt administered to dry Holstein dairy cows on the circulating concentration of immunoglobulin G in the calves

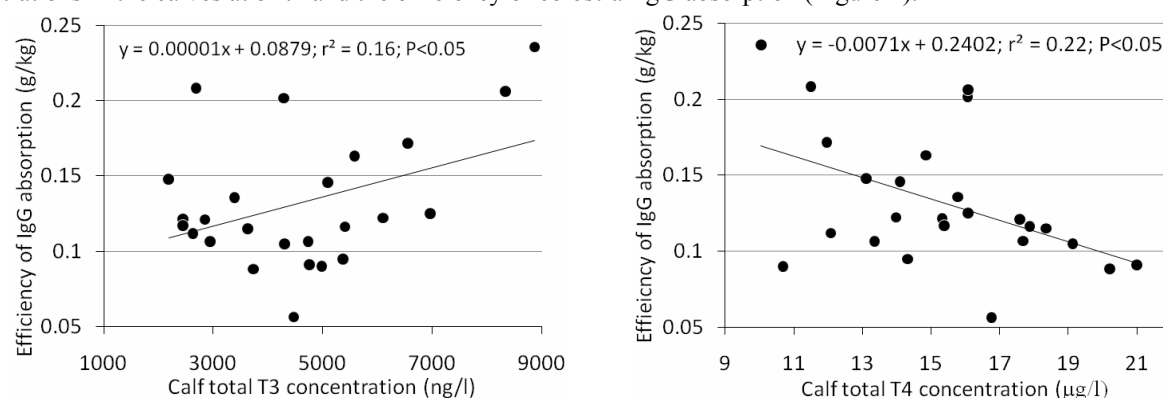
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**Introduction** It has been shown that excessive consumption of iodine by pregnant sheep causes impaired post-natal absorption of immunoglobulin G (IgG) from colostrum by lambs (Boland *et al.* 2005). We have shown that this effect occurs in the lambs of pregnant ewes fed additional iodine as low as 14.8 mg/day (equivalent to 0.64 mg/kg<sup>0.75</sup> BW/d; Rose *et al.* 2007). The aim of the present experiment was to determine if a bolus treatment containing iodine, selenium and cobalt administered to dry dairy cows, used in accordance with the manufacturer's instructions, impaired the concentration of IgG in calves 24 hours after birth.

**Material and Methods** Twenty-five multiparous, dry Holstein-Friesian cows were allocated to one of two groups (Control, n=12 and Bolus, n=13). All cows were on the same diet, the iodine concentration of the components of which ranged between 1.8 and 2.6 mg I/kg DM). Forty days before calving was due to begin, cows in the Bolus group were given 2 boluses containing a total of 6800 mg iodine, 1000 mg selenium and 1000 mg cobalt. Assuming boluses decay in the rumen constantly over 120 days (manufacturer's information), the iodine delivery was estimated to be 56 mg/d (equivalent to 0.47 mg/kg<sup>0.75</sup> BW/d). Following calving, before the calf was able to suckle, the calf was weighed, and a 10 ml jugular vein blood sample was taken. The calves were then bottle fed a total of 5 litres of colostrum (containing 73.4 g IgG/l), over the first 12 hours of life. This colostrum was bulked from the first milking colostrum produced by 12 cows. At 24 hours after birth a further jugular vein blood sample was taken from the calves. All plasma samples were analysed for total (bound and free) and free T4 and T3. Plasma samples from the calves were also analysed for bovine IgG. Hormone and IgG data were analysed by split plot analysis of variance. The fixed effect was treatment group and the split plot was time (1h and 24h of age). IgG absorption efficiency was analysed using a two-tailed Student's t-test.

**Results** There was no effect of the bolus treatment on the concentration of IgG in the plasma of the calves at 24 hours of age (15.5 vs 13.4 g/l, SED: 1.11, P>0.05, for the control and bolus calves, respectively). Equally, the efficiency of colostrum IgG absorption (total IgG in blood as a proportion of that fed, assuming 140 ml blood plasma per kg BW) was not significantly different between the Control and Bolus calves (0.139 vs 0.125, SED 0.012). While the concentrations of all thyroid hormones measured were significantly greater in the calves at 24 hours of age relative to those seen at birth, there was no significant effect of the bolus treatment of the dams on the calf concentrations of any of the thyroid hormones measured at either time. Nevertheless, there was a positive linear correlation between the concentration of total T3 in the calves at birth and the efficiency of colostral IgG absorption (Figure 1); and a negative relationship between total T4 concentrations in the calves at birth and the efficiency of colostral IgG absorption (Figure 2).



**Figure 1** Regression of efficiency of IgG absorption against plasma total T3 concentration in calf at birth **Figure 2.** Regression of efficiency of IgG absorption against plasma total T4 concentration in calf at birth

**Conclusions** Boluses containing iodine, selenium and cobalt, administered to dry cows 40 days before calving, had no effect on the plasma concentration of IgG in the calves following their consumption of a standard amount of colostrum. This is in contrast to our experiment with sheep, though that experiment delivered iodine at a rate that was approximately 36% higher when expressed on a metabolic body weight basis than the present experiment. Nevertheless, we found a positive relationship between the efficiency of IgG absorption in the calves and the plasma T3 concentration, and a negative relationship between efficiency of IgG absorption and total T4 concentration, regardless of bolus treatment. This suggests that elevated total T3 levels are associated with improved colostral IgG absorption, while elevated T4 levels are associated with the reverse.

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## Effect of concentrate build-up strategy in early lactation on production performance, health and fertility of high-yielding dairy cows

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**Introduction** The high milk production potential of the modern dairy cow frequently results in excessive and prolonged periods of negative energy balance (NEB) during early lactation. The most common approach to reduce NEB is to increase energy intake by increasing the proportion of concentrates being offered. However, offering diets containing high levels of concentrates can lead to rumen acidosis, impaired rumen function and reduced intakes, the latter exacerbating NEB. Introducing concentrates into the diet of fresh calved cows at a slower rate is likely to improve rumen function. Furthermore, diet crude protein (CP) content is likely to be reduced through offering a lower concentrate diet in early lactation. Offering a lower protein diet has been shown to reduce milk output, have no effect on dry matter intake, and thus improve cow energy status (Law *et al.*, 2009). The objective of this study was to compare two very different strategies by which to introduce concentrates into the diet in early lactation, namely a rapid build-up (RBU) or a delayed build-up (DBU) strategy.

**Material and methods** From calving onwards, sixty autumn-calving Holstein Friesian cows (mean parity 3.1) were offered a basal diet (via feeder wagon) containing 35% concentrate and 65% forage on a dry matter (DM) basis (150 g CP/kg DM and 12.0 MJ metabolisable energy (ME)/kg DM). Cows were allocated to one of two post-calving concentrate allocation strategies (via out-of-parlour feeders), namely a rapid build up of concentrates (RBU) or a delayed build up of concentrates (DBU). With the rapid build-up treatment cows were offered 2.0 kg concentrate/cow/day on the day of calving, and this was then built up incrementally (0.5 kg/day) to a maximum of 7.0 kg/cow/day at day 10 post calving. Cows allocated the delayed build-up treatment received no additional concentrate via out-of-parlour feeders until day 28 of lactation, and thereafter received incremental concentrate levels (0.5 kg/day) to a maximum of 7.0 kg /cow/day at day 42 post calving. Once these concentrate feed levels had been achieved, diets were designed to have a CP and ME content of 180 g/kg DM and 12.4 MJ/kg DM respectively. Cows remained on these two dietary treatments until day 150 of lactation. Data were analysed using the residual maximum likelihood procedure via Genstat.

**Results** Total dry matter intake was unaffected by concentrate build-up strategy (Table 1). However, forage intake was significantly higher for cows allocated to DBU treatment ( $P<0.001$ ; Figure 1), while concentrate intakes were lower ( $P<0.01$ ). Neither milk yield nor milk composition was affected by concentrate build-up strategy ( $P>0.05$ ). Despite the lack of treatment effects on milk production, cows on DBU returned to positive energy balance earlier (week 7 post-calving) than those on RBU (week 19 post-calving). From weeks 3-7 post-calving, cows allocated to DBU produced 3.5kg less milk/day than those allocated to RBU ( $P<0.001$ ). Reproductive performance was unaffected by treatment ( $P>0.05$ ).

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

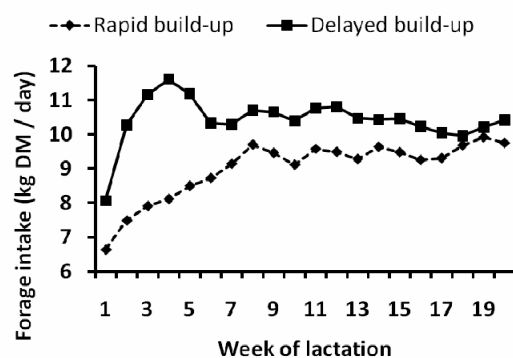
	Rapid Build-Up	Delayed Build-Up	SED	P value
Dry matter intake (kg / day)	20.5	21.4	0.53	NS
Forage intake (kg DM/ day)	9.0	10.4	0.34	***
Concentrate intake (kg DM / day)	11.5	11.0	0.19	**
Milk yield (kg / day)	38.9	37.9	1.61	NS
Milk fat (g / kg)	40.5	41.6	1.43	NS
Milk protein (g / kg)	34.2	33.3	0.71	NS
Energy balance (MJ / day)	-21.3	-6.3	7.91	P=0.06

**Conclusions** Adopting a delayed concentrate build-up strategy in early lactation improved forage intake while having no detrimental effect on production performance. This resulted in a trend towards improved energy status of the cows on this treatment, although reproductive performance was unaffected.

**Acknowledgements** This study was co-funded by DARDNI and AgriSearch.

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**Figure 1** Forage intake during the first 140 days of lactation

## Effect of exogenous long chain fatty acids on cytosolic triacylglycerol content of bovine mammary epithelial cells

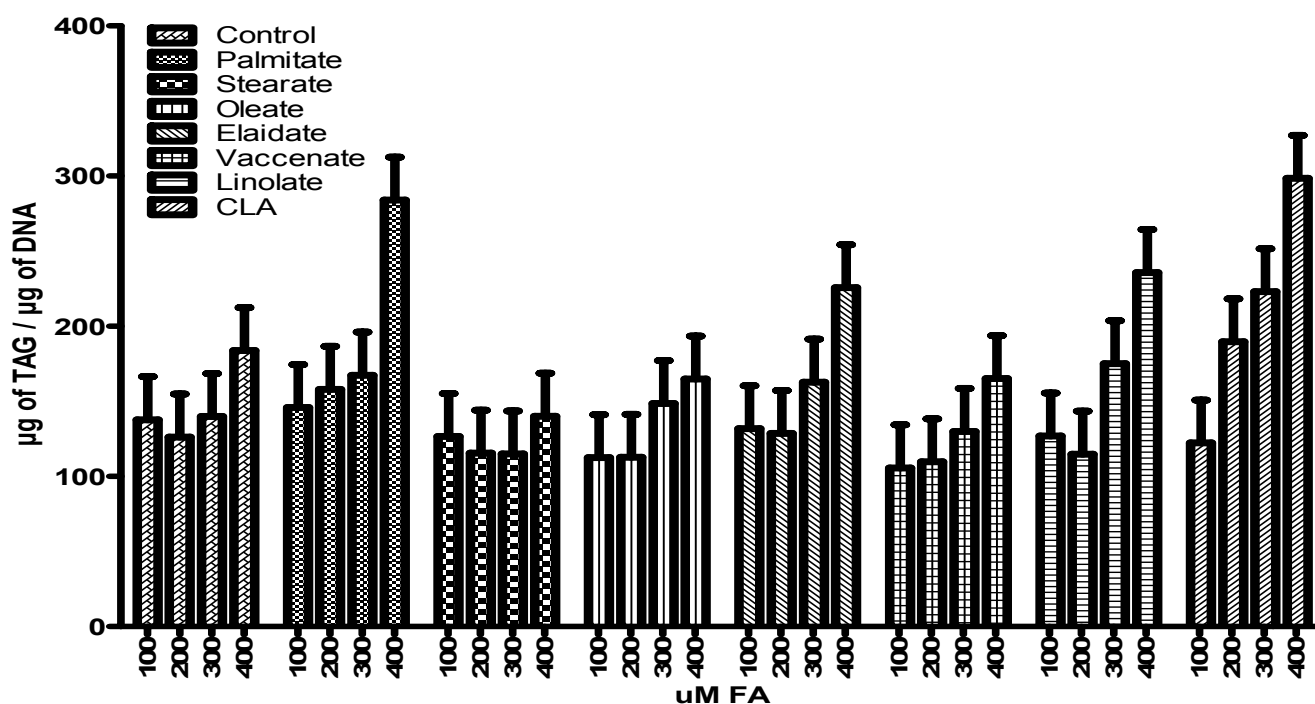
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**Introduction** There is increasing evidence to suggest that specific fatty acids (FA) can impact on the fat content of ruminant milk. The objective of this study was to compare effects of specific fatty acids on the concentration of triacylglycerol (TAG) produced by cultured bovine mammary cells.

**Material and methods** Bovine mammary epithelial (MAC-T) cells were grown supplemented with foetal bovine serum (10%), penicillin and amphotericin B in sterile 75 cm<sup>2</sup> flasks. Cultures were maintained in an incubator at 37°C in the presence of 5% CO<sub>2</sub>. Treatments were individual saturated fatty acids (palmitate and stearate), monounsaturated fatty acid (oleate), polyunsaturated fatty acid (linoleate), *trans* fatty acids (elaidate and vaccenate) and conjugated linoleic acid (cis-9, *trans* -11, rumenic acid). Absolute ethanol was used as a FA solvent and control. Concentrations of FA in cell media were 0, 100, 200, 300 and 400 mM. Cells were incubated for 24 hr and then lipid was extracted using hexane:isopropanol (3:2 v/v). TAG content of the extract was determined enzymatically using a commercial kit. DNA content of the cells was determined by the Hoechst fluorometric method and this was used to normalize TAG concentrations to the number of cells present. Data were analysed as a Linear Mixed Model design with fixed effects of FA treatments and concentrations.

**Results** DNA content was not affected by concentration or FA x concentration interaction; however vaccenate-treated cells had higher ( $P < 0.05$ ) cytosolic DNA (3.7 µg/ml), and palmitate-treated cells lower (1.9 µg/ml), compared with control (3.0 µg/ml) and other treatments (mean 2.8 µg/ml). When adjusted for DNA, cytosolic TAG (µg TAG/µg DNA; Figure 1) was increased by palmitate, elaidate, linoleate and CLA, compared with control, but was not affected significantly by stearate, oleate or vaccenate.



**Figure 1** Accumulation of TAG (µg/µg DNA) in mammary cells cultured with different FA

**Conclusions** The results demonstrate that cytosolic TAG accumulation is stimulated by increasing supply of LCFA (long chain FA). Palmitic acid appeared to have a strong cytotoxic effect, as indicated by reduced cytosolic DNA content. Effects on cytosolic TAG accumulation are not consistent within FA classes (tFA, SFA and UFA) but depend on individual FA structure.

**Acknowledgments** We thank Linda Sheldrick for technical advice and assistance with cell culture. Einar.V.B.P. thanks Consejo Nacional de Ciencia y Tecnología (CONACYT- México) for a PhD studentship.

## Calving ease and the subsequent fertility and calving performance of the dairy cow: retrospective analysis from a UK farm

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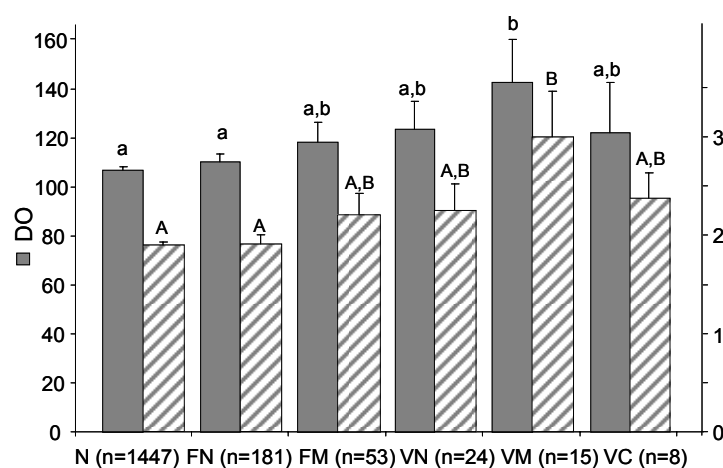
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**Introduction** Impaired fertility in the dairy cow is a major economic problem for the industry. Fertility is known to be impaired following a difficult calving but the full extent of the impact is not clear. Furthermore, a cow is at more risk of being culled following a difficult calving or poor fertility. This might be due to poor performance and health problems immediately prior to culling, but also to the anticipation of repetition of difficulty at next calving. The objective of the study was to assess the effect of various degrees of difficulty on the subsequent fertility of the dairy cow and determine if experiencing difficulty at 1st and 2nd calving puts a cow at more risk of experiencing difficulty at her subsequent calvings.

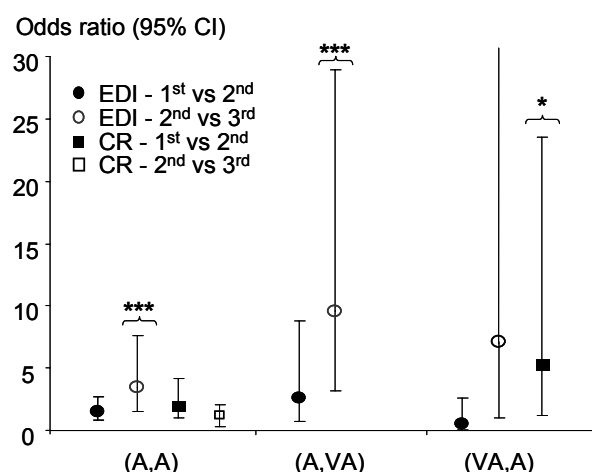
**Material and methods** Calving ease scores and subsequent fertility and calving performance of Holstein Friesian cattle were extracted from the SAC experimental farm database (UK) between 1990 and 2000 inclusive (Edinburgh herd, EDI, n=1728) and from 2003 to Sept 2009 (Crichton herd, CR, n=1041). Calving ease was scored as: no assistance (N), Farm assistance without/with malpresentation (FN/FM), Veterinarian assistance without/with malpresentation (VN/VM) and caesarean section (VC). Cows were allowed up to 12 services. In both herds, fertility performance was assessed by conception at first service (%), C1S), number of services to conception (NSERV), number of days open (DO) and calving interval (CI/days). Each herd was analysed using linear mixed models following a REML procedure in Genstat, including the cow identity nested within sire in the random model. For subsequent analysis, calving performance (CP) was defined as: abortion (AB); assistance (A) grouping all scores of difficulty but N; vet assistance (VA) grouping VN, VM and VC together. To assess replication of CP at subsequent calving, CP at 1<sup>st</sup> and 2<sup>nd</sup> calving, and at 2<sup>nd</sup> and 3<sup>rd</sup> calving was tabulated for the following combinations (CP, subsequent CP): (A, A); (A, VA); (A, AB); (VA, A). For each combination, odds ratio and relative risk (RR) were calculated and statistical significance assessed through  $\chi^2$  square tests.

**Results** In the EDI herd, VM cows had higher DO and NSERV (Figure 1,  $P < 0.05$ ) compared to N animals, but C1S and CI were not different ( $P > 0.05$ ). There was also no significant differences in the fertility between N calvings and any other scores for DO, CI, NSERV and C1S ( $P > 0.05$ ). There was no statistical evidence of impaired fertility in the CR herd, but the dataset size was smaller.

Cows needing assistance or vet assistance at 1<sup>st</sup> calving were not more likely to require assistance at next calving for the EDI cows (Figure 2,  $P > 0.05$ ). However, in the CR herd, VA cows at first calving were more likely to be assisted at subsequent calving (RR=3.7). Nevertheless, in the EDI herd, cows having experienced assistance in their 2<sup>nd</sup> calving were more likely to require assistance at 3<sup>rd</sup> calving (RR=2.7), and particularly more likely to require veterinary assistance (RR=8.0) (Figure 2,  $P < 0.05$ ). There was also a tendency of VA cows at 2<sup>nd</sup> calving to be assisted at 3<sup>rd</sup> (RR=4;  $P < 0.10$ ). In both herds, there was no evidence that assistance at 1<sup>st</sup> or 2<sup>nd</sup> calving was linked to higher risk of abortion during the subsequent pregnancy ( $P > 0.05$ ).



**Figure 1** means  $\pm$  standard error from raw data for NSERV and DO (EDI herd). Means without a common letter differ ( $p < 0.05$ ).



**Figure 2** Odds ratio (95% confidence interval) of how CP affects subsequent CP. \*:  $P < 0.05$ ; \*\*\*:  $P < 0.001$ .

**Conclusion** In line with previous findings, cows needing assistance at calving may suffer from subsequent impaired fertility. Moreover, previous dystocia can increase risk of assistance at subsequent calving. It may be due to underlying predisposition, carry-over effects or lower intervention thresholds. Therefore, calving difficulty has a long-term impact on the performance of the cows.

**Acknowledgements** Many thanks to Defra, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR for funding under the Sustainable Livestock Production LINK Programme as well as to farm staff and technicians for data collection.

## Predisposing factors for culling in first lactation Holstein-Friesian heifers

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**Introduction** The high culling rate in dairy herds is one of the most important problems for dairy farmers (Tena-Martinez *et al.*, 2009). Culling in first lactation dairy heifers can be as high as 33% (Wathes *et al.*, 2007), and both culling and infertility reduce farm profitability. Reasons for culling of dairy cows have been well documented (Bascom and Young, 1998). However, studies examining the predisposing factors and characteristics of first lactation heifers that do not progress to subsequent lactations are sparse. The objective of the current study was to identify traits that predispose first lactation heifers to an increased risk of culling.

**Material and methods** Data were obtained from the Langhill herd of Holstein Friesian cows, which are on a long-term genetic and feeding systems project (Bell and Roberts, 2006). Cows were either of the genetic line selected for kilograms of fat plus protein (select line) or selected to remain close to the average genetic merit for fat plus protein production for all animals evaluated in the UK (control line). Both lines were fed either a high-or low-forage diet as a total mixed ration. The high forage cows were also at grass during the summer. Data were collated from September 2003 to August 2010. A total of 175 cows were culled, of which 56 were first lactation heifers. Of the culled first lactation heifers, 23 were due to infertility. In the analysis, the culled first lactation heifers were paired with their cohorts that survived to at least the second lactation. The pairing was based on age at first calving, feeding system and genetic line. In order to determine the predisposing factors for those cows that were culled in the first lactation due to infertility, logistic regression was used. The traits included in the model were: age at first calving, metabolic calving weight, calving body condition score, calving ease, service body condition score, 60-day milk protein, production system and mastitis incidence. The response variable Y had the value 1 for culled first lactation heifers and value 0 otherwise. Some of the traits are detailed in Table 1.

**Table 1** Descriptive statistics of some of the traits evaluated for increased risk of culling due to infertility in 1<sup>st</sup> lactation heifers

Variable	Mean	SD	N	Minimum	Maximum
Age at first calving (Months)	26	2.8	46	23	36.5
Metabolic calving weight (kg)	113	11.7	46	94	146
Calving body condition score	2.4	0.3	45 <sup>a</sup>	1.8	3.3
Calving ease <sup>b</sup>	1.6	0.8	46	1	3
Service body condition score	2.3	0.4	43 <sup>a</sup>	1.3	3
60-day milk protein (kg)	1.2	0.4	46	0.1	2.04

<sup>a</sup> With missing value(s), <sup>b</sup> Ranks: 1=Normal, 2=Malposition and 3=Assisted

**Results** Multiparous cows tended to be culled due to fertility and udder problems. First lactation heifers were culled predominantly for fertility reasons (Table 2). Of the tested traits, body condition score at service was the only trait that had significant influence ( $P < 0.05$ ) on likelihood of cows to be culled in their first lactation due to fertility reasons. The regression estimate was positive (2.6) and the culling probability was 0.87 indicating a high probability of culling for cows with high body condition score at service. Likelihood estimates and odds ratios are in Table 3.

**Table 2** Herd level reasons for culling

Reason for culling	Total	1st Lactation
Fertility	48	23
Udder problems	47	13
Accidents	32	11
Other/Unknown	20	5
Foot/Leg problems	22	2
Died	6	2

**Table 3** Characteristics of traits in fitted model

Variable	Estimate	s.e.	Odds Ratio	Confidence limit
				Lower Upper
Age at first calving (Months)	0.08	0.14	1.08	0.8 1.4
Metabolic calving weight (kg)	-0.03	0.04	0.97	0.9 1.0
Service body condition score	2.64	1.16	13.9	1.4 139.9
Production system	-0.37	0.38	0.69	0.3 1.5

**Conclusions** The results indicate that first lactation heifers with higher than average body condition score at service were at risk of not progressing into their second lactation, because they were at an increased risk of being culled for infertility.

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## The response of grazing dairy cows to a 'flat rate' or a 'feed-to-yield' concentrate allocation strategy

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**Introduction** In order to meet the nutrient requirements of high yielding dairy cows while grazing, concentrate supplementation is normally considered essential. However, concentrate feed costs represent a significant proportion of variable costs on most farms, and as such, concentrates should be used efficiently. The efficient feeding of concentrates at farm level is made more difficult in herds with a spread calving pattern, and on many Northern Ireland dairy farms cows now calve for at least nine months of the year. Thus most herds comprise cows in early, mid and late lactation. As there is relatively little information available on optimum concentrate allocation strategies for herds with a wide range of milk yields, this study was designed to compare the effects of two allocation strategies, namely 'flat rate' and 'feed-to-yield', on the performance of grazing dairy cows.

**Material and methods** Two concentrate allocation strategies ('flat rate' and 'feed-to-yield') were compared in a continuous design study involving fifty-six Holstein Friesian cows (20 primiparous, 36 multiparous). Multiparous cows had a mean calving date and pre-experimental milk yield of 27 November (s.d. 65 days), and 33.2 kg/cow/day, respectively, while the respective values for the primiparous cows were 8 January (s.d. 22 days) and 26.7 kg/cow/day. Concentrate feed levels with the 'flat-rate' treatment were calculated based on the difference between the assumed milk yield potential of grazed grass as the sole feed (25.0, 20.0, 20.0, 16.0 and 12.0 kg/cow/day (multiparous cows) and 20.0, 16.0, 12.8 and 9.6 kg/cow/day (primiparous cows), during May, June, July, August and September), and the average yield of cows on that treatment, with concentrates offered at 0.6 kg/litre milk to make up the difference. Feed rates were determined separately for primiparous and multiparous cows, with concentrates being offered on a 'flat rate' basis (Table 1). The same total quantity of concentrates offered within the 'flat rate' treatment was also offered within the 'feed-to-yield' treatment. This was achieved by firstly calculating the difference between the milk output of each cow within the 'feed-to-yield' group and the milk potential of grass, and summing this difference for all cows (separate calculation for primiparous and multiparous cows). The total concentrate input within the 'flat rate' treatment was then divided by this total milk yield 'difference' to get a concentrate requirement per litre of milk being produced above what grass alone could sustain. This value was then used to calculate concentrate requirements for individual cows, subject to a minimum (1.0 kg/cow/day) and a maximum daily allocation (8.0 and 10.0 kg/cow for primiparous and multiparous animals, respectively). All concentrates were offered through the milking parlour, divided between two equal feeds. Concentrate feed levels were reviewed monthly throughout the study. The study commenced on the 12 May and concluded on 11 September with the cows grazing full time throughout. Both treatments involved rotational paddock grazing systems, with fresh herbage offered after each evening milking. Average stocking rates (5.7 cows/ha) and fertiliser nitrogen inputs (165 kg/ha) were constant across both treatments. Cows were milked twice daily with milk yield recorded at each milking. During two successive milkings each week, a milk sample was collected from each cow and analysed for fat and protein content. Average live weight was recorded at the beginning and the end of the study. All data were analysed by REML component analysis, with parity and appropriate pre-experimental data used as covariates within the model.

**Results** Total concentrate inputs were equal within each concentrate allocation strategy. Concentrate allocation strategy had no effect ( $P>0.05$ ) on average milk yield, milk composition or milk fat + protein yield (Table 2). End of study liveweight of the cows on the 'flat rate' treatment (560 kg) was not different ( $P>0.05$ ) from that of cows on the 'feed-to-yield' treatment (559 kg). These results suggest that with moderate yielding cows in mid lactation, a simple flat rate concentrate feeding strategy is as effective as a more complex 'feed-to-yield' strategy. However, within this study concentrates were fed to match the requirements of the 'average' animal within the 'flat-rate' treatment, thus concentrates were not being overfed, in contrast to practise on many farms.

**Table 1** Concentrate feed levels during the study

	Concentrate feed level (kg/cow/day)				
	Multiparous		Primiparous		
	Flat rate	Feed to yield (range)	Flat rate	Feed to yield (range)	
May	5.5	1.0 – 10.0	4.9	2.5 – 8.0	-
June	4.1	1.0 – 8.9	3.8	1.4 – 8.0	-
July	3.6	1.0 – 7.3	4.1	1.0 – 8.0	-
Aug	2.6	1.0 – 5.0	4.3	1.0 – 8.0	-
Sep	1.5	1.0 – 2.5	4.3	1.0 – 7.9	-
Total	451	451	508	508	

**Table 2** Effect of concentrate allocation strategy on the performance of grazing dairy cows

	Allocation strategy		s.e.d	Sig.
	Flat rate	Feed to yield		
Total milk output (123 days) (kg/cow)	2711	2798	102.5	NS
Milk yield (kg/cow/day)	22.4	23.0	0.81	NS
Milk fat (g/kg)	39.6	40.1	1.06	NS
Milk protein (g/kg)	33.2	33.5	0.33	NS
Milk fat + protein yield (kg/cow/day)	1.62	1.67	0.061	NS

**Conclusions** Concentrate allocation strategy had no effect on cow performance demonstrating that a simple 'flat-rate' concentrate feeding strategy is as effective as a more complex 'feed-to-yield' strategy.

## The benefits of Computed Tomography (CT) scanning in UK sheep flocks for improving carcass composition

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**Introduction** Selection for carcass muscle and fat weight in sheep has traditionally been via the proxy traits ultrasound scanned muscle and subcutaneous fat depth. Significant genetic gains have been made using ultrasound scans as selection criteria (Simm *et al.*, 2002). In 2000 after a 3 year research project, Computed Tomography (CT) scans became available to the sheep industry as a more accurate way to measure the carcass composition of live animals. In addition to being highly accurate and repeatable, the CT scanned traits were effectively the breeding objective traits with prediction accuracies of 0.90 – 0.98 (Young *et al.*, 2001). However, CT scanning is relatively expensive and immobile making it unfeasible for all selection candidates to be scanned. Therefore, it was recommended that CT scanning be used as part of a 2 stage selection process with elite males identified based on ultrasound scans being CT scanned. This 2 stage approach was predicted to be economical and increase genetic progress by up to 32% compared with using ultrasound scans alone (Lewis and Simm, 2002). Now that CT scanning has been available to the UK sheep industry for a decade, the aim of this paper was to look at the benefits that have been achieved by breeders that CT scanned within their flock.

**Material and Methods** Texel performance records from the October 2009 routine genetic evaluation were used to assess benefits to flocks as a result of CT scanning. The realised increase in EBV accuracies was obtained by comparing the achieved accuracies from 2 genetic evaluations where the CT information was either included or excluded. For both these genetic evaluations the models, correlations and animals included remained constant. The theoretical increase in genetic response was estimated assuming only the accuracy changed with the selection intensity, generation interval and standard deviations remaining constant.

**Results** Since 1997, there have been more than 2,000 Texel animals CT scanned from a total of 65 different flocks. This equates to approximately 19% of all flocks that have ultrasound scanned in this time having participated in CT scanning at least once. Within the flocks that recorded CT information, approximately 30% of animals within a contemporary group (based on flock, season and sex) were CT scanned during the research phase (1997-1999). After the completion of the research project this decreased to 12-25% of animals within a contemporary group being CT scanned. Although 65 flocks in total have participated in CT scanning, few flocks have consistently CT scanned animals across the years with 31, 20, 7 and 7 flocks having CT scanned progeny from 1, 2-4, 5-7 and 8-10 different progeny year groups, respectively. Table 1 shows that including CT scan information in the genetic evaluations resulted in the EBV accuracy of CT traits increasing by between 7 and 13% for animals that have been CT scanned. Small increases in accuracy were also observed (results not shown) for the other growth and carcass traits, the exception being ultrasound scanned fat depth where the accuracy increased by 6% when CT information was included. This increase in accuracy was the result of strong genetic correlations between CT fat weight and ultrasound fat depth scan. Gigot muscularity was seen to benefit the most from CT scanning because it is a trait that can be measured on the live animal only by CT scanning. The increases in accuracy for young rams may actually be higher than those reported in this paper. This is because the comparison was unable to account for progeny (that occurred after CT scanning) of these animals and the progeny information may have inflated the observed accuracy - possibly with a greater impact when less information was available (i.e. no CT scan information). The increase in genetic response was predicted based on the observed increases in accuracy as a result of including CT information. The greatest increase in genetic response was observed for muscularity because it had a lower observed accuracy when CT information

**Table 1** The average EBV accuracy of CT scanned animals (n=2,219) when their CT information was either excluded or included in the genetic evaluation and the increase in genetic response possible as a result of the observed increase in accuracy was ignored and the increase in accuracy was the greatest when CT information was included.

	CT scans excluded	CT scans included	% predicted increase in genetic response
CT scanned Fat weight	70	77	10
CT scanned Muscle weight	76	81	7
CT scanned Muscularity	64	77	20

**Conclusions** These results clearly show that CT scanning elite rams results in significant improvements to the EBV accuracies of the CT traits and ultrasound fat depth. These increases in accuracy effectively allow for non-trivial improvements in the genetic response for carcass composition. However, for this increased genetic improvement to be maintained across years, flocks need to CT scan animals annually. The new mobile SAC CT scanner may make this easier for flocks in England.

**Acknowledgements** Thank you to the Texel breed society and Signet performance recording for the use of the data.

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## Use of farmer recorded mastitis data to improve the genetic evaluation of udder health in UK dairy cattle

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**Introduction** The udder health sub-index in the UK national profit index (£PLI) utilises test day records for somatic cell count (SCC) and an udder composite type trait. Inclusion of mastitis is expected to enhance the prediction of udder health in genetic evaluations. Some dairy herds have been recording mastitis events of their cows through milk recording organisations (MROs). Preliminary analyses on these records suggest that mastitis could be used in addition to current udder health traits (Pritchard *et al.*, 2009). The objective of this study was to estimate genetic parameters of mastitis and the genetic relationships with other traits.

**Material and methods** Mastitis events were recorded by farmers voluntarily as part of routine milk recording. Mastitis events that occurred 0 to 305 days after calving in the first three lactations were considered. Mastitis was analysed as a binary trait (0/1), with analyses including affected animals and their contemporaries from the same herd, year and season of calving. Separate datasets were created for genetic parameter estimation of mastitis (49214 cows, 85311 lactations) and bivariate analyses with other traits (377,013 cows, 566,835 lactations). The main edits for genetic parameter estimation were 1) calving ages for first, second and third lactation were within the ranges of 18-48 months, 30-62 months, and 42 to 70 months, respectively; 2) sires were born from 1992 onwards, had at least 50 eligible daughters, and up to the first 200 daughters born were selected, and 3) at least five animals per herd-year-season. Edits were less strict for bivariate analyses to allow more data on other traits to be matched. (Co)variance components and genetic parameters were estimated in ASReml (Gilmour *et al.*, 2006), via a sire model. For mastitis, fixed effects included month of calving, age at calving nested within lactation number, and herd-year-season. For the repeatability model the permanent environmental effect was also included. Genetic evaluations were also carried out on SCC and mastitis data, and proofs were obtained for bulls and cows.

**Results** Incidence of mastitis increased with lactation number, ranging from 14 to 24%. Heritability estimates for mastitis were low and significant ( $p < 0.05$ ), with the exception of lactation two. Estimates ranged from 0.02 to 0.06 for lactations one to three, and was estimated as 0.03 (0.006) using a repeatability model. Mastitis was strongly genetically correlated with SCC ( $0.65 \pm 0.07$ ) and moderately correlated with udder composite ( $0.28 \pm 0.10$ ), which are traits currently included in the udder health sub-index. Genetic correlations of mastitis with other traits, using a repeatability model, are shown in Table 1, and all were significant ( $p < 0.05$ ). For Holstein Friesian bulls (with a reliability of at least 30 %) mastitis PTAs (Predicted Transmitting Ability) ranged from -4.0 to 13.7 % and had a normal distribution. A negative PTA value is expected to reduce the incidence of mastitis in that bull's daughters compared to the mean of the population. The genetic trend of mastitis closely tracks that of SCC. There has been an increase in PTA's in both traits, in the undesired direction, but recently (since the inclusion of SCC into the evaluations from 1998) this has stabilised and both PTA's are now decreasing. Similar results were also obtained for cow proofs.

**Conclusions** The heritability of mastitis is low, but sufficient genetic variance and its inclusion together with current udder health traits in an index should help to improve mastitis resistance, as experienced by other countries (Philipsson and Lindhé, 2003). The high genetic correlation between mastitis and SCC supports the current use of SCC as a good predictor of udder health. However, a genetic correlation less than one indicate that they are genetically not the same trait. An antagonistic relationship between mastitis and production traits was found, which suggests animals genetically above average for yield traits are more susceptible to mastitis. Genetic correlations also show that increased mastitis resistance would be accompanied by reductions in lameness, lower SCC, increased fertility, and increased length of productive life. Future work should involve encouraging and increasing farmer awareness of the benefits of disease recording for their own herd management, in addition to the use of data in genetic evaluations.

### Acknowledgements

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## Use of national cattle movements data to enhance the genetic evaluation of lifespan in UK dairy cattle

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**Introduction** In the UK, lifespan evaluations are currently based on a model which uses a lifespan score calculated from the number of lactations completed plus a survival score computed from the following traits: mammary composite, legs and feet, fore udder attachment and somatic cell count. This process has been very effective, but has some limitations: the system cannot account for animals that get culled before starting the next lactation and the lifespan score of animals which leave the milk recording system are predicted from their last recording date which remains static. In 1996 cattle passports were introduced into the UK in order to improve the traceability of cattle. This was followed in 1998 by a computer based system to register all cattle. The British Cattle Movement Service (BCMS) database contains records on all cattle within the UK, which includes information on births, movements and deaths. This information is valuable for calculating the actual duration an animal is alive and productive. The aim of the present study was to utilise BCMS data to (i) estimate genetic parameters for two new lifespan traits: days of life (DL, days from date of birth to date of death) and days of productive life (DP, days from date of first calving to date of death) and (ii) estimate genetic correlations of DP with production, mastitis, lameness, lactation somatic cell count (LSCC) and fertility of the cow.

**Material and Methods** Data from the BCMS database has been validated, matched and combined to national milk record databases to obtain accurate information on actual dates of birth and death thereby providing accurate data on DL and DP. Univariate analyses of DP and DL were carried out on a dataset containing 73981 records from 2472 sires. The data were analysed using ASReml (Gilmour *et al.* 2006) with a linear sire model to estimate genetic parameters of DP and DL. Sire was fitted as a random effect and further non-genetic effects fitted in the model included year of birth, age at first calving (months), deviation of milk yield (kg) from the mean of the contemporary group herd-year and herd-year. Furthermore, a bivariate sire model was applied to a dataset of 577916 to estimate genetic correlations of DP with production, mastitis, lameness, lactation somatic cell count (LSCC) and fertility. The same non-genetic factors were applied to DP as in the univariate analysis. For mastitis, LSCC, lameness, production and fertility the cow (permanent environmental effect) was fitted because the three lactations were treated as a repeated trait. The following effects were also included: For mastitis, LSCC, lameness, production: age at calving (months) nested within lactation, month of calving and herd-year-season; For fertility: linear and quadratic of age at calving (months) nested within lactation, month of calving, and herd-year.

**Results** From the univariate analyses heritability estimates for DP and DL were low (0.05) but significant ( $p$ -value  $< 0.05$ ). Table 1 outlines the genetic correlations of DP with production, mastitis, lameness, LSCC and fertility. All genetic correlations were significant and negative, indicating that cows that produce higher yields, have lower fertility, higher LSCC, and higher disease incidence of mastitis and lameness and are likely to have a reduced productive life. All genetic correlations shown in Table 1 were significant ( $p$ -value  $< 0.05$ ), although some traits, particularly lameness, had relatively few numbers of records.

**Table 1** Significant genetic correlations between DP and other traits

Traits	$r_g$ (SE)
Mastitis	-0.64 (0.062)
Lameness	-0.30 (0.131)
LSCC	-0.52 (0.030)
CI	-0.40 (0.063)
DFS	-0.39 (0.058)
Milk	-0.66 (0.021)
Fat	-0.23 (0.034)
Protein	-0.46 (0.028)

Abbreviations: LSCC, lactation average somatic cell count; CI, calving interval; DFS, days to first service

**Conclusions** Utilisation of BCMS data has been valuable in obtaining data on actual date of death of UK dairy cows. This has allowed for evaluation of longevity as a direct measure (DL and DP). In the present study, the estimated heritabilities for DL and DP are low (0.05) but significant and consistent with reports in the literature of dairy cows. Furthermore, DP has shown good correlations with mastitis, LSCC, lameness, production and fertility. Current lifespan evaluations have been successful in improving genetics of cow longevity in the UK. Incorporating lifespan as a direct measure as days of life or days of productive life may improve on the current breeding value estimation. However, further work is required to validate this.

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## Genetic relationship of lameness with milk yield, body condition score and reproductive traits in primiparous Holstein cows

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**Introduction** Direct (treatment cost and involuntary culling) and indirect (reduced milk yield and compromised fertility) economic losses, animal welfare implications and increasing prevalence have placed lameness among the most important problems for the dairy industry to solve. Green *et al.* (2002) concluded that there was a decrease in milk yield from 4 months before up to 5 months after a cow was diagnosed as clinically lame. Lameness heritability estimates vary from 0.10 to 0.22 depending on the model of analysis used, the trait definition and the size of the dataset (Koenig *et al.*, 2005), suggesting that lameness and its adverse effects could be partially addressed through genetic selection. Appropriate inclusion of lameness in the breeding goal presupposes knowledge of its genetic correlation with other important traits. The objective of this study was to calculate the genetic correlation of lameness with milk yield, body condition score (BCS) and reproductive traits.

**Material and methods** The study was conducted in a large commercial dairy farm located in Northern Greece and included 518 first lactation Holstein cows that calved between 2005 and 2009. Cows were examined for lameness weekly throughout the lactation period. If a cow exhibited lameness at any stage of lactation she was scored with 1, otherwise with 0. Milk yield was recorded weekly and these measurements were used for the estimation of total lactation milk production. Body condition was scored during the first week post-calving (BCS\_calv) and then weekly for the first 13 weeks of lactation, using a five-point scale with increments of 0.25 (Ferguson *et al.*, 1994). From these records, minimum BCS (BCS\_min) was determined and the rate of decline from BCS\_calv to BCS\_min (BCS\_rate) was calculated. Furthermore, the following reproductive traits were recorded: number of insemination per conception (NINS) and NINS for conception in the first 305 days (NINS\_305), interval (days) from calving to conception (CALV\_CONC) and conception rate (0/1) in the first 305 days of lactation (CONC\_305). Heritability and genetic correlation estimates were derived from a series of 4-variate analyses, each considering lameness, lactation milk yield, a BCS trait and a reproductive trait. Models included year and season of calving, age at calving, and genetic effect of the animal (including all known pedigree relationships). Models for reproductive traits also included the effects of synchronisation of oestrus, and days in milk and season of first artificial insemination. Where multiple estimates per trait were derived, an average value was taken as the final result.

**Table 1** Genetic (rg) and residual (re) correlation estimates with lameness and corresponding P-values.

Trait	rg	P-value	Re	P-value
Lactation milk	0.75	0.02	-0.63	0.05
BCS_calv	-0.45	0.09	0.21	0.45
BCS_min	-0.27	0.37	-0.01	0.95
BCS_rate	0.31	0.69	0.00	0.99
NINS	-0.47	0.24	0.30	0.17
NINS_305	-0.12	0.81	0.04	0.88
CALV_CONC	-0.51	0.43	0.09	0.66
CONC_305	0.38	0.36	-0.34	0.09

**Results** Lameness prevalence was 52.7% and heritability was 0.32 ( $P=0.08$ ). The relatively high heritability estimate is attributed to the controlled environment in the farm of the study; similarly higher than usual estimates were derived for the other traits. Genetic and residual correlations of lameness with other traits are shown in Table 1. Lactation milk yield had a positive (unfavourable) genetic correlation with lameness indicating that cows of high genetic merit for milk production are also genetically predisposed to suffer from lameness. Interestingly, a negative (favourable) residual correlation was observed between these two traits, suggesting that high producing cows may be more likely to receive preferential care leading to reduced lameness incidence. There was a negative, albeit non-significant, correlation between BCS\_calv and lameness. The low P-value ( $P=0.09$ ) associated with this estimate indicates the possibility for cows with the genetic potential for high BCS during the first week post-calving to be genetically more resistant to lameness. All other genetic and residual correlations derived in the present study were statically no different from zero.

**Conclusion** Continuation of selection without considering lameness in the breeding goal will lead to a deterioration of hoof health, as attested by the unfavourable genetic correlation with milk yield. More research is needed on the association of lameness with other economically important traits.

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## Genetic correlations between calving ease and fertility traits in UK Holstein Friesian heifers

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**Introduction** In dairy cattle, genetic selection has historically focussed on increasing milk, fat and protein yields, although more recently information on fitness has been included. This suggests awareness is rising that, in order to maintain or improve economic efficiency, genetic emphasis on fitness traits is needed (De Maturana *et al.*, 2007). Due to its unfavourable genetic correlation with milk production and direct effect on the profitability of the herd, fertility is an important fitness trait. Calving ease (CE) is important to the dairy cattle industry being linked to impaired performance, loss of animals, compromised animal welfare and high additional costs. There is a lack of understanding on the genetic relationships between calving and fertility traits (De Maturana *et al.*, 2007). This study aims to estimate the direct and maternal genetic relationships between CE and fertility traits in UK Holstein-Friesian heifers

**Material and methods** First parity CE records were provided by the Cattle Information Service (CIS) and National Milk Records (NMR) and recorded on a 4 -grade and 5 -grade scale respectively. Categories were defined by CIS as: 1-ease, 2-assisted, 3-difficult, 4 -vet assisted, and by NMR as: 1- normal (not assisted), 2- moderate assistance (farmer), 3- moderate assistance (vet called as precaution) 4 - difficult (extraction by farm staff), 5- very difficult calving (vet assisted). To harmonise scales, category 2 and 3 of the NMR scale were merged; both referring to 'moderate assistance required'. Merging the CE and fertility data led to a total of 27845 cow performance records, respectively, originating from 1,751 herds. Fertility data consisted of calving interval (CI), number of inseminations (NINS) and days to first service (DFS), in the first lactation following the recorded calving. CI was restricted between 300-600 days. In all datasets, age of cow was restricted between 18-40 months to ensure data included only first parity records. Data were analysed using bivariate linear mixed models in ASREML (version 2.00, 2006) fitting fixed effects of month\*year of calving, age of cow, data source, sex of the calf and herd. Random effects fitted were contemporary group of herdyear, sire of the cow (maternal grandsire (MGS) of the calf) and sire of the calf to account for the direct genetic effect of CE. Sire and MGS variance components were subsequently transformed into direct and maternal variance components.

Trait	$h^2$	$r_{gCEdir}$	$r_{gCEmat}$
CI	$0.022 \pm 0.01^*$	$-0.57 \pm 0.25^*$	$0.70 \pm 0.24^*$
NINS	$0.027 \pm 0.01^*$	$-0.47 \pm 0.17^*$	$0.65 \pm 0.20^*$
DFS	$0.069 \pm 0.02^*$	$-0.33 \pm 0.20$	$-0.23 \pm 0.22$
CE dir.	$0.077 \pm 0.02^*$	-	$-0.46 \pm 0.18^*$
CE mat.	$0.033 \pm 0.01^*$	$-0.46 \pm 0.18^*$	-

\* $P < 0.05$

**Table 1** Genetic parameters of calving ease and fertility traits in UK dairy cattle

**Results** Table 1 presents the estimated genetic parameters for fertility traits and CE of UK dairy cattle heifers. All heritability estimates are similar to previously published estimates for dairy cattle in the UK (Wall *et al.*, 2003, 2010). Estimates heritabilities for fertility traits are low to moderate and consistent with literature where estimates range from 0.01-0.12 for CI, 0.035-0.09 for DFS and 0.02-0.04 for NRINS (Wall *et al.*, 2003; De Maturana *et al.*, 2007). Direct and maternal CE heritabilities concur with literature as well (De Maturana *et al.*, 2007; Hickey *et al.*, 2007), with the maternal heritability being approximately half of the direct heritability. Estimated genetic correlations show that a longer CI is genetically associated with a more difficult calving. Supporting this estimate is the positive genetic correlation of maternal CE with NINS, suggesting that cows with more difficulty at first calving tend to need more inseminations to conceive. DFS was not affected by CE. Direct CE is negatively correlated with CI and NINS, indicating that heifer calves born from more difficult calvings tend to have lower NINS and shorter CI in their first lactation. Literature on direct and maternal correlations between CE and fertility traits is scarce and statistical methods differ highly. However, results here relate to reports by Muir *et al.*, 2004 and Roughsedge *et al.*, 2005.

**Conclusions** Calving ease is genetically related to fertility, however direct and maternal relationships differ. This should be addressed when implementing CE in national breeding indices, to ensure genetic selection moves in the desired direction. Estimated genetic correlations show that maternal CE is favourably associated with NINS and CI, but not with DFS. This suggests that, genetically a difficult calving does not delay time of breeding but rather impairs the ability to conceive and prolongs the calving interval. Direct CE is negatively related to NINS and CI. Estimation of genetic correlation between CE, fertility traits and other traits of interest, e.g. age at first calving and milk production, might aid in the understanding of this relationship.

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## A model for deriving economic weights for calving ease in UK dairy cattle

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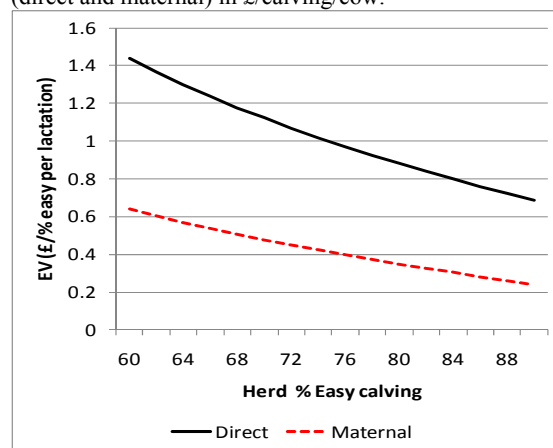
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**Introduction** Calving ease is an important trait in dairy cattle. Calving difficulties in daughters caused by genes with either direct or maternal effects for calving ease are likely to result in higher farm labour and veterinary costs, as well as poorer performance, survival and welfare for both the cow and calf. In January 2010, genetic evaluations for direct and maternal calving ease became available for UK dairy cattle but these have not yet been incorporated in national selection indices (Wall *et al.*, 2010). Direct calving ease predicts the effects of the genes carried by the calf, while maternal calving ease predicts the effects of the genes carried by the dam giving birth. To be included in selection indices economic values need to be calculated. The aim of this study was to develop an economic model to develop appropriate selection index weightings for both direct and maternal breeding values calving ease in UK dairy cattle

**Material and Methods** A model was developed which specifically focuses on the economic value of calving ease. The model predicts changes in incidences of calving difficulty levels (e.g. slight assistance, vet assistance, caesarean) as the herd mean incidence of severe or worse calvings increases. Based on previous work (Eaglen *et al.*, 2010, McGuirk *et al.*, 2007), the impacts on production and functional traits of different classes of calving ease were used to parameterise the economic model. The economic assumptions of the different classes of calving ease and subsequent impacts were taken from a range of industry sources. Two separate economic values are computed, to address issues of double counting which occurs when effects of calving difficulty on traits already in the genetic evaluation are counted. A full economic value includes farm labour and veterinary costs as well as the economic impacts of difficult calvings on cow fertility, cow survival, cow milk yield, and calf survival. A daughter independent economic value, includes farm labour and veterinary costs only. The model also took account of the cumulative discounted genetic expressions using the approach of Berry *et al.*, 2006 and reparameterised for the UK to account for the different timing and frequency of expression of direct and maternal calving ease genes from a bull relative to other breeding goal traits expressed once per lactation.

**Results** Figure 1 shows the economic values for the direct and maternal calving ease traits as a function of herd mean incidence of easy calvings. The economic weights give the £ per calving per cow from a 1 unit increase in calving ease where calving ease is defined the percentage of calvings requiring no assistance. At the industry average incidence of easy calvings of 77%, the economic weights of calving ease direct and calving ease maternal are £0.94 and £0.38 respectively. It should be noted that these values are based on the impacts and industry reports and will vary when these assumptions are changed. The economic weighting for direct calving ease traits was approximately twice that of maternal calving ease. This is because the economic effects of direct calving ease expressions affecting mates are currently not accounted for in UK dairy breeding goals and therefore receive a significantly higher weighting in the index. Economic weights would be modestly higher in herds with a higher incidence of difficult calvings (Figure 1). However, this effect is probably under-estimated, because the variance of true breeding values on the incidence scale would be much higher in higher incidence herds, but this is unlikely to be reflected in national proofs which will be standardised to the national average incidence scale. Results also suggest that it is unnecessary to model cow parity and sex of calf interactions separately when computing the economic value (not shown).

**Figure 1** The effect increasing the level of easy calvings (%) in a herd on the economic weights for calving ease (direct and maternal) in £/calving/cow.



**Conclusions** The model developed in this study can be used to derive economic weights for direct and maternal calving ease for a range of scenarios (economic, animal and herd). These weights can be used by the dairy industry to add calving performance traits to the wider selection index for UK dairy cattle, £PLI (profitable lifetime index). This index already includes a range of production and functional traits and the addition of calving performance would broaden it further and make it more complete.

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## Threshold analysis of type traits in dairy cattle

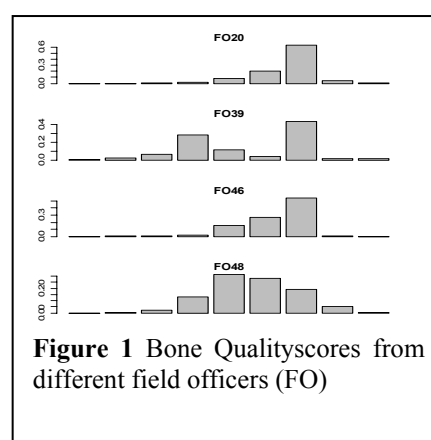
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**Introduction** Breed organization Holstein UK carries out routine type classification of animals in the UK. They have several independent field officers who classify animals for around 28 traits. The current method of analyzing the type traits is by treating them as continuous data and fitting an animal model, which gives breeding values for animals that are scored and for their relatives. These breeding values are used in selection decisions for bulls. It is important that scoring and analysis are as accurate as possible. The type classification scores represent ordinal data which are quasi continuous and in theory the ideal method of analysis is by fitting a threshold model (Gianola, 1982). In threshold analysis an underlying normal distribution of phenotype (liability) is assumed and the observed scores are then determined by a set of fixed thresholds. The aim of this study was to investigate whether threshold models offer any advantage over linear models in practice. This was done by fitting both models to the Holstein UK data and by comparing the results.

**Material and methods** Data was type classification records scored by field officers coded as 20, 39, 46 & 48 in the years 2006 to 2009 and comprised 80783 first lactation cows. Three representative traits were selected for use in the study: condition score (1=thin, 9=fat), bone quality (1=coarse, 9=refined) and milking ease (1=slow, 9=fast). The cows were progeny of 5060 sires and a sire pedigree with 12765 animals over a maximum of 15 generations was included in all models. The data comprised 3047 distinct herds and 6148 herd visits, where herd visit groups cows classified on a farm at a particular time. The cows were of age 20 to 50 months at the time of scoring and were at a stage of lactation of 1 to 13 months. The analysis was performed using ASReml 3.0 (Gilmour *et al.*, 2009). A mixed model was assumed with fixed and random effects. The fixed effects were age of the animal at the time of scoring in months, stage of lactation in months, effect of field officer and whether the animal was Holstein ( $\geq 12.5\%$  Holstein genes) or Friesian ( $< 12.5\%$  Holstein genes) (0 or 1). Random effects were sire and herd visit. The dams were assumed to be unrelated. A probit model was used for the threshold analysis. The heritabilities from the linear model were transformed to the underlying scale so that the heritabilities from the two models could be directly compared (Gianola, 1982). In addition, to make model comparison easier data was simulated with a sire variance of 0.1 and a phenotypic variance of 1. This data were analysed with both models which included one random effect (sire) and no fixed effects.

**Results** The scores given to cows by the field officers were predicted for various explanatory variables keeping the other factors constant. These probabilities when plotted as a bar plot showed that probabilities of scores given by the field officers differ for all 3 traits analysed in the study (see Figure 1 for a bar plot for bone quality). These results indicate that the field officers were using different thresholds while scoring the animals. Heritabilities from the two models are given in Table 1. If the threshold model is considered as the “gold standard”, the results would indicate a tendency for the linear model to overestimate heritability. However, given that the method of fitting the threshold model involves approximations to the likelihood (Gilmour *et al.*, 2009), it is also possible that the linear model is closer to the correct answer, whilst the threshold model underestimates heritability. However, the results from the simulation study show that heritabilities from the threshold model are closer to the parameters used when simulating the data. The estimated breeding values (EBVs) calculated for the animals by the different models are highly correlated (Table 1).



**Figure 1** Bone Quality scores from different field officers (FO)

**Table 1** Heritabilities from the two models, estimated from both real data and simulated data

Traits	Real Data		Correlation between EBVs	Simulation study	
	Linear $h^2 \pm s.e$	Threshold $h^2 \pm s.e$		Linear $h^2 \pm s.e$	Threshold $h^2 \pm s.e$
Condition score	0.34 $\pm$ 0.03	0.27 $\pm$ 0.02	0.976	0.47 $\pm$ 0.08	0.38 $\pm$ 0.06
Bone quality	0.31 $\pm$ 0.03	0.28 $\pm$ 0.02	0.985	0.46 $\pm$ 0.06	0.41 $\pm$ 0.06
Milking ease	0.13 $\pm$ 0.01	0.09 $\pm$ 0.01	0.969	0.40 $\pm$ 0.06	0.38 $\pm$ 0.06

**Conclusions** There are notable differences in the scoring between field officers. There is little difference between the linear and threshold models in genetic parameter estimates and EBVs. However, in the simulation study results from the threshold model are closer to the simulated values than those from the linear model.

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## Genetic and non-genetic factors influencing *Fasciola hepatica* antibodies in milk in UK Holstein Friesian cattle

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**Introduction** Infection by *Fasciola hepatica*, a parasite of cattle and sheep, is widespread in temperate regions. Infection in cattle generally causes subclinical disease with negative effects on production such as lowered growth rates and weight gain, lowered milk yield and reduced fertility (Charlier *et al.*, 2007). Genetic selection in dairy cattle for production traits is commonplace but now increasingly animals are selected for improved health and welfare traits. Research in sheep has been carried out into selecting animals for resistance and/or resilience to nematode infection however similar research for helminths in cattle is limited (e.g. Morris *et al.*, 1997). The aim of this work was to estimate the genetic variation, heritability and effects of other non-genetic factors on *F. hepatica* specific IgG concentration in milk.

**Material and methods** Milk samples were collected from 1244 Holstein-Friesian cattle in 223 commercial dairy herds (2002-2003) during their first (82%) or later lactations (2-12). Milk samples were assayed for IgG antibodies against *F. hepatica* by ELISA (Salimi-Bejestani *et al.*, 2007). The results were expressed as percent positivity (PP) (PP = mean OD of sample / mean OD of positive control sample x 100). The PP value was log transformed to improve the normality of the distribution. A univariate mixed animal model was fitted to the data using ASREML software and the variance components estimated. The inclusion in the model of various fixed, random and nested effects was investigated (herd, area of country, year, month and season of sample, sample time, lactation number and days postpartum). The final model included the fixed effects of days postpartum, lactation number (1, 2, 3, 4 & ≥5), year (n=2), season of sample (n=4), season within year, month (n=12), herd (n=223) and the random effect of the animal by way of the pedigree file (n=11,298; Edinburgh Genetic Evaluation Service, Scottish Agricultural College, Penicuik, UK).

**Results** The fixed effects of days postpartum, lactation number, year, season, season nested within year, month and herd all effected PP ( $P < 0.05$ ). The highest PP values were seen during October and November. This pattern is thought to reflect the ingestion of infective metacercariae, which are present on pasture and whose concentrations typically are at their highest between September – November which agrees with seasonal patterns of infections found in other studies (Charlier *et al.*, 2008). The PP was highest in lactation 1 and declined with lactation. PP was not found to be heritable in this study ( $h^2 \pm \text{s.e.}; 0.01 \pm 0.07$ ) which is in contrast to a previous study of the same data investigating the antibody response to *Ostertagia ostertagi* which was found to be heritable ( $h^2 \pm \text{s.e.}; 0.13 \pm 0.12$ ;  $P < 0.05$ ; Hayhurst *et al.*, 2010) this may be due to lower genetic variation in *F. hepatica* immunity or to it being harder to elucidate amongst the many significant environmental factors affecting it. *F. hepatica* specific IgG is a measure of exposure to the parasite, but not a good measure of protective immunity in cattle; by selecting a better correlate of protective immunity, it may be possible to quantify genetic variation in the development of the immune response to *F. hepatica* infection in cattle.

**Conclusions** This study found that *F. hepatica* IgG levels in milk are significantly affected by many non-genetic factors which is of great importance for future genetic studies on responses to this trematode in order to minimise unwanted variation. Although in this study genetic variation was not apparent, further investigation of a larger dataset with fewer environmental variables is needed to accurately quantify the genetic variation in the immune response to *F. hepatica* in cattle.

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## Review of current knowledge of equine demography and movements within Great Britain

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**Introduction** Increased international movement of horses, horse products (plasma, semen), industry personnel and worldwide climatic changes represent an increased risk to the worldwide horse population of exposure to exotic, novel or notifiable diseases (Herholz *et al.* 2008). The recent outbreaks of Equine Infectious Anaemia (EIA) and Equine Influenza (EI) and the threat of African Horse Sickness (AHS), mandate the need for data to improve disease preparedness within Great Britain (GB). Understanding the potential for the spread of infectious diseases at the national level depends both on importation (the risk of introducing disease) and population demographics and movements (the potential to spread disease); optimal epidemic preparedness requires both. The objective of this study is to collate and describe existing equine demography data and identify data gaps in the current knowledge of equine demography within GB.

**Material and methods** Existing data on equine demography and equine movements were requested and obtained from identified equine stakeholders, agricultural census data and Trade Controls and Expert System (TRACES). Data on the distribution of 'leisure' horses, which are not routinely recorded, were obtained from horse owners via an ongoing online questionnaire. Data on equine owner location were also obtained from the National Equine Database (NED). All data obtained were anonymous and summarized at the resolution of postcode area and geographical region within GB and subsequently mapped for each population subgroup (agricultural horses, donkeys, racehorses, competition horses and leisure horses). For the purposes of this study, the term horse is used to represent all horses, ponies, donkeys and other hybrids.

**Results** Preliminary results suggest that horses are concentrated within the southwest (18.7%), south central England (12.5%) and East Anglia (12.2%). Leisure horses are also concentrated within these areas; agricultural horses are mainly located in the southwest, whereas the location of racehorses is highly clustered in south central, northeast England and East Anglia. The recorded destination for imports of foreign horses is also highest for East Anglia and central England.

The majority of questionnaire respondents either kept their horses on their own premises (36%) or DIY livery yards (35%). The majority of horses not kept on their own premises are kept within 5 miles of home (22%). 11% of owners kept their horse more than 50 miles from where they lived. Of the questionnaire respondents, 23% of horse owners had a foreign passport for equidae residing in GB. Unless owners register correct address details for these horses in the NED, the geographical location of these animals (within GB) is unknown.

**Conclusions** Preliminary analyses of the data collected so far provide valuable insights into previously unknown attributes of the equine population of GB. Inclusion of this completed dataset with all other available data on equine demography and movements from multiple sources will be combined to parameterize mathematical models that will be used to predict the impact of disease incursion into different regions of GB. The online survey is ongoing and will greatly improve the validity of models of equine disease spread in GB.

**Acknowledgements** Defra and the Institute for Animal Health for funding this study. RRK is funded by a Wellcome Trust Senior Research Fellowship.

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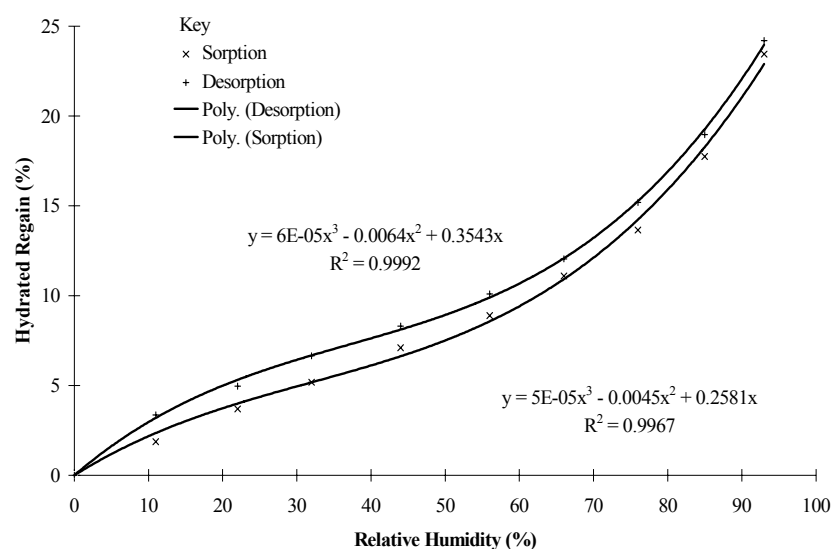
## Determination of the sorption and desorption isotherms of donkey hoof horn

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**Introduction** The moisture content of hoof horn is believed to affect its function, quality and mechanical properties. To investigate the hydration of any substrate the first step is to determine the water vapour sorption and desorption isotherms, which reflect the interaction of the chemical structure of the material and the water molecules at a given temperature and relative humidity (RH). These sigmoidal shaped isotherms have been determined for other keratinous materials but not for donkey hoof horn. The aim of the present study was therefore to identify the sorption and desorption isotherms for donkey hoof horn.

**Material and methods** Hoof clippings were obtained from the midline dead centre (MDC) of the left fore limb of 10 donkeys from The Donkey Sanctuary, Sidmouth, Devon. Samples were divided into 10 full hoof wall depth (HWD) sections of 1.5 mm x 1.5 mm and labelled 1 (most lateral) -10 (most medial) according to the position at the MDC. Samples 1-5 were used for sorption tests. Samples 6-10 were used for desorption tests. A variety of RH environments (11%, 22%, 32%, 44%, 56%, 66%, 76%, 85% and 93%) were produced by saturated salt solutions and were kept at room temperature (23°C). Phosphorus pentoxide was used to produce the environment at 0% RH. Sorption samples were weighed to ascertain their *in vivo* mass, dried over phosphorus pentoxide for 10 days and reweighed to ascertain dry mass. One sample from each was allocated to the different RH environments and left for 10 days in order for equilibrium to be achieved. Samples were then reweighed to ascertain hydrated mass. The hydrated regain was calculated for all the samples. The desorption samples were weighed to establish their *in vivo* mass and were then placed in their respective



**Figure 1** Sorption Desorption Isotherms for Donkey Hoof Horn

NB: The polynomial curves have been added for ease of reference only

environments. The masses were recorded daily for 10 days until an equilibrium hydrated mass was reached. These were dried over phosphorus pentoxide for 10 days and, again, the hydrated regains were calculated. The mean values determined at each RH for both sorption and desorption data were then plotted against the respective RHs to produce a sorption and desorption isotherm. The results were analysed statistically using Minitab (version 10.51xtra, Minitab Corporation, USA) and the normality of data was established using the Minitab normality test. Subsequently the probability of there being significant differences was tested by the non-parametric Mann-Whitney *U* Tests as the majority of data were non normally distributed. Relative hysteresis was calculated on a dry matter basis as (desorption - sorption) x 100 and then divided by sorption (Jeffries 1960).

**Results** The sorption and desorption isotherms are shown in Figure 1. An increase in RH produced an increase in hydrated regain. Mann-Whitney *U* tests revealed there were significant differences between sorption and desorption samples for all environments ( $P < 0.05$ ) except for those at 93% RH ( $P > 0.05$ ) resulting in the relative hysteresis decreasing as the RH increased *e.g.* there was a 79% relative hysteresis for 11% RH and a 3% relative hysteresis for 93% RH.

**Conclusions** The results for donkey hoof horn showed typical sigmoidal shaped isotherms and were similar to that previously shown for other keratinised tissues. It is important to know whether sorption or desorption has occurred when testing as differences in moisture content result in a very different mechanical testing results (Hopegood 2002). This study reflects implications for management of the donkey's environment as, in nature, hysteresis may be considered as a built-in protective mechanism against extremes, such as loss of water due to a dry atmosphere (Kapsalis 1981). This may therefore be extremely useful for donkeys living in hot climates.

**Acknowledgements** The authors gratefully acknowledge funding for studentships for LH and SC.

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## The validation of infrared thermography as a non-invasive tool to assess welfare in the horse (*Equus caballus*)

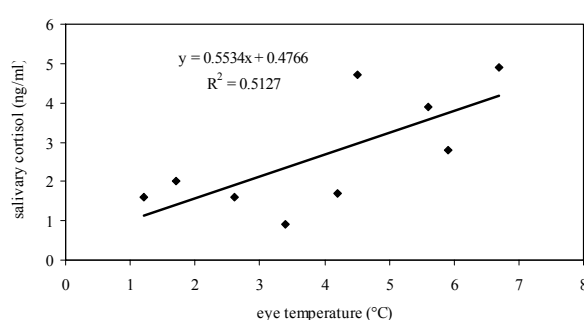
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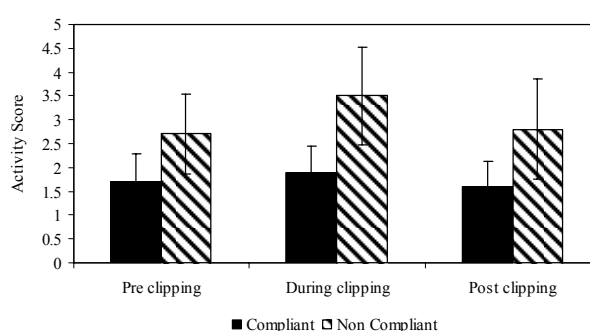
**Introduction** Domestication has removed horses from their natural environment and placed them into situations they may find distressing. The resultant physiological response has a protective role however research suggests that if this response becomes repetitive or chronic, stress related disease and behavioural problems may emerge. Existing methods to identify and assess the stress response have limitations. The accepted measure of the stress hormone cortisol can be expensive and time consuming and behavioural assessment is often subjective. Recent studies using infra-red thermography (IRT) have shown that eye temperature may be an indicator of distress in humans, monkeys, cows and wapiti. The principle aim of this study was to investigate the thermal response of the equine eye to a short term aversive stimulus when compared with a currently accepted measure (cortisol response) in order to validate IRT as a non-invasive and reliable measure of the physiological stress response.

**Material and methods** Clipping was used as a routine potentially aversive husbandry procedure. Horses (n=10) included five who were consistently behaviourally compliant with the clipping procedure and five known to consistently show behavioural signs of distress during clipping. Each horse was exposed to ten minutes of sham clipping. Clippers were placed on the cranial crest of the neck and underbelly (two minutes each side), flanks (two minutes each side) and each front leg (one minute per leg). The horses could feel and hear the clippers but no hair was removed. Both the handler and the operator remained silent for the duration of the sham clip and at no time verbally or physically comforted, coaxed or rewarded the horse. Thermal images were taken every five minutes from ten minutes pre onset of clipping until thirty minutes post onset of clipping. Images were captured at a 90° angle, 1metre from the horse. Saliva was sampled for cortisol analysis at ten and five minutes pre clip and then every ten minutes until forty minutes post onset of clipping. The procedure was video recorded for each horse for behavioural analysis. The footage was divided into three segments which were pre clipping, during clipping and post clipping. A five minute sample from each section for each horse was extracted from the footage and a pre determined activity score assigned. A mean activity score for the compliant and non-compliant groups was calculated for pre-clip, during clip and post-clip. A control study was carried out on a separate day using the same horses and same experimental design however; the potentially distressing stimulus (presence of the clippers) was removed.

**Results** There was a significant increase in both eye temperature ( $p<.001$ ) and salivary cortisol ( $p<.005$ ) in response to the aversive stimulus with a positive correlation between the two measures ( $p<0.5$ ) (Figure 1). These physiological changes occurred in both groups with no significant difference between the compliant and non compliant horses. There was a significant difference in activity level between the compliant and non compliant horses for the pre clipping ( $p=.03$ ), during clipping ( $p=.008$ ) and post clipping video segments ( $p=.01$ ) with the non compliant horses displaying higher activity (Figure 2). The results of the control study showed no significant change in eye temperature or salivary cortisol in either group. In addition there was no significant difference in activity level for the pre clipping, during clipping or post clipping video segment in either group.



**Figure 1** Positive correlation between increase in salivary cortisol and increase in eye temperature



**Figure 2** Activity score of compliant and non compliant horses

**Conclusion** IRT is able to identify eye temperature change as a result of the physiological stress response in horses. Both compliant and non compliant horses displayed physiological changes that indicated they were distressed. However, activity levels in the compliant horses did not change at onset of clipping and were lower than the non compliant horses. It is possible that the compliant horses were masking distress as a survival mechanism or have been trained not to respond behaviourally to procedures they find aversive despite being physiologically compromised. IRT offers a non-invasive, instant measure of the stress response and is able to measure physiological changes that cannot be masked. It can offer a more reliable assessment of how domestic horses perceive management practices and training procedures and allow us to alter them if necessary in order to improve welfare.

## Comparison of the zonal moisture content of the *Stratum medium* of donkey hoof horn to the zonal moisture content of the *Stratum medium* of horse hoof horn

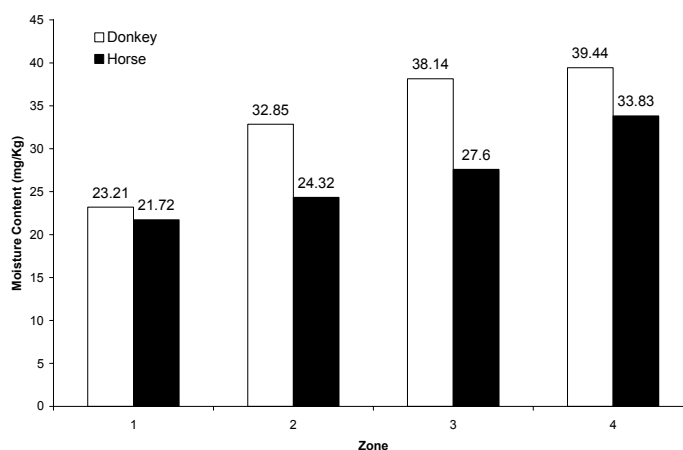
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**Introduction** The moisture content (MC) of hoof horn is believed to affect its function, quality and mechanical properties. The zonal MC of donkey hoof horn has not been identified. Various authors have found that this changes for horse hoof horn (Leach 1980; Douglas *et al.* 1996) but comparisons are difficult as methods vary. The aims of the present study were therefore to identify the zonal MCs across the *Stratum medium* (SM) for donkey hoof horn and compare it to those found for horse hoof horn.

**Material and methods** Hoof clippings were obtained from the midline dead centre (MDC) of the left fore limb of 10 donkeys. Horse samples were obtained from the left forelimb from 16 animals. One sample of 1.5 mm x 1.5 mm by the hoof wall depth was removed from each MDC section. The white line was removed and samples were then wrapped immediately in Parafilm (Parafilm "M" Laboratory Film, American National CanTM, CT 06836, USA) to maintain water content and stored at 4°C. Each sample was then divided equally into four zones. Zone 1 being the outer SM, zone 4 being the inner SM. The MCs for each zone were assessed following drying of the samples over phosphorus pentoxide. Again, MCs were calculated as a percentage of fresh mass. The results were analysed statistically using Minitab (version 10.51xtra, Minitab Corporation, USA) and the normality of data was established using the Minitab normality test. Subsequently the probability of there being significant differences in MCs obtained from the different methods was tested by the non-parametric Kruskal-Wallis and Mann-Whitney *U* Tests as the majority of data were non normally distributed.

**Results** The zonal MC results for donkey hoof horn (Figure 1) were non normal ( $P < 0.05$ ) and therefore median values were used to describe the data set. There was a median MC of 35 mg/Kg for the full data set. There was a dorso-palmar increase in median hoof MC. There were significant differences between MCs for individual zones ( $P < 0.01$ , Kruskal-Wallis) (Figure 1). Mann-Whitney *U* tests indicated there were significant differences between the combination of all zones ( $p < 0.01$ ) except for between zones 3 and 4 ( $P > 0.05$ ). For horse hoof horn the full HWD MC was 25 mg/Kg with zonal MCs for zones 1-3 being normally distributed but zone 4 was non-normally distributed. There was a dorso-palmar increase in median hoof MC (Figure 1). There was no significant difference between zones 1 and 2 ( $P > 0.05$ , Mann-Whitney *U* test). There were, however, significant differences between all other combinations of zones ( $P < 0.05$ , Mann-Whitney *U* test). However, there was a significant difference between the median MCs of zone 2, zone 3 and zone 4 for both donkey and horse hoof horn ( $P < 0.01$ , Mann-Whitney *U* test) but not for zone 1.



**Figure 1** Comparison of Zonal Moisture Contents for Donkey Hoof Key: \*\* denotes significant differences between zones at  $P < 0.01$

**Conclusions** Significant differences exist in MC between the zones of the SM of donkey and horse hoof horn. The more upright hoof in the donkey may cause greater stress in the donkey hoof wall compared to the horse. This problem may be counteracted by the mechanism of the higher MC in the inner hoof wall of the donkey when compared to the horse. Both of these facts may indicate that donkey hoof horn receives more moisture from the underlying dermis than horse hoof horn. However, reasons for this are unclear. Perhaps it is a self-regulating mechanism by which the possible concussive effect of a more upright hoof of the donkey is counteracted by the actual moisture, as this factor is known to influence the mechanical properties of hoof horn. However, it must be borne in mind that the zonal boundaries were slightly different between the two data sets as the wear of the samples of horse hoof had not been taken into account. These results should also be viewed with caution as the samples were taken from different yards.

**Acknowledgements** The authors gratefully acknowledge funding for studentships for LH and SC.

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## Identification of the influence of moisture content on the mechanical properties across the *Stratum medium* of donkey hoof horn

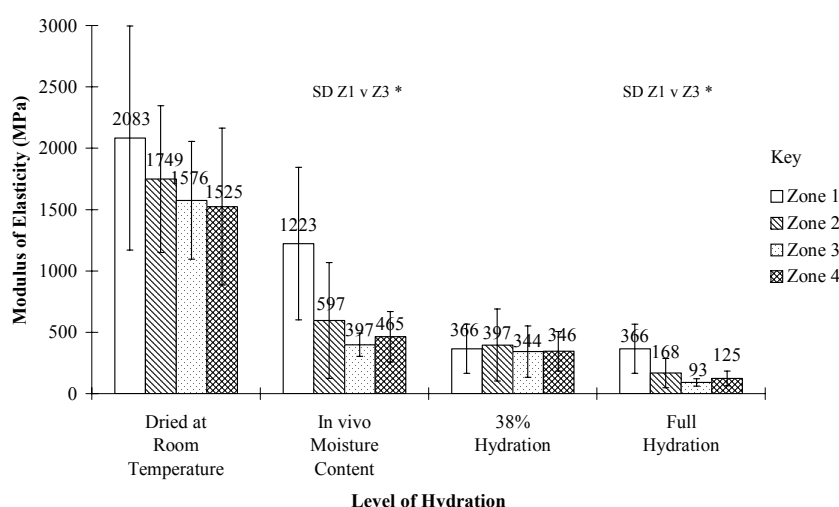
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**Introduction** Moisture content (MC) is known to affect the mechanical properties of horse hoof horn (Douglas *et al.* 1996). However, the mechanical properties of the different regions of the hoof wall depth (HWD) of donkey hoof horn, together with the influence of moisture content on these properties, have not been investigated previously. Moisture contents have also been shown to be lower in the outer hoof wall compared with the inner hoof wall. It is likely therefore that the resultant moduli for each zone will also be different. Mechanical properties of neither donkey or horse hoof have been assessed by taking the effect of MC out of the equation. The aims of the present study were therefore to assess the influence of moisture content on the mechanical properties of donkey hoof horn and to assess whether the mechanical properties across the hoof wall depth are solely related to moisture content.

**Material and methods** Hoof clippings were obtained from the midline dead centre (MDC) of the left fore limb of 5 donkeys from The Donkey Sanctuary, Sidmouth, Devon. The white line was removed and samples were cut to 15 mm in length, milled to 1 mm depth and wrapped immediately in Parafilm (Parafilm "M" Laboratory Film, American National CanTM, CT 06836, USA) to maintain water content and stored at 4°C. Samples were divided at 25%, 50% and 75% HWD and then tested by 3 point bending at the following moisture contents: *in vivo* moisture content, following full hydration, at 38 mg/Kg hydrated regain and following drying at room temperature. The hydrated regain previously found for zones 1-4 was 38, 57, 69 and 75 mg/Kg (Hopegood 2002). As the samples were fully hydrated it would not have been possible to reproduce an hydrated regain of 75 mg/Kg in zone 1 as the maximum hydrated regain had been shown to be 38 mg/Kg in that zone. It was therefore decided to reproduce an hydrated regain of 38 mg/Kg for all 4 zones by equilibrating and dehydrating zones 2-4 to appropriate levels. The results were analysed statistically using Minitab (version 10.51extra, Minitab Corporation, USA) and the normality of data was established using the Minitab normality test. As the data were normally distributed ( $P>0.05$ ), subsequent analyses were carried out using one way ANOVA and Student's *t* tests.

**Results** The mean moduli of elasticity at the different levels of hydration are shown in Figure 1. The force extension curves for the zones showed a Hookean relationship with a decrease in moduli with increasing hydration as previously shown for full HWD samples (Hopegood 2002). There was a significant difference for some of the zones for those at an *in vivo* MC and those fully hydrated ( $P<0.05$ ) (Figure 1) but not at the other levels of hydration ( $P>0.05$ ). The large standard deviations should be borne in mind when assessing the data.



**Figure 1** Mean moduli of elasticity of donkey hoof horn at different levels of hydration. Error bars = standard deviations

**Conclusions** The force extension curves showed a Hookean relationship with the moduli generally decreasing in a dorso-palmar direction with increasing MC. This mechanism may afford the contents of the hoof a protective gradient of stiffness by these levels of hydration contributing towards the transfer of stress across the HWD. The moduli for samples tested at 38 mg/Kg hydrated regain were normalised for MC and the

results indicated that the actual mechanical properties, when MC has been removed from the equation, across the HWD did not vary. Again, this has not been reported for horse hoof horn. The standard deviations for the samples should, however, be noted as this may explain the lack of a difference between zones. This indicates that MC does indeed have a great role to play in influencing the mechanical properties, and thus the functioning, of the hoof wall. The results should, however, be interpreted with caution owing to the limited number of samples available.

**Acknowledgements** The authors gratefully acknowledge funding for studentships for LH and SC.

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# A comparison between industry standard method of semen analysis and the use of SpermVision™ for the evaluation of the measureable parameters of chilled stallion spermatozoa

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**Introduction** The efficient use of chilled semen in equine breeding programs is reliant upon correct evaluation of spermatozoa prior to cooling and insemination (Squires, 2009). The industry standard method of semen assessment employs a light microscope to determine motility (M), progressive motility (PM), concentration (CON) and the morphology of ejaculates (Varner, 2008). However, this method is naturally very subjective and results vary between laboratories and individuals (Iguer-ouada and Verstegen, 2001), highlighting the need for an objective method of evaluation. Computer assisted sperm analysis (CASA) systems, such as SpermVision™ (Minitüb, Germany), have been developed to provide repeatable, objective, accurate and timely results (Schäfer-Somi and Aurich, 2007). One of the unique features of SpermVision™ is the three calibrations available for the evaluation of equine semen, each using different values to assess motility parameters. CASA systems are costly and as such are limited to use by researchers in a laboratory setting, thus making them unavailable to the breeder, who must have confidence in the routine evaluations they perform on the semen from their stallions, prior to shipping for artificial insemination. The aim of the present study was to compare the SpermVision™ (SV) system with the industry standard, visual method for the determination of concentration, motility and progressive motility of chilled stallion semen.

**Material and methods** Ejaculates (n=6) were collected from a healthy fell pony stallion and extended 3:1 with Kenneys skim milk extender before chilling in a neopor disposable semen shipper until 5°C. All equipment was heated to 38°C prior to assessments which were conducted on a heated stage. Manual determination of concentration was carried out using the gold standard improved Neubauer haemocytometer and a phase-contrast Zeiss Axioskop 40 microscope (Carl Zeiss Ltd., Hertfordshire) and viewed at x200. At least 500 cells were viewed over 8 fields of a 20µm Leja chamber (Leja Products B. V., Nieuw-Vennep, the Netherlands) and graded as immotile, motile or progressively motile. CASA evaluations were carried out using SpermVision™ version 3.5.6.3 following manufacturers guidelines, in under 60 seconds at 30 frames/second over 8 frames. At least 500 cells were counted by SV for each sample using each equine calibration. All methods of evaluation were repeated three times for each ejaculate. One way ANOVA was performed alongside the Bonferroni *post hoc* test, using SPSS for Windows v. 16.

**Results** No significant differences were seen between SV and a manual determination of CON and PM (Table 1) but a difference was noted in M ( $p<0.05$ ). A highly significant correlation ( $r=0.76$ ,  $p<0.01$ ) was found between a visual analysis and SV for CON however no correlations were found between the motility analyses.

**Table 1** Means  $\pm$  SEM for the measurable parameters for SV calibrations (A,B and C) and the manual (MAN) analysis

Method of analysis	CON (bil/ml)	M (%)	PM (%)
A	0.16285 $\pm$ 0.0263	36.92 $\pm$ 3.34	22.30 $\pm$ 2.16
B	0.1593 $\pm$ 0.0254	45.31 $\pm$ 3.65	32.81 $\pm$ 3.00
C	0.1581 $\pm$ 0.0295	41.43 $\pm$ 3.63	27.74 $\pm$ 2.77
MAN	0.1680 $\pm$ 0.0120	52.01 $\pm$ 3.05	30.19 $\pm$ 3.33

**Conclusion** The difference found between the subjective motility analysis and that of SV was expected due to the limitations of the human eye and the high number of cells involved. These results show that the use of the gold standard haemocytometer is just as accurate as the CASA system in the concentration estimate of an ejaculate. The results from the present study are comparable to other studies conducted on similar CASA systems (Klimowicz *et al.*, 2008; Rijsselaere *et al.*, 2003). Progressive motility is used by the majority of the stud industry as a measure of the quality of semen and these results confirm that it is acceptable for experienced technicians within the stud industry to continue to evaluate samples visually, until a more commercially available, objective method is developed.

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## **A comparative antibody study of the potential susceptibility of Thoroughbred and non-Thoroughbred horse populations in Ireland to Equine Influenza Virus**

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**Introduction** In Ireland horses may be protected against Equine Influenza Virus (EIV) as a result of natural exposure or vaccination. Current mandatory vaccination programmes are targeted at highly mobile horses. A correlation between antibody levels as measured by single radial haemolysis (SRH) and protective immunity against EIV has been established (Newton *et al.*, 2000; Mumford, 2001).

**Material and methods** Blood samples were collected from Thoroughbred weanlings, yearlings, racehorses and broodmares, teaser stallions and non-Thoroughbred horses. Antibodies against EIV H3N8 and H7N7 were measured by SRH. SPSS version 16.0 for Windows (Chicago, Illinois, USA) was used to analyse the data. Mean H3N8 antibody values were calculated from SRH results obtained against the subtype 2 antigens A/eq/Kildare/92, A/eq/Newmarket/2/93 and A/eq/South Africa/4/03. A significance level of  $p < 0.05$  was used for all statistical tests.

**Results** The order of susceptibility to Equine Influenza (EI) in the populations examined in Ireland was as follows: Thoroughbred weanlings > Teasers > non-Thoroughbred horses and ponies > Thoroughbred yearlings > Thoroughbred horses in training > Thoroughbred broodmares. The H3N8 antibody levels of the weanlings, yearlings, broodmares and horses in training were similar to their H7N7 antibody levels suggesting that their antibodies were primarily vaccinal in origin. The teasers and non-Thoroughbreds had higher H3N8 antibody levels than H7N7 antibody levels suggesting that the majority of seropositive horses in these populations had been exposed to H3N8 by natural infection.

**Conclusions** Weanlings, teasers and non-Thoroughbred horses were identified as most susceptible to EIV. The results suggest that it would be advisable that weanlings are vaccinated prior to attendance at public sales, that teaser stallions are vaccinated prior to each breeding season and that mandatory vaccination be implemented for participation in non-Thoroughbred events.

**Acknowledgements** All of the experimental work was funded by the Department of Agriculture under the National Development Plan and carried out at the Irish Equine Centre. The results will be submitted as part of a PhD thesis by Sarah Gildea to the University of Limerick.

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## Modulation of endothelial cell signalling and function by parstatin, a putative anti-angiogenic peptide

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**Introduction** The ability to manipulate angiogenesis is of great therapeutic importance in a number of disease settings. Angiogenesis requires Endothelial cells (EC) proliferation, migration and differentiation, functions which are orchestrated by angiogenic factors produced by ECs and other cell types. The cyclooxygenase-2 (COX)/prostanoid axis in ECs has recently been highlighted as an important signalling pathway in angiogenesis as the induction of COX-2, and consequent prostanoid production, is noted in response to many pro-angiogenic stimuli. Parstatin, a peptide generated upon cleavage of the G-protein coupled receptor protease-activated receptor-1 (PAR1) has recently been reported to inhibit agonist-induced EC signalling and angiogenesis. The studies described herein have tested the hypothesis that parstatin suppresses key signalling events in human umbilical vein ECs (HUVEC) stimulated by exposure to a range of pro-angiogenic mediators.

**Material and methods** HUVEC were isolated as previously described.

**Western blotting** was used to determine the effect of parstatin on EC signalling. For incubation periods of less than 3 hours cells were initially pre-treated with parstatin (MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLDPR) or scrambled parstatin (LRTNASLLVPFLTARAKSSGTREAADPPRLMCLRLPLARRCG) for 1 hour and subsequently treated for 10 minutes with vehicle, thrombin (0.1-10 U/ml), PAR1-agonist peptide (AP) TFLLR-amide (10 µM), PAR2-AP 2-furoyl(2f)-LIGKV-OH (10 µM), PAR4-AP AYPKGF-amide (50 µM), VEGF (25 ng/ml), PGE<sub>2</sub> (0.5-10 µM) or the stable PGI<sub>2</sub> analogue, iloprost (0.1-1 µM). Preparation of cell lysates was subsequently carried out and blots were probed for p-Akt (ser473), panAkt, p-ERK1/2, ERK1/2, p-VEGFR2 (vascular endothelial growth factor receptor 2) and VEGFR2. For treatment periods of over 3 hours, co-incubation studies were carried out. Blots were probed for COX-1, COX-2 and B-actin. Densitometric analysis of blots was carried out using Quantity One software.

**Matrigel assay** was used to assess induction of capillary-like tube formation in ECs. 15 000 HUVEC were plated into each well of a 96-well plate which had been pre-coated with 40 µl growth-factor reduced Matrigel. Tube formation was quantified 6-8 hours after exposure to the appropriate treatment. Tubes were then fixed with paraformaldehyde, stained and images were captured using a Leica DMIRB inverted light microscope and IM500 imaging program. Interbranch number, total branch length, and total tube area were taken as indicators of tube formation and were quantified using a tailor-made macro (Leica QWin Imaging Software).

**PGE<sub>2</sub> release assay:** Cells were plated on 24-well tissue culture plates and cultured to confluence. Appropriate treatments were added and incubated for 1 or 8 hours. The supernatants were then removed and analysed for PGE<sub>2</sub> using a Homogeneous Time-Resolved Fluorescence assay.

**Statistical analysis:** A paired student's T-test or a one-way repeated measures ANOVA with Bonferroni post-hoc test was used, as appropriate, to assess statistical significance within pre-treatment/treatment groups using graphPad prism version 5. *p* values of less than 0.05 on two-sided tests were considered statistically significant.

**Results** Parstatin was shown to significantly decrease capillary-like tube formation on growth-factor reduced Matrigel. Parstatin, but not scrambled control peptide, inhibited VEGF- and thrombin-, but not PAR-AP-induced ERK1/2 phosphorylation and COX-2 induction. Reduced PGE<sub>2</sub> synthesis was also noted. Parstatin was further shown to decrease activation of ERK1/2 in iloprost- but not PGE<sub>2</sub>-stimulated cells. Parstatin treatment additionally modulated Akt phosphorylation resulting in two distinct HUVEC-isolate dependent responses. In some EC isolates parstatin alone strongly enhanced Akt phosphorylation and did not modify agonist-stimulated Akt activation. Other cell isolates showed no basal activation of Akt following parstatin treatment but the peptide suppressed VEGF-stimulated Akt phosphorylation. Further studies showed that parstatin reduced VEGF-mediated phosphorylation of VEGF receptor (R) 2 on tyr<sup>1175</sup>, a modification required for downstream activation of ERK1/2 and PI3k/Akt pathways and subsequently COX-2 induction. VEGFR2 phosphorylation (tyr<sup>1175</sup>) was not detected in cells exposed to thrombin, PAR-APs, PGE<sub>2</sub> or iloprost.

**Conclusions** Together, these studies show that parstatin limits pro-angiogenic signalling events in an agonist-selective manner. Inhibition of VEGFR2 activation could, in part, explain parstatin's suppressive actions on VEGF-stimulated signalling but this mechanism cannot account for its ability to reduce signalling by other agonists, suggesting multiple targets. Further study of these mechanisms may ultimately reveal precisely how parstatin regulates pro-angiogenic signalling and whether it has potential as a novel therapeutic.

**Acknowledgements** The authors would like to acknowledge Elaine Shervill for her skilled technical assistance and the midwives and patients at the Royal London NHS trust

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## Muscle fibre profile number and size distribution changes with age in *mdx* mice

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**Introduction** The *mdx* mouse is used extensively to investigate the pathogenesis of and potential treatments for Duchenne muscular dystrophy (DMD). Many studies investigating treatments to restore the missing dystrophin protein use estimates of muscle fibre number to calculate the proportion of dystrophin-positive fibres. In contrast to DMD boys it is thought that *mdx* mice do not exhibit significant muscle fibre loss. This study investigated whether muscle fibre profile number changes throughout the lifetime of the *mdx* mouse.

**Material and methods** *Tibialis anterior* (TA), *Rectus Femoris* (RF), *Extensor Digitorum longus* (EDL) and *soleus* muscles were collected from *mdx* mice of a range of ages (n=5-7 in each age group). Muscles were frozen in liquid nitrogen cooled isopentane and 10µm sections were cut on a manual cryostat. To facilitate counting, sections were immunostained for the extracellular matrix (ECM) proteins perlecan (MAB 1948, Millipore) or Laminin 1,1,1 (Sigma L9393). Fibre profiles were counted manually using Image J software and differences between age groups were compared using a one way ANOVA and Bonferroni's post-hoc comparison.

**Results** All 4 muscles exhibited significant changes in fibre profile number but in different patterns. Fibre profile number in the EDL dropped progressively from 3 weeks to 24 months, and was significantly lower at 12 and 24 months than at 3 weeks. Fibre profile number in the soleus was significantly higher at 6 months than at 3 weeks, 12 months or 24 months. At 24 months the number was significantly lower than at 3 weeks. Fibre profile number in the TA increased progressively between 3 weeks, 3 months, 6 months and 12 months then fell dramatically between 12 months and 24 months. Fibre profile number was significantly higher at 12 months than at 3 weeks, and significantly lower at 24 months than at 12 months. In contrast fibre profile number in the RF shows little variation until 12 months of age with a significant drop between 12 and 24 months. There was no significant difference between counts obtained with perlecan immunostaining and those obtained using laminin immunostaining. The distribution of muscle fibre profile sizes (minimum Feret's diameter) shows increased variation after the onset of pathology. The distribution of fibre profile diameters also varies with age and analysis of fibre loss relative to fibre size will be presented.

**Conclusion** In contrast to popular assumptions *Mdx* mice do in fact show changes in muscle fibre profile number with age, with all 4 muscles showing significant fibre loss in a muscle-specific pattern. These changes should be taken into account when assessing the effects of interventions such as exon-skipping to avoid under- or over-estimating their significance. Staining for ECM proteins facilitates fibre counting yielding higher and more accurate counts than conventional haematoxylin and eosin staining.

**Acknowledgement** This work was supported by a Wellcome Trust CRVT Fellowship.

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## Proteomic analysis of the early osteoarthritic equine cartilage secretome

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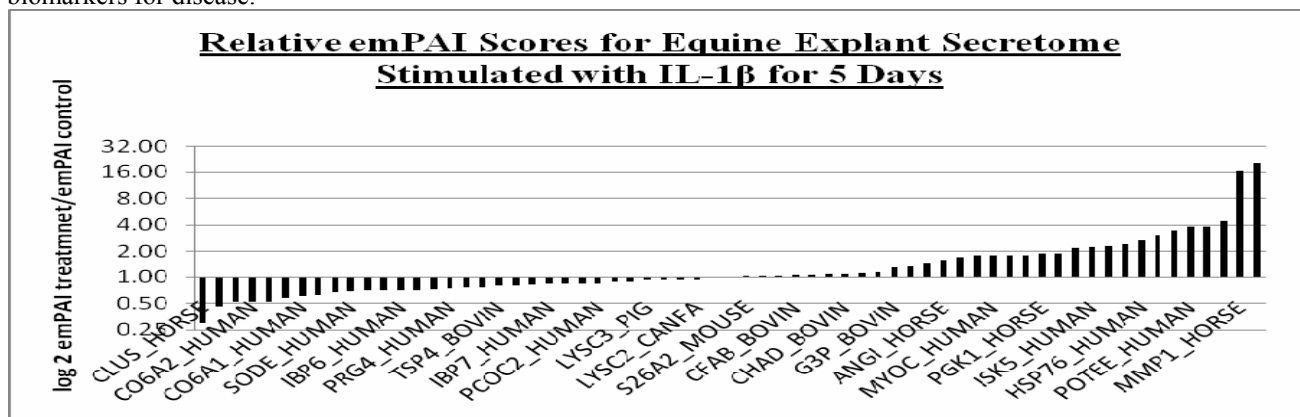
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**Introduction** Articular cartilage is composed of a single cell type, the chondrocyte, embedded within an extracellular matrix (ECM). Osteoarthritis (OA) is characterised by the slow degeneration of cartilage ECM. Pro-inflammatory cytokines such as IL-1 $\beta$  induce matrix degrading enzymes and these are significant in the pathogenesis of OA (Goldring and Goldring, 2004). The use of catabolic stimulants on explants, combined with proteomic analysis can offer an important approach to elucidate cellular mechanisms in cartilage degradation. In this study we conducted a comparative proteomic analysis of the cartilage explant secretome in the presence or absence of catabolic stimulation in order to ascertain the cellular mechanisms involved in early OA. Mass-spectrometry based proteomics provides a highly sensitive tool for exploration of this cartilage secretome.

**Material and methods** Cartilage explants were obtained from the distal metacarpal bones of horses acquired from an abattoir. Explants (n=8) were incubated for 5 days at 37°C in serum-free Dulbeccos' modified Eagles medium, either alone or in the presence of human recombinant IL-1 $\beta$  (10ng/ml). The explants media was analyzed for sulphated glycosaminoglycans (sGAGs) using a dimethylmethylene blue assay. In addition the media was reduced, alkylated and digested with trypsin. Digests were then resolved by liquid chromatography-mass spectrometry (LC-MS) using a linear ion-trap Orbitrap mass spectrometer (LTQ-Orbitrap Velos) with triplicate or quadruplicate technical replicates per donor. In addition media from 4 donors was separated by 1D SDS-PAGE and analysed using LC interfaced with a linear ion trap quadrupole (LTQ) following in-gel tryptic digestion. Data obtained was used to search for protein identifications against mammalian entries in the SwissProt database using MASCOT. Relative abundance data was provided using exponentially modified protein abundance index (EmpPAI) (Ishihama *et al.*, 2005) in order to estimate relative protein abundance in early OA compared to control secretomes.

**Results** Protein fractionation using both 1D SDS PAGE and LC-MS elucidated both qualitative differences and treatment induced changes in relative protein abundance between the secretome protein profiles of explants models of early OA versus controls. For more comprehensive protein profiling in solution digests of explant media were performed. Identifications were accepted when identified more than once in different study cohorts and in 3 or more donors. Additionally, proteins had a MASCOT score of greater than 28 with 2 or more identifying peptides, a confidence interval of 95% within a mass accuracy of 10ppm. By using the target-decoy sequence search strategy, the overall false discovery rate from the runs were estimated to be <1.8% which validates our MS/MS results. Although treatment for 5 days with 10ng/ml IL-1 $\beta$  did not augment significant GAG release there was a considerable difference in the profile following IL-1 $\beta$  treatment. The comparative emPAI analysis resulted in evidence of OA specific over-expression of several protein groups. 65 proteins were identified in both conditions allowing a relative quantitation using emPAI (Graph1). When emPAI treatment/emPAI control ratios were calculated, there was a significant increase in a number of proteins including vimentin, MMP1, fibulin-7, TR11B. A relative reduction was also evident in a number of proteins including clusterin, COL6A3, fibronectin and TIMP1. Additionally a number of proteins were identified that fulfilled our criteria in the secretome of control (36) and IL-1 $\beta$  treatment (139) only.

**Conclusions** This study enabled the characterisation and relative quantitation, at the protein level of the early equine OA secretome. We demonstrate some of the proteins altered in early OA including ECM structural proteins, pericellular matrix interaction proteins, inflammatory proteins and cartilage proteases. Our results improve the understanding of the equine OA secretome and provide a useful tool to identify candidate proteins for further study in the pathogenesis of OA or as biomarkers for disease.



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## Validation of a real-time PCR to detect *Coxiella burnetii* in placenta and abortion samples from ruminants, and application of the PCR as a tool to monitor an infected UK goat herd

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**Introduction** Q fever is a zoonotic disease caused by *Coxiella burnetii*, with ruminants providing the main reservoir for the bacterium. Infection in ruminants is mainly sub-clinical but can be associated with abortions. Infected animals excrete the bacteria in placenta, vaginal mucus, faeces and milk, causing environmental contamination where the bacterium survives in an infectious form. Infection is particularly associated with the inhalation of contaminated aerosols. Consequently, rapid diagnosis of ruminant abortions caused by *C. burnetii* is essential to provide early notification of related zoonotic risks and enable suitable control measures to be implemented. We have therefore undertaken validation of a real-time PCR to detect *Coxiella burnetii* in ruminant (sheep, goat and cattle) abortion material, including placental cotyledons. The validated real-time PCR has subsequently been applied as a tool to monitor an infected goat herd over a 31 month period.

**Material and Methods** Real-time PCR methods targeting the multi-copy insertion sequence IS1111 and the single copy *com1* gene were used to detect *C. burnetii* in ruminant abortion material. The primer and probe sequences had previously been applied to the detection of *C. burnetii* in human tissue samples (Harris *et al.*, 2000; Marmion *et al.*, 2005). Determination of the analytical sensitivity of the IS1111 real-time PCR was undertaken by cloning the target sequence of the PCR into a plasmid vector. A preparation of plasmid DNA was made, and this was used to introduce a known number of template copies into the real-time PCR reaction. A panel of organisms, including some known to be common causes of ruminant abortion, were used to check the analytical specificity of the real-time PCR. DNA extracts of the panel, which included representatives of the *Campylobacter* spp., *Brucella* spp, *Salmonella* spp., *Yersinia* spp., *Listeria* spp, *Toxoplasma gondii* and *Chlamydomphila abortus* were used as the template for the real-time PCR targeting IS1111. To determine the performance of the assay in the field situation, a panel of samples were collected through the VLA Regional Laboratory network between 2004 and 2008. The panel included 35 samples from ovine fetuses, 8 caprine and 8 bovine fetuses. The panel included placental cotyledons, fetal fluid, and fetal tissue samples. Additionally, samples were collected from a goat herd which had experienced an abortion storm, where the involvement of *C. burnetii* had been confirmed by immunohistochemistry. There was also widespread serological evidence of infection within the herd. Placental samples were collected from aborting and non-aborting goats, and fetal fluid and fetal tissue samples were also examined. Nucleic acid extractions were made from the samples using the Roche MagNA Pure LC robot and dedicated buffers contained within the MagNA Pure LC DNA isolation kit II. Each nucleic acid extract was tested with triplicate real-time PCR reactions targeting IS1111 and triplicate reactions targeting *com1*.

**Results** The IS1111 real-time PCR produced a standard curve that was linear over a range from 10<sup>8</sup> to 10 template copies. As this insertion sequence is present in at least 19 copies in the *C. burnetii* genome, the real-time PCR should be able to detect a single copy of the bacterial genome. No cross-reactivity was seen when the IS1111 PCR was used to test nucleic acid extracts from a panel of other micro-organisms. A total of 23 (25%) of the 93 field samples examined were positive using the IS1111 assay, and for 19 of these samples, the presence of *C. burnetii* DNA within the sample was confirmed using the *com1* assay. The positive samples included placental cotyledon, fetal fluid and fetal tissue samples. 62 placental cotyledon samples collected from the goat herd were tested using the IS1111 assay, and 57 (92%) of these tested positive for *C. burnetii*. Seven fetuses were also examined and 10 of 33 samples from these were positive, mainly from fluid and liver.

**Conclusion** The real-time PCR targeting IS1111 has been experimentally shown to be both a sensitive and specific method of detecting *C. burnetii* DNA. The test has been shown to be suitable for detecting the bacterial DNA in samples of caprine, bovine and ovine origin, and in placental cotyledon, fetal fluid and fetal tissue samples. When applied to field samples, the results obtained using the IS1111 real-time PCR could be confirmed for the majority of the samples using a PCR targeting an alternative region of the *C. burnetii* genome, the *com1* gene. The results of the goat herd study demonstrated a high prevalence of infection, regardless of the abortion status of the animals. This real-time PCR provides a useful diagnostic tool to aid in the rapid detection of ruminant abortions due to *C. burnetii*, allowing the zoonotic risk posed by these cases to be swiftly alerted to the appropriate authorities.

### Acknowledgements

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## Acute phase protein measurements in metabolic profiles in UK dairy herds.

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**Introduction** Metabolic profiles are used as a method for the routine evaluation of nutritional adequacy in dairy herds with the purpose of identifying production-limiting factors. However subclinical disease may also be present within the herd limiting production, and such issues may also confound the interpretation of metabolic profile analysis. The aim of the present study was to evaluate the diagnostic value of using Acute Phase Protein (APP) assays in metabolic profile screening programmes.

**Material and Methods** Blood samples were obtained from 12 commercial dairy herds as part of a routine nutritional monitoring programme using metabolic profiles as described by Macrae *et al.* (2006). To summarise, blood samples were taken from early lactation cows (EARLY: mean 21.7 days calved  $\pm$  1.1 SEM), mid lactation cows (MID : mean 102.9 days calved  $\pm$  2.4 SEM) and transition dry cows (DRY : mean 9.9 days from predicted calving date  $\pm$  1.2 SEM). Blood samples were taken from the coccygeal vein into oxalate (for glucose analysis), lithium heparin vacutainers (all other metabolites) or with no anti-coagulant (for APP analysis). Samples were analysed for metabolites within 24 hours of collection using an Instrumentation Laboratory IL600 wet chemistry system using reagents supplied by Randox (BOHB and NEFA) and Instrumentation Laboratory (all other metabolites). Samples were analysed for:  $\beta$ -OH butyrate (BOHB), glucose, NEFA, urea-N, albumin, globulin, magnesium and phosphate. Aliquots of serum were frozen and stored at  $-20^{\circ}\text{C}$  and subsequently analysed for the APP haptoglobin and Serum Amyloid A (SAA) using a haemoglobin binding method and an ELISA respectively in the laboratory of ReactivLab Ltd. (Eckersall and Bell 2010).

**Results.** Blood samples were received from 388 individual cows, and a summary of the APP results is given in Table 1. There was no significant difference in haptoglobin between the three groups, although the EARLY and MID lactation groups had significantly higher SAA results compared to the DRY group (Kruskal-Wallis test;  $P < 0.01$ ). Spearman Rank correlations showed a significant positive correlation between both APPs tested (haptoglobin and SAA) and NEFA, total protein and globulin ( $P < 0.01$ ). Haptoglobin and SAA were also significantly positively correlated ( $P < 0.01$ ). Haptoglobin was significantly negatively correlated with albumin and magnesium, and SAA was significantly negatively correlated with magnesium and phosphate ( $P < 0.01$ ).

**Table 1** Summary of haptoglobin and SAA results in the three groups of cows sampled.

Group	Number of samples analysed for haptoglobin	Mean Haptoglobin (g/l) $\pm$ SD	Median Haptoglobin (range) (g/l)	Number of samples analysed for SAA	Mean SAA (mg/l) $\pm$ SD	Median SAA (range) (mg/l)
EARLY	156	0.135 $\pm$ 0.29	0 (0 – 2.025)	157	78.4 $\pm$ 161.4	24 (1 – 1340)
MID	120	0.071 $\pm$ 0.16	0 (0 – 1.27)	121	59.8 $\pm$ 93.3	18 (1 – 520)
DRY	110	0.064 $\pm$ 0.17	0 (0 – 1.16)	110	36.9 $\pm$ 88.4	8 (2 – 815)

**Conclusions** These results show that there are significant positive correlations between the APPs haptoglobin and SAA, and existing crude measures of disease conditions in dairy cows such as albumin and globulin. Although there were no significant differences in haptoglobin concentrations between the three groups (in contrast to the study by Bionaz *et al.* 2007 which showed a rise in haptoglobin concentrations during the perparturient period), there was a significant rise in SAA in the milking cow groups compared to the dry cows. Correlations between APPs and measures of nutritional status are likely to reflect reduced Dry Matter intakes (observed by the low magnesium and phosphate results) and altered metabolism (increased NEFAs) in diseased cows, and highlight the benefit of early identification and treatment of diseased cows. Future work will investigate the potential of APP measurements to predict disease and reduced production in dairy cows when used as part of a metabolic profile.

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## A metabolomic investigation of milk in bovine mastitis

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**Introduction** Metabolomics is one of the new ‘omic’ technologies that have recently gained interest as a means to identify the total composition of a cell, tissue or biological fluid. Metabolomics has been used to identify the wide range of small molecule metabolites in samples such as milk (Boudonck *et al.* 2009). The aim of this study was to further investigate the metabolites present in bovine milk and especially to compare milk samples from healthy cows to milk from cows with clinical mastitis.

**Material and Methods** In the study, 10 healthy milk samples were compared with 10 milk samples from udder quarters infected with *E. coli* and another 6 milk samples from quarters infected with *S. aureus*. Bacteriological investigation confirmed the presence of the pathogens. Chloroform/methanol extraction was performed on the milk samples prior to analysis (Alvarez-Sanchez *et al.* 2010). Following centrifugation, the supernatants were analysed using liquid chromatography followed by mass spectrometry (LC-MS) on an Orbitrap (Thermo Scientific Ltd) mass spectrometer with data being processed using MzMine 2.1 software for peak detection. Multivariate statistical tools were also used to compare the metabolite profiles amongst the groups of milk samples.

**Results** The LC-MS analysis was able to identify 512 metabolites in negative ion mode and 814 metabolites in positive ion mode. Among these, many metabolites were at lower levels in milk from healthy cows compared to those from animals with clinical mastitis. It was noticeable that di- and tri-peptides were prominent among the molecules which were more abundant in milk samples from the dairy cows with mastitis caused by infection with either *E. coli* or *S. aureus*. Analysis using multivariate statistical tools indicated that it was possible to differentiate between the healthy, *E. coli* and *S. aureus* groups on the basis of the metabolite profile determined by LC-MS.

**Conclusion** Several hundred metabolites can be identified in milk using LC-MS for metabolomic investigation. Preliminary analysis has revealed that milk samples from quarters with clinical mastitis show an increase in metabolites compared to healthy milk samples. Many of the metabolites which increase in mastitis, are peptides probably caused by degradation of casein and other milk protein by bacterial proteases. With further validation, this approach may lead to improved diagnostic capabilities for bovine infectious diseases of the mammary gland.

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## Newly emerging veterinary diagnostic technologies: exploring attitudes, behaviours and wider social impacts

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**Introduction** Rapid/point-of-care (POC) devices allow tests to be run at veterinary surgeries or even on farm rather than sending samples to a lab. POC devices could therefore provide opportunities for earlier diagnosis and hence reduce welfare, economic and social impact of animal disease. This study explored stakeholder attitudes to potential adoption of POC devices in a range of different farm animal species examining both likely barriers to adoption and potential impacts of adoption.

**Material and methods** The research undertaken was qualitative in nature and sought to understand how different constituencies understood the role of POC devices. The aim was to sample a diversity of stakeholders but they were not viewed as statistically valid samples and hence generalising these results to larger populations would require additional quantitative analysis. Data were gathered systematically using semi-structured interviews lasting approximately one hour with 41 stakeholders (28 government vets, 10 vets in practice, industry or representing veterinary bodies, 8 farming/food chain professionals) during Sept-Dec 2009. Interviews were recorded and transcribed. Subsequent analysis proceeded on an inductive basis with specific themes identified from the interview transcripts. Interviews covered a range of animal species (cattle, sheep, pigs, poultry, horses and aquaculture species) and included notifiable and non-notifiable diseases and exotic as well as endemic diseases.

**Results** A number of potential advantages were identified from the use of POC devices, if they were available, e.g. devices that could be used on farm to identify animals infected with Bovine Viral Diarrhoea at birth and allow the eradication of the disease, devices to allow rapid, on-farm discrimination between mastitis caused by *Strep. Uberis* and coliform mastitis resulting in appropriate use of antibiotics. However a number of issues of concern were also raised, notably around lack of confidence in the results from POC devices, loss of control of information regarding disease status and its likely consequences and potential for over-reliance on POC devices with concomitant reduction in valuing of clinical knowledge.

Use of POC devices in the context of notifiable diseases poses presents particular opportunities but also challenges. Most opportunities identified for use of these devices were in the context of preliminary indicative screening tests that would later be confirmed by standard laboratory tests. In the context of endemic diseases speed was seen to be less of an advantage (with some exceptions) than the ability to conduct tests on farm. However several vets preferred to use the POC devices in more controlled conditions in their surgeries rather than on farm. Faster was not always better in terms of diagnostic test. The magnitude of some of the decisions made with the aid of diagnostic tests meant that confidence in the results was paramount. Vets in practice stressed the wider perspective of their relationship with their client and the need to incorporate POC devices in this context, providing a clear framework for the use of the devices as part of a package of animal health measures taking into account herd history and an understanding of the regional animal health situation. Potential wider impacts on disease surveillance and veterinary lab networks were identified, both potentially negative but also positive ways in which POC devices could be harnessed to different systems of practice.

Loss of control in the context of notifiable diseases centred around the potential to fail to recognise and act on potential notifiable disease cases quickly. However, some notifiable diseases (e.g. Classical Swine Fever) are currently difficult to distinguish from other endemic diseases. The decision to suspect a notifiable disease is not always clear cut and can be influenced by social context. Loss of control was also an issue with endemic diseases with vets very concerned about any potential for POC devices becoming available on the internet. The few farmers interviewed for this project indicated their willingness to adopt POC devices subject to cost and usability. Farmer attitudes to POC devices need to be understood in the context of their attitudes to disease and biosecurity and interviews reflected themes found elsewhere of the stigma associated with endemic disease and frustration at lack of government action to control exotic diseases, such as preventing illegal meat imports.

**Conclusions** Those wishing to promote the adoption of POC devices will need to demonstrate their use to gain confidence in them in practical situations. The potential deployment of POC devices is in the context of a complex and changing farming and veterinary environment with a wide range of different forms of livestock production and with different degrees of commitment to animal disease control. Control of animal disease in the UK is under flux with different jurisdictions adopting different strategies, with increasing stress on Responsibility and Cost Sharing agenda by Defra and likely shifts in relationships among governments, vets and livestock keepers. Many respondents identified these relationships as key to how POC devices would be adopted in practice. Unpredictable wider impacts are therefore very possible.

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## Estimating diagnostic accuracy of the tuberculin skin test and abattoir meat inspection from bovine tuberculosis surveillance data

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**Introduction** Bovine tuberculosis (TB) is a serious infectious disease of cattle caused by *Mycobacterium bovis*. Bovine TB control in the UK and Ireland is based on diagnosis using the single intradermal comparative tuberculin test (SICTT), supported by active abattoir surveillance (De la Rua-Domenech *et al.*, 2006). The heritability on the liability scale of SICTT responsiveness has been estimated to range between 0.14 and 0.16 in Republic of Ireland and Great Britain Holstein-Friesian dairy cows, respectively (Bermingham *et al.*, 2009, Brotherstone *et al.*, 2010). However, imperfect diagnostic performance of the SICTT may result in the misclassification of risk within tuberculosis herd breakdowns, resulting in underestimation of heritability of responsiveness to the SICTT test. Results from a recent theoretical exploration of the genetic parameters of disease data have accordingly demonstrated that imperfect test sensitivity and specificity result in a severe underestimation of the heritability on the liability scale (Bishop and Woolliams, 2010). The aim of this study was to extend the Hui-Walter latent class model using a Bayesian framework, a method which enables the estimation of diagnostic test properties in the absence of a gold standard diagnostic test (Hui and Walter, 1980), to estimate diagnostic test parameters and true prevalence of bovine TB from surveillance data.

**Material and methods** Data from 73,003 Holstein-Friesian dairy cows across 409 TB herd breakdowns in Northern Ireland (NI) between 1995 and 2010 were available for inclusion in the analysis. To address the fact that some, but not all SICTT animals undergo post-mortem inspection at abattoir inspection, the Hui-Walter no gold standard latent class model methodology was utilised and extended by including two additional multinomial counts, viz. the probabilities that a SICTT positive animal and a SICTT negative animal are not inspected at abattoir. To allow for breakdown-specific variation in sensitivity of the SICTT and abattoir inspection, the sensitivity of the two diagnostic tests was modelled for each breakdown, as the population specific test sensitivity plus a breakdown-specific residual variance. Population subset Bayesian bootstrap analysis was conducted to provide a predictive distribution of the diagnostic parameters. A simulation study, using the Hui-Walter parameter estimates from the surveillance data, was conducted to test the predictive ability of the model. All analyses were conducted using WinBUGS and the R2WinBUGS package.

**Results** Estimates with narrow credibility intervals were obtained for the sensitivity and specificity of the two diagnostic tests. Both diagnostic tests were highly specific, although abattoir inspection had poorer sensitivity than the SICTT. The model also provided estimates of the true within herd prevalences which were higher than the apparent prevalences. The simulation study demonstrated that the extended model had good predictive power to estimate the diagnostic parameters of the SICTT and abattoir inspection, and to estimate true prevalence within herd breakdowns from surveillance data. The Hui-Walter parameter estimates were robust to removal of population subsets; the 95% bootstrap confidence intervals overlapped widely with the posterior distributions of the diagnostic parameter estimates. The Pearson's correlation coefficient between the posterior true prevalence from the surveillance data and estimated true prevalence from the simulated data set was 0.93 (95% CI 0.92-0.95;  $P < 0.001$ ).

**Conclusions** This study provides an extended Hui-Walter latent class model that allows for the unbiased estimation of diagnostic test parameters and true prevalence of bovine TB from surveillance data. This facilitates estimation of diagnostic test performance at the population level. Accurate estimates of test performance are crucial in the design of control programmes, and in the modelling of potential interventions. The model suggests that current estimates of test performance are within the published range, although current estimates of prevalence are likely to be underestimated. Furthermore, after correcting the heritability estimates using the diagnostic test parameters estimated in this study, lead to predicted true heritability estimates for SICTT responsiveness in the GB and Irish studies of 0.16 and 0.19 respectively; indicating that true genetic variation is likely to be greater than estimated from surveillance data. Furthermore, the extended methodology developed in this study has applications in the genetic epidemiological analysis of other human and animal diseases for which incomplete surveillance data on two or more diagnostic tests are available.

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## The prevalence of helminth infestations in hedgehogs admitted to wildlife centres across England

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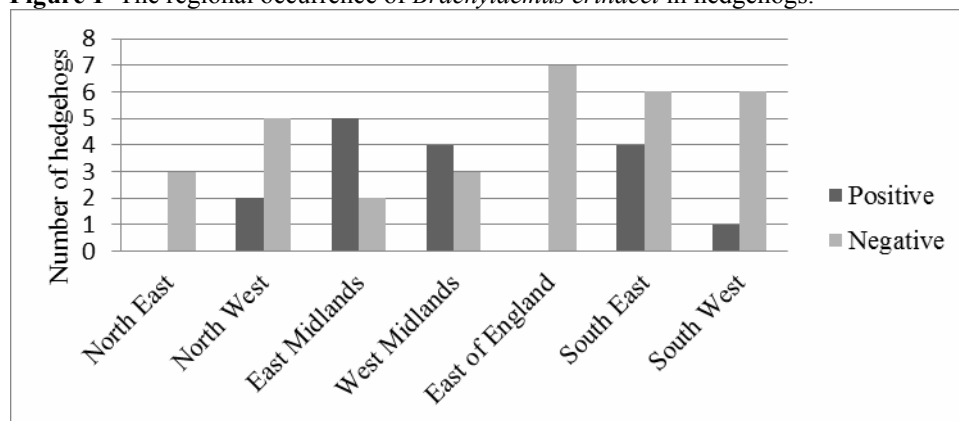
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**Introduction** The hedgehog (*Erinaceus europaeus*) accounts for an estimated 54% of wildlife mammalian rescues (Churchfield, 2008). This makes it one of the most common mammalian wildlife species to be presented to rescue centres and veterinary practices in the United Kingdom. The species is susceptible to a range of endoparasites including lungworms, intestinal threadworms and intestinal fluke. Healthy hedgehogs are able to cope with this endoparasitic burden, but in compromised hedgehogs, infestation may prove fatal. The aim of the study reported here was to determine prevalence of hedgehog helminth infestation to help improve the captive health and wellbeing of this mammal.

**Material and methods** The study was conducted between October and November 2009. One faecal sample was taken from each newly rescued hedgehog (n=48) prior to any endoparasitic treatment from eight rescue centres. Rescue centres were based in Northumberland, Cheshire, Worcestershire, Derbyshire, Avon, Bedfordshire, Kent and Buckinghamshire. Each rescue centre provided a faecal sample from between three to seven hedgehogs. Samples were stored in a refrigerator prior to analysis. All faecal samples were analysed using the Baermann funnel, McMaster technique and faecal sedimentation. Direct faecal smears were used to assess endoparasite burden where sample volumes were inadequate, and therefore unsuitable for other techniques. Assessment sheets were completed by centre workers in relation to each hedgehog. These sheets recorded sex, weight, body condition, any presenting clinical signs, diagnosis and treatment. Rescue workers were also asked to state the outcome of the case and estimate an age for the hedgehog. Statistical analyses included Chi square test. Significance was indicated by  $P \leq 0.05$ .

**Results** 9/48 hedgehogs tested negative for endoparasite infestation. 17/48 hedgehogs tested positive for infestation with one endoparasite specie, of which 16 hedgehogs were infested with *Capillaria* spp. Mixed infestation with two to three species of helminth were present in 22/48 hedgehogs. The most prevalent mixed helminth infestation was with *Crenosoma striatum*, *Capillaria* spp. and *Brachylaemus erinacei* (13/22 hedgehogs). Chi square test revealed no significant association between helminth specie infestation and life stage of hedgehogs affected ( $P=0.173$ ).

**Figure 1** The regional occurrence of *Brachylaemus erinacei* in hedgehogs.



**Conclusions** Mixed helminth infestation was more prevalent than single infestations in the sample population used for this study. This may affect decisions as to which treatments to use in practice. Life stage is not a useful predictor of helminth infestation in hedgehogs. This study has shown *Brachylaemus erinacei* to be more widely distributed than previously thought; Robinson and Routh (1999) state that it is less common or absent in Northern England and East Anglia. Further research could include identifying factors that may affect helminth burden in hedgehogs.

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## A cross sectional study on the point prevalence and risk factors for *Habronema* in working equids in central Ethiopia

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**Introduction** Adults of the nematode *Habronema* spp. attach to the mucosa in the stomach of equids. The adults are not considered to be highly pathogenic, although heavy infection can lead to gastritis, colic and gastric perforation. Larvae are passed in the faeces and ingested by maggots of the flies *Musca domestica* and *Stomoxys calcitrans*; co-development occurs and adult flies carry the L3 in their mouth parts. Habronemiasis occurs when the L3 are deposited on the animal and burrow into the skin, causing an eosinophilic hypersensitivity reaction characterised by extensive, non-healing proliferative granulomatous lesions. Typical predilection sites for these lesions are the male genitalia, the medial canthus of the eye, wound edges and the perineal and axillary regions. The lesions are difficult to treat, requiring extensive debridement and long courses of treatment, which are not readily available or affordable in Ethiopia. Consequently, the lesions become debilitating; impeding wound healing, vision and urination. This predisposes affected animals to further infection and wasting and raises welfare concerns.

**Material and methods** A sample of 210 donkeys from market sites, water points and the DebreZeit clinic were examined. This sample size was the maximum that could be achieved within the time available for the study and allowed the results obtained to be analysed with a confidence of 95% and precision of 7%. The donkeys included animals that were apparently healthy or had lesions, which were brought to markets and water points from up to a 25 km radius with their owners. The owners were asked questions in local language on donkey use, housing, feeding and co-grazing/housing. Each animal was examined clinically and any lesions were recorded and described. Photographs were taken of wounds and a faecal sample was collected for parasitology. Faeces was processed as described by Traversa *et al.* (2004) and sent to Italy for PCR. A drop of processed faecal material was also examined microscopically for qualitative detection of other parasite eggs for comparison with PCR results for the presence of *Habronema* spp.

**Results** There was no significant relationship between the presence of lesions and the age ( $t$ -test  $P = 0.078$ ) or sex of donkey. Donkeys from highland regions had a 3.4 times increased risk of lesions (Chi square  $P < 0.001$ ). The presence of a lesion elsewhere on the body decreased the risk of having an ocular lesion to one eighth (Chi square  $P < 0.001$ ). Housing inside/with shelter also increased the risk of having a lesion by 2.1 times (Chi square  $P = 0.013$ ) but this was confounded with region. There was no significant association between the occurrence of lesions and whether donkeys were housed tied/untied or with/without cattle.

**Conclusions** Housing donkeys inside and location in the highland area of Ethiopia increased the likelihood of having a lesion typical of habronemiasis. This could be due to the altered climate, abundance of flies and possible exposure to greater numbers of infected flies inside. Furthermore, having a lesion elsewhere on the body appeared to be protective against developing an ocular lesion; this could be due to the fact that an exudative body lesion attracts the flies away from the ocular area, thus reducing exposure around the eyes. If control measures were put into place to reduce overall fly exposure, the prevalence of non-healing debilitating lesions may be reduced. For control of habronemiasis overall the results on gastric prevalence need to be realised and analysed with the manageable risk factors so potential control methods could be implemented at a more feasible, possible, and manageable level.

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## Assessment of risk factors for antimicrobial resistance diversity in dairy cattle

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**Introduction** Antimicrobial resistance (AMR) in cattle production systems is of economic and health importance in terms of increased morbidity and mortality of livestock. In epidemiological studies of AMR in cattle, it has been identified that calves generally demonstrate a greater prevalence of AMR than older animals, which declines with animal age (Hoyle *et al.* 2004; Berge *et al.* 2010). The effect of antimicrobial treatment on AMR prevalence in cattle has also been examined; whilst some studies indicated that treatment leads to a transient increase in AMR prevalence (Lowrance *et al.* 2007), others have concluded that resistance does not increase in treated animals (Singer *et al.* 2008). The majority of studies examining the association of animal age and treatment with AMR have looked at prevalence; here, these factors were examined with respect to AMR diversity.

**Material and methods** The data for this analysis were of phenotypic antimicrobial resistance profiles from faecal *E. coli* isolates obtained from calves and cows on seven dairy farms in the Western United States. Animals were repeatedly sampled over a period of 14 days. Information was available on the treatment status, age (cow or calf), farm of origin, and sampling days for each animal. A multivariable risk factor analysis was undertaken to assess the association of predictor variables with AMR diversity. The unit of analysis was the number of unique profiles found within a single animal's sample on a single sampling day; the data were restricted to sampling days where 16 isolates per sample were analysed. A proportional odds logistic regression model was used to model the associations between the outcome and predictor variables. To account for repeat sampling of the same animal, the unique animal identity was included as a clustering variable in the model.

**Results** The dataset included 775 sampling days, from 216 animals (114 cows, 102 calves) on seven farms. The number of unique profiles per animal per sampling day ranged from 1 – 12. The final model included farm, age, and a farm/age interaction term as predictor variables. The significant farm/age interaction term indicated that the effect of age on AMR diversity was dependent upon the specific farm. On two farms, the odds of cows having a higher AMR diversity was significantly lower than that of calves; conversely, on three farms, the odds of cows having a higher AMR diversity was significantly higher than that of calves, compared to the referent farm. Sampling day and treatment were not significantly associated with the outcome variable.

**Conclusions** Antimicrobial treatment, which was primarily ceftiofur in this study, was not significantly associated with AMR diversity. Sampling day was also not associated with the outcome, indicating that AMR diversity within an animal did not significantly change over the 14 day sampling period. The significant interaction of farm and animal age suggests that there may be particular animal husbandry or farm management practices that influence AMR diversity. Further investigation into these farm level factors will assist in identifying the practices which are associated with increased or decreased AMR diversity.

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## The influence of interview on the selection of students into a veterinary medicine degree programme

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**Introduction** A major challenge in admissions to training programmes in veterinary medicine is to select those students with most suitability for clinical training and careers from a large and diverse pool of applicants with very high academic ability. In an English Medical School study, 20 of the 22 schools in the study used interview in some format as part of the selection process (Parry and others 2006). In the UK veterinary sector, all 7 of the Schools utilise the selection tool of interviews in some format after an initial short-listing process (Hudson and others 2009). Therefore, the interview process is considered to be an important part of veterinary and medical admissions processes. However, there is some debate on the validity and reliability of interviews (Kogan and McConnell 2001). The aim of this study was to evaluate the influence of the interview on veterinary selection decisions at the University of Edinburgh by investigating a cycle of interviews of school-leaving applicants to the Veterinary Medicine course.

**Methods** This study evaluated candidates applying for Government (Scottish Funding Council, SFC)-funded places. In the 2008/9 admissions cycle, the interview process was analysed. On the basis of academic and non-academic criteria, applicants were short-listed to 180 for interview and further final selection of 72 for entrance. Short-listed applicants were invited to attend a 20 minute panel-based interview at the School. The interview panel comprised 2 members: usually academic members of staff but some panels involved an external member. The selection panel for each group of interviews (consisting of a chair and one other selector) was asked to independently evaluate the applicants before interview on the basis of their paper-based application. Applications were assessed on an individual basis taking into account all of the supporting material (references, academic history, career exploration, motivation and extra-curricular activities). Selectors were asked to rank the candidates in descending order of merit. Usually, there were 8 applicants interviewed in any given session; accordingly the top candidate was given a rank of 1, the next, 2 and so on. Selectors were also asked to make a decision on the applicant prior to interview. Categories of decision were: 'accept' (the applicant should be offered a place), 'reject' (the applicant should not be offered a place), or 'hold' (not a clear 'accept' or 'reject' but may be offered a place depending on numbers). The applicants were then interviewed and the selectors then repeated their evaluations independently of each other i.e. ranked the candidates and made a selection decision. During the interview, selectors evaluated various attributes and performance of the applicants using a 4-point scale ('outstanding', 'good', 'average' or 'poor'). At the end of each interview, the selectors then discussed the applicants and agreed a consensus evaluation of the applicants, again ranking the candidates and making a final decision ('accept', 'reject' or 'hold'). The results of the pre- and post interview evaluations of the applicants were captured and analysed; levels of agreement were evaluated using the Kappa statistic. Analysis was carried out in R (V2.10.1 © The R development core team), and  $P < 0.05$  was taken to indicate statistical significance. The admissions research study was approved by the College of Medicine and Veterinary Medicine Ethics Committee at the University of Edinburgh.

**Results** There was 57.7% agreement between the chair and co-interviewer on selection decisions taken before interview and 77.5% agreement after interview. There was 67.0% agreement for chairs between their pre and post interview decisions and 64.4% agreement for co-interviewers. The agreement between post interview decisions and consensus decisions (after consultation) post interview was 84.1% for chairs and 87.4% for co-interviewers. Pre-interview rankings of the candidates showed a moderate level of agreement between chair and co-interviewer (Kappa: 0.448,) whereas post-interview rankings showed a substantial level of agreement (Kappa: 0.727). There was almost perfect agreement between post-interview rankings and post consensus discussion rankings (Kappa: 0.906 for chairs and 0.891 for co-interviewers). There was moderate agreement between pre and post interview rankings for both chairs (Kappa: 0.514) and co-interviewers (Kappa: 0.442). The impact of candidate attributes and performance was further explored.

**Conclusions** These results show that there was significantly more agreement possible between interviewers with regard to selection decisions based on interview assessment compared to pre-interview assessment of written applications. The interview process did influence selection decisions and rankings of candidates. Once interviewers have made their decisions independently after interview, there was no particular influence of post interview discussions on the final selection decisions. This study suggests that the interview process for selecting veterinary students is important in facilitating decision making and is a useful selection tool.

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## Predictors of success on the veterinary undergraduate course: Evaluating variables within the student intake and correlating with performance

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**Introduction** Predicting the success of students in an undergraduate veterinary course is challenging and relatively few studies have been undertaken to objectively identify factors that will facilitate this educational experience. According to the Schwartz Report on Fair Admissions to Higher Education (Schwartz 2004), prior educational attainment data remain the best indicators of success at undergraduate level and accordingly, evaluation of academic history remains central to the admissions process. Previous academic performance (e.g. A-Level results) is considered by some to be the best predictor of the outcome at medical school (McManus *et al.* 2003). Research from the US has shown that in the veterinary course, academic difficulty experienced by veterinary students was associated with a low pre-requisite Grade Point Average [GPA] achieved prior to admission (Rush *et al.* 2005). In a study from South Africa, previous academic performance was positively correlated with academic performance at veterinary school (Van der Walt and Pickworth 2007). The aim of this project was to analyse student intake data such as educational history and demographic factors of students entering the Royal (Dick) School of Veterinary Studies at the University of Edinburgh and to investigate possible correlations between these data and academic performance in the first year at veterinary school.

**Methods** Data were collected and collated for three consecutive veterinary student intakes numbering 130, 147 and 171 students (total 448). Data included: school qualification (e.g. A-Level, Highers, Advanced Highers), subjects taken including grades, institution, domicile, whether gap year taken, gender and age. Particular data collected for graduate applicants included degree classification, degree subject, year graduated, GPA and the calibre of the applicant's academic institution as profiled by reference sources (Barron's Profiles of American Colleges and University league tables). The student cohorts were tracked and the results of the examinations for their first year were collected. Performance was measured by the marks achieved in major first year examinations. The univariate relationships between absolute marks and the putative influence of intake variables were statistically analysed using analyses of variance. All analysis was carried out in R (V2.10.1 © The R development core team), and  $P < 0.05$  was taken to indicate statistical significance. The admissions research study was approved by the College of Medicine and Veterinary Medicine Ethics Committee at the University of Edinburgh.

**Results** For school leaving entrants to veterinary school, the presence of straight As in school subjects (A-Level and Advanced Higher and Higher) was linked to a better exam performance in end of first year examinations compared to students with grades less than A ( $P = 0.001$ ). Students with an A in Chemistry or Biology (A-Level and Advanced Higher) performed better than students with grades below A in these subjects ( $P = 0.001$ ). There was no statistical significance when similar comparisons were made evaluating Mathematics and Physics. For all entrants, there was no significant effect on exam performance at veterinary school from the type of school attended, gender, domicile or calibre of previous university. There was no effect of taking a gap year or number of years elapsed since previous study. Increased age ( $> 21$  years of age) at the onset of veterinary studies was associated with a reduced exam performance ( $P = 0.001$ ). A higher GPA was associated with a better exam performance ( $P = 0.016$ ) as was a higher degree classification ( $P = 0.026$ ).

**Conclusions** This study shows that intake variables are associated with subsequent academic success or otherwise on the veterinary course. Straight As in high school subjects are correlated with better exam performance in the first year at veterinary school. Biology and Chemistry seem to have more of an effect on exam performance than Mathematics and Physics. For graduate entrants, prior educational attainment such as GPA and degree classification is correlated with exam performance and increased age was negatively associated with exam performance. It is anticipated that this study will inform and facilitate longitudinal improvements in the School's selection and teaching processes as they relate to transition to university and the first year experience. An important part of this project will be to provide the platform for longitudinal studies tracking the success or otherwise of the cohorts of veterinary students as they progress through the entire 4-5 year BVM&S course and on into their postgraduate careers.

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## What are our final year veterinary students seeing? Finding the evidence

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**Introduction** Final year veterinary students spend the last year of their course participating in rotations through various veterinary disciplines, which are either species-based or subject-based. It is expected that during these rotations, students will gain the clinical experience they need to put the knowledge they have acquired during the first four years of the course into practice. However, it is somewhat difficult to assess the caseload seen by students during this final year to ensure that they have had the opportunity to participate in all facets of veterinary medicine prior to graduating. There is also little published evidence outlining the types of cases commonly presented to vets in the UK, or the body systems that are affected. Similarly, there is little published information regarding how many of these cases result in diagnostic testing, whether definitive diagnoses are made and what actions are taken at the end of the consultation. In order to improve the evidence-base within veterinary practice, it is crucial that this information is gathered from a range of practice types. Therefore, the aim of this study was to collect information relating to the cases seen during rotations by final year veterinary students at the School of Veterinary Medicine and Science (SVMS) at The University of Nottingham.

**Material and methods** Information was collected by final year students during rotations through the Clinical Associate practices involved with the SVMS commencing May 2010. This information excluded any general or compulsory extramural studies (EMS) or elective practice that students saw during this time. A clinical coding booklet was created to enable students to record information relating to each veterinary case whose treatment they observed or participated in. These pocket-sized booklets were designed to make recording rapid and easy; it was expected that each case would take no longer than one minute to record. A range of closed questions were asked, in addition to several open questions allowing more detailed information to be recorded if necessary. Questions related to the type of case, species seen and body systems involved. If multiple body systems were affected, this was also recorded. Additional information was requested regarding any tests, diagnoses or actions undertaken. The case information within these booklets was transferred to an electronic database by students, at either an allocated time within the rotations or at a time of the student's choosing. This database was located within a site already used by students, tutors and other staff members for general communication, feedback and assessment whilst on rotations. Questions in the database were structured in an almost identical way to the questions in the booklet, using drop-down menus instead of tick boxes where necessary. Students received the booklets prior to rotations starting, and were instructed on the procedure for recording the information via an initial presentation and then again via email. Reminder emails clarifying the procedure were sent approximately 6 weeks after the rotations began. After approximately 25 weeks of rotations, information was downloaded and descriptive statistics calculated using Microsoft Excel 2007 (Microsoft Inc.) and Minitab Version 15 (Minitab Inc). Minitab was also used to determine normality of student entries.

**Results** Seventy two of 87 final year students documented case information electronically, with a total of 3920 cases recorded over the rotation period investigated. The number of cases recorded was not normally distributed. The median number of cases per student was 33 (interquartile range 15-75), with 305 the maximum number recorded by one student. Just under 80% of the cases were classified as first opinion, and just under 90% were recorded as individual animal cases (versus as a group). Of the 3824 recorded entries for species, just over a third of animals recorded were canines (35%) followed by equines (27%), bovines (14%) and felines (12%). Of the total number of entries for body system (n=4829), the majority involved the 4 main systems outlined below (Table 1).

**Table 1** Predominant body systems recorded for cases seen by final year veterinary students

Body system	Number of cases	Percentage of total cases
Musculoskeletal	972	20
Gastrointestinal	746	15
Integumentary	606	13
Reproductive	562	12

Most cases resulted in some form of diagnostic testing (n=2280; 58%). Of the recorded entries selecting one of the outcome options (n=2116/3920; 54%), the majority resulted in treatment (n=980/2116; 46%). Interestingly, of the recorded cases stating whether a diagnosis was reached (n=1757/3854; 46%), the majority of consultations did not reach a definitive diagnosis (n=1412; 80%).

**Conclusions** These results show that a broad range of conditions and species were recorded by final year students during the first six months of rotations through the Clinical Associates associated with the SVMS at The University of Nottingham. This reflects well the curriculum that is being taught in the preceding 4 years of the course. In addition, the information recorded here adds to the limited knowledge base that exists on what vets are commonly presented with in veterinary practice and how these cases are managed. Further understanding of common issues in clinical practice is important for highlighting relevant areas for future veterinary research.

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## The use of a blackboard wiki as a tool for teaching evidence-based veterinary medicine

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**Introduction** Evidence-Based Veterinary Medicine (EBVM) is a growing area of interest to all veterinary practitioners. Therefore, university institutions have an increasing responsibility to teach undergraduates effective techniques in article appraisal as described by Sackett *et al* (1997). We used an e-learning environment to train students in standardised EBVM techniques via a Powerpoint™ presentation and an excel™ calculator. The students used an online Wiki tool to record a case chosen from their own experience in first opinion farm animal practice. They performed a database search relevant to a patient, intervention, comparison and outcome (PICO) approach, explained by Cockcroft and Holmes (2003). The students appraised the resulting articles and recorded this on the Wiki. The Wiki tool also allowed them to discuss their results with other groups online before attending case rounds with experienced clinicians.

**Material and methods** There were 3 groups of 4 students, whom rotated through a 3 week cycle of EBVM searches and first opinion practice. So far, a total of 60 students have experienced this format of teaching. The searches were interspersed with time exposed to real cases to allow students to ask a clinically relevant PICO format search. They could then produce a Critically Appraised Topic (CAT) to obtain a “clinical bottom line”, introduced by Hardin *et al* (2006). This would be a best-evidence solution to their intervention plan. They could then return to the case and have a chance to apply their findings in practice. We used Microsoft powerpoint™ and excel™ software to train the students to create PICO format questions and appraise articles using evidence-based statistical formulae such as Number Needed to Treat (NNT), SpINS and SnNOUTs, Positive Predictive Values etc. This allowed them to enter such data as recovery rates (often published in articles) and convert this, without manually calculating, to NNTs etc. The Blackboard online learning environment had a built-in Wiki tool which we designed for students to enter the case history, presentation, intervention plans, prevention and monitoring plans. They could then enter their article search history and appraisal (with study weaknesses found) and finally their clinical bottom line. This converted the Wiki to a CAT. Other groups could enter comments after reading their CAT, permitting the original group to answer the questions in a CAT club at the end of their 3 week rotation. The important use of CAT clubs is documented by Phillips and Glasziou (2004).

**Results** The rotation was assessed using student feedback on a Blackboard questionnaire. Replies total 54% so far, with students answering in 86% positive agreement when asked if the EBVM exercise was useful and relevant to their clinical experience. A more stringent evaluation of their feedback is currently being conducted via Turning Point™. Using the computer-based software was successful, with all groups creating CATs and discussing scientific background of case histories online. The Wiki allowed them to enter specific data in free-typing boxes but to make the case history more interesting to the reader, they could also upload web links to relevant websites, video, sound or image files. All groups included photo images to illustrate their cases.

**Conclusions** Overall, students found the EBVM Wiki CAT creating exercise to be both interesting and relevant to their future clinical careers. They understood the principles of PICO patient question formulation and applied this to a database search for articles relevant to their intervention plan. They were able to use an excel™ calculator to obtain EBVM-related statistical values and appraise articles for their reliability. From this, they could create a best-evidence answer to their case intervention and apply this in practice later in their rotation.

This project has great potential to the clinician in practice. If a database of peer-reviewed CATs was created, the clinician may be able to search this and quickly see a clinical bottom line to proposed interventions based on the best scientific evidence.

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## Development of the Animal Welfare Associated Reflective Exercise (AWARE): a self-directed learning tool to promote ethical reflection in pre-clinical veterinary students

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**Introduction** Anecdotally, participation in Extra Mural Studies (EMS) has a major influence on veterinary students' understanding of normal husbandry practice and may have an informal role in their ethical development. Encouraging ethical reflection in the early stages of the veterinary course is desirable as this is likely to better prepare students for what is increasingly recognised as an ethically challenging profession.

The aim of this study was to develop and test a prototype self-directed learning tool, to promote ethical reflection, for use by veterinary students during pre-clinical EMS (PC-EMS). The tool, named the Animal Welfare Associated Reflective Exercise (AWARE), was designed to encourage the students to reflect on the ethical content of an animal welfare issue they witnessed during an EMS placement on a cattle, sheep or horse unit.

**Material and methods** A prototype reflective tool was trialled with 25 first year veterinary students (19 female and 6 male, age range 18-28). Students were given a one hour explanatory teaching session prior to their EMS placements and they completed the exercise within two weeks of finishing their EMS. The AWARE tool comprised 15 questions (plus demographic information) divided into five sections. After completing their demographic information, students were invited to identify, and give details of, an issue or event that through human action impacted animal welfare. Students were then encouraged to describe their emotional reaction and consider the factors contributing to the event, before reflecting on its ethical basis. The final section asked students to describe their overall experience.

A focus group was held after EMS to give students an opportunity to discuss their experiences on their placements and using the AWARE. An online questionnaire was also used to evaluate students' attitudes towards the tool.

The content of the AWAREs (n=25) was qualitatively compared to unstructured PC-EMS reflections sourced from students in the previous cohort (n=17) using NVivo Software. All sources were coded into 12 distinct categories using questions from the AWARE as a guideline and the percentages coded to each category were compared using Mann-Whitney U tests.

**Results** The majority of the students (92%) recounted an experience on a sheep farm and 80% of the students chose an experience that negatively impacted animal welfare. The results of the qualitative analysis revealed that the AWAREs had significantly less descriptive content ( $p<0.001$ ) than unstructured reflections, while expression of 'why they felt that way' ( $p=0.002$ ) and 'why they thought the action was taken' ( $p<0.001$ ) were higher in the AWAREs. Ethical reflection was virtually absent in the unstructured reflections, with only two students displaying any and there was no inclusion of ethical frameworks; this was significantly higher in the AWAREs ( $p<0.001$ ) with 22 of 25 students displaying some ethical reflection. Despite this however, four students failed to list the animal as an affected party.

Results from the online feedback survey (n=22) demonstrated that all students liked the self-directed aspect of the exercise but 32% found it difficult to identify an issue to reflect on. More than 85% of the students agreed that the AWARE prompted them to think more about animal welfare issues and the pressures on farmers and over 80% agreed that they thought more about their feelings on the issue as a result of the exercise. In addition, 82% of the students felt that completing the AWARE improved their ability to recognise animal welfare issues and to reflect on their experiences at least a moderate amount, while 77% felt it improved their ability to recognise ethical issues and respect others viewpoints to this same degree.

**Conclusions** Students engaged with the AWARE and used it to consider relevant welfare issues. Ethical reflection was virtually absent in the unstructured reflections, but was reliably elicited by the AWARE. Feedback was very positive with more than 75% of students reporting that it improved their ability to recognise and reflect on welfare and ethical issues. Collectively, the results suggest that this approach has value and provides a structure in which students may constructively reflect on ethically challenging situations experienced during EMS. The tool has been further refined and will undergo validation this academic year.

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## Measuring ethical reasoning ability in first year veterinary students using the Defining Issues Test

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**Introduction** As veterinary medicine becomes increasingly recognised as an ethically challenging profession, the inclusion of specific ethics tuition in the veterinary undergraduate curriculum is gaining momentum. Vets regularly face a range of dilemmas, resulting from a need to balance a range of interests including those of animals, clients and practices. Ethical reasoning can therefore be regarded as a vital skill in veterinary graduates to improve the ease and quality of their decision making. In this study we aimed to establish the level of ethical reasoning ability in first year veterinary students using a well established approach, the Defining Issues Test (DIT) (Rest *et al.*, 1974). The DIT measures the level of post-conventional moral reasoning used to solve a series of ethical dilemmas. Post-conventional reasoning is the most advanced level of moral reasoning and is the level of reasoning expected to be predominant in graduates of professional courses. The test was applied twice to investigate the effects of continuing veterinary education including an EMS placement and to confirm test re-test reliability.

**Material and methods** The DIT was applied to first year veterinary students. The students completed the 3-story DIT, firstly in second term (Test 1) and then again in third term (after EMS, Test 2), 9 weeks later. During testing, students were given written instructions and additional information was collected (gender, age, nationality, upbringing and whether participants already held a degree). The test took around 30 minutes to complete. DIT scores, expressed as P or N scores, both give the percentage of the respondent's answers that demonstrate post-conventional reasoning. The Test 1 DIT was completed by 51 students and the Test 2 DIT by 48, with 38 students sitting both tests. Tests were purged if they did not meet the reliability criteria set out in the DIT guide (Rest, 1987). After purging, the number of Test 1 DITs was reduced to 43 (15.7% purged), Test 2 DITs to 35 (27.1%), and both tests to 25 (34% purged). Normality assumptions were met for all scores and General Linear Models were carried out using Minitab Statistical Software to investigate effects of test number, gender, nationality, educational level and age.

**Results** Descriptive statistics for the DIT scores (Tests 1 and 2) are shown in Table 1. No difference was found between the Test 1 and 2 scores but strong positive correlations were seen (P scores,  $r=0.60$ ,  $p=0.002$ ; N scores,  $r=0.78$ ,  $p<0.001$ ). The mean scores were not affected by completion of EMS. Compared to studies carried out on students of other professions, the mean P scores (38.9 to 40.0) of the veterinary students were similar to those found in first year pharmacy students (Mean=38.5,  $n=244$ ) but lower than first year American veterinary students (Mean=45.7,  $n=54$ , Latif, 2000). Educational level has a strong influence on DIT scores (Thoma, 1986) and this may explain this difference given that American students are college graduates on entering professional degrees. P scores of senior high school students average in the 30s and in general, undergraduate students average in the 40s so these scores are towards the lower end of the expected range.

**Table 1** Descriptive statistics for the DIT scores for Test 1 and Test 2

DIT score	Test 1 mean $\pm$ SD (n=43)	Test 1 range (%)	Test 2 mean $\pm$ SD (n=35)	Test 2 range (%)
Post Conventional (P score)	38.9 $\pm$ 17.3	0.0 – 76.7	40.0 $\pm$ 18.5	13.3 – 80.0
N score	35.8 $\pm$ 16.0	8.7 – 66.7	41.6 $\pm$ 13.9	16.8 – 71.4

Although not seen in Test 1, a significant difference was found for Test 2 P and N scores between those holding a degree and those that did not ( $p=0.029$  and  $p=0.003$  respectively) with non-degree holders having a lower score, and between nationalities with North American students scoring higher than other nationalities ( $p=0.003$  and  $p=0.013$  respectively). These two factors are linked as only North American students had degrees and the variation is most likely explained by educational level. The post N score also showed a gender difference ( $p=0.037$ ) with females scoring higher. Neither the Test 1 nor 2 P and N scores were affected by age.

**Conclusions** These are the first available DIT data for UK veterinary students and reveal a wide range of ethical reasoning ability among those tested. This is important information and emphasises the need for careful planning when designing suitable educational approaches for teaching ethical skills. In future work, we plan to measure students' ethical development as they progress through their undergraduate training and the effect of the Animal Welfare Associated Reflective Exercise, a novel self directed learning tool to promote ethical reflection during pre-clinical extra-mural study placements.

**Acknowledgements** This study was made possible by funding from the British Veterinary Association Animal Welfare Foundation.

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## Ten years of Post-weaning Multi-systemic Wasting Syndrome in England: Retrospective study on morbidity, breeds used and farmers' perception

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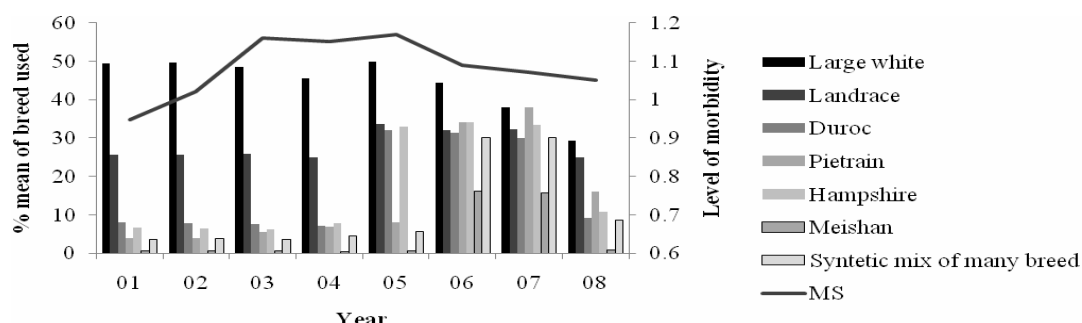
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**Introduction** Post-weaning Multi-systemic Wasting Syndrome (PMWS) emerged in England in 1999. The necessary pathogen, porcine circovirus type 2 (PCV2), has now been shown to be present on most English farms. Until the appearance of PCV2 vaccines, farmers struggled to control the disease and it is widely believed that some breeds are more susceptible than others. Other ideas on how PMWS can be controlled have been circulating and may have affected farmer behaviour in the past. The present study aimed to 1) describe the changes of PMWS morbidity over time and to explore potential association with the changes of breeds used from 2001-2008, and 2) to assess farmer's perception on disease risk factors and its association with PMWS severity.

**Material and Methods** A retrospective study on 147 English pig farms, recruited through the BPEX PCV2 vaccination programme and veterinary practitioners, was conducted in 2008-09. Using the protocol developed by Alarcon *et al.* (2011) a morbidity score (MS) for each farm and each year from 2001 to 2008 was derived based on morbidity observed in different age groups and collected through farmers' interview. To minimize errors in PMWS morbidity estimation, only farmers that passed a misclassification process were analyzed. Data on breeds and commercial name of parents and grand-parent stock were collected. The proportion of each breed present in a typical finishing pig of a farm was then calculated for each year. Farmers' perceptions on PMWS and possible risk factors were investigated with open and closed questions. Association between breeds and MS was tested using Generalized Estimating Equation (GEE) linear models with an autoregressive correlation structure to adjust for farm and year correlation. Association between farmers' perception and PMWS severity categories was investigated using multivariable ordinal logistic regression.

**Results** Figure 1 shows that during 2001-04 the majority of farms used almost exclusively a combination of Large White (LW) and Landrace. From 2005 onwards the proportion of Hampshire and Duroc in finishing pigs significantly increased followed by Meishan, Pietrain and other synthetic breeds a year later. Analysis of the MS over time showed that the epidemic peak of the disease occurred in 2003 and remained at the same level until 2005 before it slowly decreased. Outcomes of the GEE linear model suggest that an increase in the proportion of LW increased the risk of MS ( $p=0.007$ ).

**Figure 1** Mean percentage of each breed present in a finishing pig and mean PMWS morbidity score for the period 2001-08.



Analysis of data on farmers' perception showed that 46% believe that the disease was introduced onto their farm through breeding stock. Movement (56%), housing (44%) and breed (42%) were believed to be associated with PMWS. Statistical analysis showed evidence that those farmers aware of pig stress in weaning practice ( $OR = 0.38$ ,  $p=0.02$ ) and those farmers that believe that PMWS was introduced through mixing pigs from different sources ( $OR = 0.15$ ,  $p=0.05$ ) had lower odds of being in a high severity category. Farmers who believe that PMWS can be treated had higher odds of being in a high severity category ( $OR = 3.24$ ,  $p=0.03$ ).

**Conclusion** Although other reasons for the change in breeds should be considered, the time of change and its impact on PMWS morbidity suggest that this was part of an industry reaction against PMWS. Especially noticeable is the reduction in morbidity in 2006 that could be due to the higher use of a third breed. However, statistically, only Large White was found associated to MS. Farmers' perception highlights the importance of breed management on the introduction of PMWS on the farm. Understanding how an emerging disease could change an industry is essential in order to predict and deal with future food security issues.

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## Pigs and the H1N1 pandemic: Innate immune responses in experimentally infected pigs

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**Introduction** The current human influenza pandemic caused by H1N1 arose in early 2009, and has since caused worldwide morbidity on a large scale, with a proportion of clinical cases suffering serious disease and even death. The virus continues to cause disease in both the northern and southern hemispheres and has the potential to mutate to cause additional challenges to human health. In view of the potential importance of pigs in the genesis of the pandemic virus, a multi-institutional team (combating swine influenza consortium; COSI) was constructed to provide a scientific evidence base on which to inform policies aimed at minimising the impact of the pandemic virus in both animal and human populations. One of the aims of the consortium was to study the disease pathogenesis and early host responses of pigs experimentally infected with an influenza virus isolate from an early UK human case (A/England/195/09).

**Material and Methods** The study used 12-week old grower pigs which had previously tested negative for influenza virus by real-time PCR for matrix gene and by haemagglutinin inhibition for antibody. Pigs were infected by intra-nasal inoculation (total dose  $10^{5.7}$  EID<sub>50</sub>) and four animals were mock-inoculated with egg fluid. Two infected pigs were subjected to post mortem examination at each of days 1, 2, 3, 5, & 7 of the study. Post-mortem examinations on mock-inoculated pigs were carried out on days 1, 3 & 7 post-challenge. A range of tissues were collected for histopathological examination and immunohistochemical detection of influenza A nucleoprotein and detection of viral RNA by matrix gene RT-PCR. Tracheal mucosa was removed and homogenised in lysis buffer using a gentleMACS™ dissociator (Miltenyi Biotec, Germany). Alveolar macrophages were collected by washing the left caudal/diaphragmatic lung lobe with PBS followed by centrifugation of bronchoalveolar lavage fluid and then homogenised by shearing through a needle and syringe. Following total RNA extraction and cDNA synthesis, cytokine gene expression was analysed by qPCR using TaqMan® primers and probes. Cytokine targets (IFN $\alpha$ , IFN $\beta$ 1, IL1 $\beta$ , IL6, IL8 and TNF $\alpha$ ) were determined using the standard curve method, normalised to 18S RNA.

**Results** Infection of the pigs with H1N1 A/England/195/09 resulted in mild clinical signs in infected pigs, with infection restricted to the respiratory tract. Virus antigen and RNA were detected in the upper respiratory tract of pigs between 1 and 7 days post infection. Levels of virus in the lower respiratory tract were lower and less widespread in their distribution. Pulmonary lesions consistent with influenza infection were only observed at dpi 5 & 7. Analysis of host gene expression showed low levels of interferon alpha expression, which exceeded that of mock infected pigs in only one animal. Interferon beta expression showed transient elevation in the tracheal mucosa of day 1 pigs but was undetectable in all other samples. IL1 $\beta$  expression appeared elevated in alveolar macrophages at dpi 3, while IL8 expression was higher in the tracheal mucosa and alveolar macrophages of some of the animals culled at dpi 2-5. IL6 expression in tracheal mucosa was highest at dpi 2 & 5 although there was considerable biological variation between individual animals on these days. No IL6 expression was observed in alveolar macrophages. Day 1 expression levels of TNF $\alpha$  in samples of tracheal mucosa were similar to those observed in the mock animal samples. However, between dpi 2-7, TNF $\alpha$  levels appeared higher than seen in the mock-infected animals. In alveolar macrophages, there was considerable variation in TNF $\alpha$  expression, both between individual animals and between days post infection. Considerable biological variation was also observed between the mock-infected animals.

**Conclusion** Experimental infection of grower pigs infected with H1N1 A/England/195/09 resulted in minor clinical signs, with viral pathogenesis restricted to the lower respiratory tract. Given the limited number of animals in this study, the significance of the cytokine expression data is difficult to assess. The absence of a significant up regulation of the type 1 interferons and the extent of pathological changes, suggest that infection in the group was relatively mild. Despite this limitation, the cytokine responses are in broad agreement with that previously reported for swine influenza in pigs (Van Reeth *et al.*, 2002).

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## Epidemiological investigation of the efficacy of PCV2 vaccination on PMWS severity in English pig farms

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**Introduction** Since the first appearance of post-weaning multi-systemic wasting syndrome (PMWS) in 1991 in Canada, the disease has spread widely throughout the world. From both an economic and welfare point of view, PMWS has become one of the most significant diseases for the pig industry. High levels of mortality and poor performance parameters due to PMWS continue to contribute to production losses. Consequently, looking for effective control measures has become a priority for the pig industry and the development of PCV2 vaccines had a huge uptake in the industry. In England, commercial PCV2 vaccines for piglets and sows are available since 2008. The objective of the study presented here was to assess the effect of PCV2 vaccination on changes in PMWS severity over time.

**Material and methods** Fifty farms which took part in a related cross-sectional study on PMWS were revisited between May 2009 and March 2010. Farm recruitment took place through a BPEX PCV2 vaccination programme and veterinary practitioners. The first visit to the farms took place before the start of PCV2 vaccination followed by a re-visit 8-12 months later. During both visits, an on-farm questionnaire was used to collect data on farm management, health status, vaccination programme, breeding performance, production parameters, genetics, and PMWS morbidity. In addition blood samples were collected and tested by ELISA for antibodies to PCV2 and for presence of PCV2 through a semi-quantitative PCR. Based on the protocol described by Alarcon *et al.* (2011), a PMWS severity score ranging from 0 to 10 was calculated for each visit, which allowed for classification as non/slightly affected, moderately affected or highly affected. Changes in PMWS severity score between the first and second visit were assessed for PCV2 vaccinating and non-vaccinating farms. A One-way ANOVA test and an appropriate paired t-test were used to compare mean values across different PMWS severity categories, and post-hoc analysis (Bonferroni test) was used to make further conclusions about different PCV2 vaccination groups (non vaccinated, piglet vaccinated and sow vaccinated).

**Results** The sample included 14 (28%) PCV2 non-vaccinated farms and 36 (72%) PCV2 vaccinated farms. Amongst the PCV2 vaccinated farms, 8 farms (22.22%) used a sow vaccine and 28 farms (77.78%) used a piglet vaccine. For six farms, some data were missing, and had therefore to be excluded from further analysis. There was a significant difference in change of PMWS severity score between non-vaccinated, piglet vaccinated and sow vaccinated farms ( $p < 0.01$ ). Further post-hoc analysis showed that there was a significance difference in change in PMWS severity score between both: (1) non vaccinated and piglet vaccinated farms ( $p < 0.01$ ); and (2) piglet vaccinated and sow vaccinated farms ( $p < 0.01$ ). Overall, the highest reduction in PMWS severity score mean (MD) was observed in “highly affected” farms ( $p < 0.01$ ), resulting in most farms moving from “highly affected” to “moderately affected”, followed by “moderately affected” farms ( $p = 0.01$ ). No statistically significant difference in PMWS severity score was observed amongst “non/slightly affected” farms ( $p = 0.99$ ). Table 1 below summarise the changes in PMWS severity score by severity category and by PCV2 vaccination group.

**Table 1** Summary of changes in PMWS severity score by PMWS severity category and by PCV2 vaccination status

Farm (N=44)	MD	SE	SD	95% CI
Non/Slightly affected (n=16)	0.01	0.44	1.79	-0.94; 0.96
Moderately affected (n=15)	2.54	0.48	1.83	1.50; 3.58*
Highly affected (n=13)	3.97	0.59	2.14	2.68; 5.27*
Non vaccinated (n=10)	- 0.66	0.45	1.44	-1.69; 0.37
PCV2 vaccinated (n=34)	2.84	0.37	2.19	2.07; 3.60*
- Piglet vaccine (n=26)	3.47	0.40	2.04	2.64; 4.29*
- Sow vaccine (n=8)	0.78	0.41	1.17	-0.19; 1.76

\* statistically significant:  $p$ -value  $< 0.05$

**Conclusions** These results demonstrate that “moderately affected” and “highly affected” farms have successfully managed to reduce the severity of PMWS through PCV2 vaccination. On the other hand, no statistically significant changes in PMWS severity were seen amongst PCV2 non-vaccinated or “non/slightly affected” farms. This may, however, be due to the fact that these farms had low severity scores at the time of the first visit and had therefore little room for improvement.

**Acknowledgements** The authors would like to acknowledge the BBRSC (grant BB/FO18394/1), BPEX, Biobest Ltd. and Pfizer Animal Health. We would also like to thank all the farmers and veterinarians for their help.

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## Dynamics of herd infection: Multiple enteric pathogens in young pigs

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**Introduction** Incidence of enteric disease is high in young pigs, particularly during the post-weaning period due to environmental and nutritional stressors. Infections with various enteric pathogens can occur simultaneously; the presence of a second pathogen in an already infected gut can result in increased pathogen adhesion and invasion and, as such, elevated disease symptoms (Di Biase *et al.*, 2000; Melin *et al.*, 2004). Although checks are in place to aid control of zoonotic pathogens such as the ZNCP scheme for *Salmonella*, these have been established on an individual pathogen basis. Co-infections with multiple pathogens may increase overall susceptibility to enteric infection and hence control of one pathogen may aid control of another. The objective of the current study was to establish the presence of Rotavirus and *Salmonella* in piglet excreta around the period of weaning, to identify potential periods of co-infections.

**Methods** Excretion of *Salmonella* and Rotavirus were tracked in pre- and post-weaning pigs from a commercial herd. Six batches, each containing 60-80 pigs, were tracked from birth to four weeks of age. Five time points around the period of weaning were selected for pathogen testing including two weeks prior to weaning, at weaning then one, two and four weeks post weaning. Ten faecal samples were selected at random from the pen floor of each batch, pooled and subjected to pathogen tests. *Salmonella* testing utilised a semi quantitative assessment where the sample was serially diluted prior to testing providing an indication of the pathogen levels present in the pen (Wales *et al.*, 2006). Rotavirus was detected using an ImmunoCard STAT Rotavirus kit for Group A Rotavirus by VP6 antigen detection.

**Results** Rotavirus and *Salmonella* were detected in sequential faecal excretions within all batches of the pig herd. Rotavirus was most predominant at weaning and preceded the excretion of *Salmonella*, which then always occurred post-weaning (Table 1).

**Table 1** Faecal samples testing positive for Rotavirus and *Salmonella* across the period of piglet weaning

Week number							
Batch number	-2	0 (Weaning)	+1	+2	+4		
1	-	+	+	+	+	+	+
2	+	+	+	+	+		
3	-	-	+	+	+		
4	+	+	+	+	+	+	+
5	-	+	+	+	+	+	+
6	+	+	+	+	+	+	+

Key	Negative	Rotavirus positive	<i>Salmonella</i> positive
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The semi quantitative results of pooled *Salmonella* tests varied across the batches from levels of  $10^1$  cfu.ml<sup>-1</sup> in batch 4 compared to  $10^4$  cfu.ml<sup>-1</sup> in batches 1,5 and 6. These higher levels of *Salmonella* positively correlated with the early presence of *Salmonella* in these batches (Table 1).

**Conclusions** The sequential nature of Rotavirus and *Salmonella* excretions within the pig herd supports the theory that early infection with one pathogen can result in a predisposition to infection by another secondary pathogen. The knowledge of co-infection dynamics will enable enhanced understanding and control of pathogens in pig herds.

**Acknowledgements** We acknowledge BBSRC and BPEX for supporting the project, and also the participating farm for their commitment and assistance.

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## British Pig Health Scheme: Use of slaughterhouse data to inform infectious disease control in pig farms

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**Background** Respiratory diseases result in considerable economic losses to the UK pig industry. Lesions resulting from respiratory disease e.g. pneumonia and pleuritis (pleurisy) are commonly found in pig lungs at slaughter and are often recorded to assess herd health. The British Pig Health Scheme (BPHS) is a voluntary monitoring system, which informs producers of the presence and levels of various respiratory lesions in their pigs at slaughter, with an emphasis on enzootic pleuropneumonia, enabling farm-level decisions to be made. The aim of this study was to assess whether the information provided by the BPHS through the monitoring of slaughter pigs is associated with the presence of respiratory pathogens on the farm, and also with farm management practices and productivity.

**Methods:** The current study used data from a cross-sectional study of postweaning multisystemic wasting syndrome (PMWS) described in detail in Wieland *et al.* (2010). Farms were recruited through the BPEX porcine circovirus type 2 (PCV2) vaccination scheme and through veterinary practitioners. Farms were visited before commencement of PCV2 vaccination and a detailed questionnaire regarding farm management was administered. Blood samples from twenty pigs (six weaners, six growers, six finishers and two sows) were collected from each farm and serologically tested for antibodies against *Antinobacillus pleuropneumoniae* (APP), swine influenza (SI), porcine respiratory and reproductive syndrome (PRRS), porcine parvovirus (PPV) and *Mycoplasma hyopneumoniae* (M. hyo) and tested for PCV2 using PCR. A total of 70 farms from the cross-sectional study were enrolled in the BPHS monitoring scheme and included in the present study. Farm-level serology, PCR and questionnaire results were combined with a single quarterly BPHS report. Principal components analysis (PCA) was performed in order to assess correlation between the proportion of pigs with different lesions and the proportion of pigs seropositive (or PCR positive, in the case of PCV2) for each pathogen. This technique allowed these variables to be condensed into a smaller number of 'components', representing patterns of pathology and infection. Finally, multivariable linear regression was used to identify management practices and respiratory pathogens associated with the principal components relating to BPHS-recorded pathology.

**Results:** Three BPHS components were identified using PCA, which together explained 71% of the total variance. Enzootic pneumonia (EP) score, severe pleurisy and acute pleuropneumonia had the highest loadings for the first component (BPHS 1), which accounted for 45% of the variation (Table 1). This component was therefore interpreted as representing farms with acute disease.

**Table 1** Components identified by PCA of BPHS variables

Components	BPHS 1	BPHS 2	BPHS 3	Unexplained variance
EP Score	0.5124	-0.2014	0.1288	38.5%
Severe Pleurisy	0.5572	-0.0446	-0.168	31.7%
Mild Pleurisy	0.1702	0.8085	0.1049	18.4%
Acute Pleuropneumonia	0.4024	-0.4229	-0.0676	45.2%
Viral-type Lesions	0.3106	0.1158	0.7917	13.4%
Pericarditis	0.3736	0.3338	-0.5592	25.7%

From the results of the blood tests, two components were retained from the PCA accounting for 80.1% of the total variance. Disease component A included farms with high levels of APP and SI. Farms with a high score for component B had high levels of APP and PCV2. Multivariable linear regression found farms with high scores for disease component A had increased BPHS 1 scores ( $p=0.02$ ). Also, an increase in farm's BPHS 1 score was associated with a decrease in the mean dead weight of slaughter pigs ( $p=0.001$ ).

**Conclusions** The results presented here indicate that, as expected, respiratory lesions identified at slaughter through the BPHS may be indicative of respiratory pathogens in the herd. In particular, increasing levels of APP and SI in the herd appeared to be associated with an increased average EP score, and increased proportion of pigs with signs of severe pleurisy and acute pleuropneumonia, and to a lesser extent, an increased proportion of pigs with viral-type lesions and pericarditis (those variables included in the BPHS 1 component). In addition, pigs from farms with higher BPHS 1 component scores were found to have lower mean slaughter weights suggesting that, providing producers take action in response to their reports, participation in the BPHS may have economic benefits. As such, further research is needed to fully validate the scheme and a full cost-benefit analysis of the BPHS is advised, including the costs and benefits of possible control options, in order to make the scheme as informative as possible for producers. Through these methods, producer participation with the scheme may be encouraged, which may result in the BPHS providing a valuable surveillance stream for identification of disease in British pigs.

**Acknowledgements** The authors gratefully acknowledge the BBRSC (grant BB/FO18394/1), BPEX, Biobest Ltd., and Pfizer for the funding this project.

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## Finding the mutation causing early calf mortality in a pedigree breed of cattle as the basis for a genetic test to identify carriers of an autosomal recessive condition

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**Introduction** A group of pedigree cattle breeders noticed an unusual number of inexplicable calf deaths in their breed. The calves appeared to be born normally but rapidly deteriorated after birth, and usually died within 10 days. The RVC was approached to see if the cause of this phenomenon could be isolated and a possible remedy devised. The initial epidemiology of the condition suggested a possible genetic cause. This paper outlines the results of combining pedigree and Mendelian analysis with the methodology of Charlier *et al.* (2008) to understand the genetics of the condition and devise the basis for a genetic test to identify carriers.

**Material and methods** Initially a survey was undertaken to investigate the extent of the condition and identify affected calves. Data were collected from 28 farms in the UK. Calf deaths were classified into 3 types relating to the reported condition; Type 1 – probable, Type 2- possible and Type 3 – not related to the condition. Pedigrees of all calves born on the farms were collected and merged with the breed society's database. Pedigree visualisation was carried out using Lineage software (J. Pollack; Personal communication). Calf mortality was analysed as a binomial trait using ASReml (Gilmour *et al.*, 2009) by fitting a model comprising sire, farm, year of birth and sex. Mendelian ratios for autosomal dominant and recessive, and sex-linked dominant and recessive, modes of inheritance were tested using a chi-squared test in SAS (2008). DNA was extracted from tail hairs of 8 Type 1 calves and 9 unaffected animals of both sexes and genotyped using the Illumina 54k bovine beadchip. Homozygosity testing was carried using Plink (Purcell *et al.* 2007) with 10kb windows and candidate areas of interest were identified within any homozygous segment where cases were autozygous for a SNP genotype which was absent in controls. Statistical significance of any candidate SNPs was calculated following the homozygosity score and core marker test methodology of Charlier *et al.* (2008). Build 4 of the Bovine Genome in the UCSC genome browser was used to identify candidate genes associated with any promising areas of the genome.

**Results** Data collected from the 28 farms comprised 881 calves (395 males, 455 females, 12 castrates and 19 of unrecorded sex), the offspring of 84 sires and 229 dams. There were 1,777 animals in the pedigree file (i.e. all animals and their ancestors, as far back as possible) developed from both the breed society's records and data collected on farms. A total of 72 calf deaths were recorded; 14 Type 1, 21 Type 2 and 38 Type 3. From the binomial analysis sire was the only significant ( $P = 0.041$ ) effect on calf mortality suggesting a genetic basis to the condition. Pedigree analysis indicated one bull in the pedigree of all Type 1 calves; this bull was considered to be the originator of the condition or very closely related to it. Chi-squared analyses of the four modes of inheritance eliminated both sex-linked and the autosomal dominant scenarios ( $P > 0.05$ ). This implicated an autosomal recessive mode of inheritance for this early-calf-death condition. The call rate for the 17 genotyped calves was 0.989 and only one contiguous run of homozygosity was identified for all 8 affected calves. A further 2 sections of DNA were identified common to 7 calves and a further 2 were common to 6 calves. SNPs from these 5 segments of DNA were inspected for the required genotypic ratios. Only the segment common to all 8 dead calves contained SNPs which were homozygous for one allele in all the dead calves, this homozygous genotype being absent in the 9 unaffected animals. Four adjacent candidate SNPs had the same genotypic pattern. This was the only region of the genome achieving significance for both the homozygosity score and the core marker test ( $P < 0.05$ ). Taking a region of the bovine genome from one extra SNP either side of the 4 candidate SNPs, two protein-coding segments of DNA were suggested as possible candidate genes for the early-calf-death syndrome. These were a phosphatase gene involved in the cell stress response pathway and an amino acid transporter gene. The DNA segments containing these two genes will be sequenced in order to identify a possible mutation causing this condition.

**Conclusions** A combination of genetic skills and methodology was used to confirm the mode of inheritance of an autosomal recessive disorder and to identify the likely site of the mutation involved. Once the mutation has been identified it should be possible to use this information to devise a relatively inexpensive genetic test to identify carriers of the condition. The results of this test could then be used to plan matings and eliminate the gene from the population.

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## Polymorphisms in mitochondrial transcription factor A (TFAM) are associated with growth and fertility in dairy cows

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**Introduction** Growth, fertility and milk production are highly important quantitative traits that are potential targets for marker assisted selection to improve the performance of the dairy cow. This study focused on single nucleotide polymorphisms in the nuclear encoded mitochondrial transcription factor A (TFAM) gene, which encodes a histone-like protein essential for transcription and replication of mitochondrial DNA (Jiang *et al.*, 2005). As this protein controls mitochondrial functionality, polymorphic variants in the gene may affect energy production and subsequently many important traits in the dairy cow. Therefore, the objective of this study was to determine whether two SNPs – TFAM1 (C/T) and TFAM3 (G/A) both located in the TFAM promoter (Jiang *et al.*, 2005, 2006) were associated with growth and fertility in UK dairy cows.

**Material and Methods** Weight, height at withers, crown rump length and heart girth were measured on Holstein-Friesian heifers (n= 509) at 1 month (28 ± 0.8d), 6 months (184 ± 0.8d, pre-pubertal) and 15 months (452 ± 3d, post-pubertal, at start of service period) of age. During the first lactation, the age at calving, number of services for conception, days to conception, the prevalence of the animal being in calf 100 days post-partum, conception rate and the calving interval were recorded. SNP genotyping was performed by Eurofins Medigenomix GmbH, Martinsried, Germany using a MassARRAY Compact Analyzer (Sequenom Inc., USA). Mixed model analyses were performed in ASREML (v2.0) to determine whether the SNPs were associated with the traits measured. For the growth analyses, fixed effects were the herd (1-21), year (2003-04) and season (1= March-May, 2= June-Aug, 3= Sep-Nov and 4= Dec-Feb) of birth. Age was fitted as a 4<sup>th</sup> order polynomial to account for changes in growth over time. For the fertility models, fixed effects were herd-year-season of calving, age at calving and milk per day. Pedigree information was collected for each animal for the preceding 3 generations (n=2251) to fit a random animal effect. A false discovery rate (FDR) was used to correct for multiple testing.

**Results** TFAM1 and TFAM3 were in close linkage disequilibrium ( $r^2 = 0.85$ ) so only TFAM3 was studied further. Allele frequencies of TFAM3 were: G= 0.71 / A= 0.29 and were distributed according to the Hardy-Weinberg equilibrium expected values. Results from the association analyses are presented in Table 1. Genotype substitution effects were calculated for traits with significant associations. TFAM3 was significantly associated with all growth parameters apart from weight, where a trend was observed. In general, GG animals were the largest and AA animals the smallest. TFAM3 was also associated with many aspects of fertility such as services for conception, days to conception, in calf by 100 days and the calving interval. Here, heterozygotes had consistently the best fertility, being more likely to conceive (by 12% cf AA) and requiring fewer services (by 0.4 cf GG) and days for conception (14 d cf GG).

**Table 1** Phenotypic measures, mean ± SE (n = 509) and SNP associations with TFAM3 (probability values)

Trait	1 month	6 months	15 months	P	Genotypic Effects
Weight (kg)	80 ± 0.2 (499)	104 ± 0.3 (482)	126 ± 0.3 (430)	0.073	
Height at Withers (cm)	56 ± 0.7 (497)	175 ± 1.7 (463)	373 ± 2.4 (438)	0.039 <sup>a</sup>	GG > AG = AA
Crown Rump Length (cm)	94 ± 0.4 (500)	135 ± 0.5 (482)	169 ± 0.5 (431)	0.036 <sup>a</sup>	GG = AG > AA
Heath Girth (cm)	89 ± 0.4 (500)	131 ± 0.4 (482)	174 ± 0.5 (430)	0.016 <sup>a</sup>	GG > AG > AA
1 <sup>st</sup> Lactation					
Age at Calving (d)	792 ± 5.3 (395)			NS	
Services for Conception (n)	2.29 ± 0.1 (355)			0.005 <sup>a</sup>	AG = AA < GG
Days to Conception (d)	132 ± 5 (374)			0.003 <sup>a</sup>	AG = AA < GG
In Calf by 100 days (%)	44 ± 2.5 (389)			0.029 <sup>a</sup>	AG > GG = AA
Conceived (%)	93 ± 1.3 (386)			0.050	AG = GG > AA
Calving Interval (d)	414 ± 5 (343)			0.009 <sup>a</sup>	AG = AA < GG

The number of records analysed are in parenthesis. <sup>a</sup> = significant after correction for FDR. NS = not significant

**Conclusion** Polymorphisms in TFAM have previously been associated with subcutaneous fat depth and marbling in beef cattle (Jiang *et al.*, 2005), however, no studies have considered associations with economically important traits in the dairy cow. These results indicate that TFAM SNPs are associated with growth and fertility parameters, whereby the heterozygotes had an intermediate growth but the best fertility. TFAM SNPs are therefore likely to be useful for marker assisted selection to improve fertility in the dairy cow.

**Acknowledgements** The authors gratefully acknowledge the support of Merial Animal Health Ltd and the BBSRC.

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## Results of genotyping UK dairy bulls with a high density DNA array

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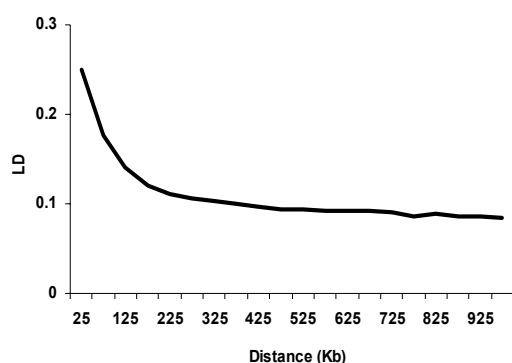
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**Introduction** The bovine high density DNA array comprising circa 50,000 Single Nucleotide Polymorphisms (SNP) became available in 2008 and is currently being used internationally to genotype dairy bulls and cows with the aim to enable early-life genomic selection. Additional uses include detection of Quantitative Trait Loci (QTL) and gene mapping. In the UK, a number of dairy bulls were genotyped with this array during 2009-2010. The objective of this exercise was to characterise the genotyping results of these bulls before they are used to calculate genomic predictions.

**Material and Methods** Data consisted of genotypes of 325 UK Holstein bulls for 53,032 SNP loci derived with the Illumina 50K DNA array; total number of records was 17,235,400. Genotypes with a GC score (provided by the genotyping lab) of less than 0.20 were considered unreliable and removed from further analysis; the GC score is a measure of genotyping quality and has a maximum value of 1 indicating highest quality and reproducibility of results. The imposed edit removed 88,629 individual genotype records from the database. The average GC score in the remaining genotypes was 0.90. Further edits removed 566 SNP loci with unknown chromosome number and/or undefined position on the chromosome, as well as 7,069 loci with no variation (i.e., the same base was present in all individual bull genotypes). In addition, loci with minimum allelic frequency lower than 0.01 were considered monomorphic and removed (1,691 loci). At the end of these edits, 43,706 SNP loci (82.4% of the original total) were kept for further analysis. Allelic frequencies and a chi-square test were used to examine the Hardy-Weinberg equilibrium (HWE) state in each locus. Linkage disequilibrium (LD) was calculated between pairs of syntenic SNP loci using the software programme PLINK (Purcell *et al.*, 2007). The squared correlation value proposed by Hill and Robertson (1968) was used as an LD estimate.

**Results** The four bases (A, T, G and C) were found in relatively equal frequencies. HWE was confirmed in all but 3.4% of the analysed loci. Six hundred and ninety one loci were associated with extreme chi-square values (>300). Although such loci may be linked with important traits in the selection index, the possibility of bias in the animal genomic prediction needs to be investigated. Average expected and observed heterozygosity levels were practically the same (35% in each case), except for loci not in HWE where a considerable heterozygote deficit (average of 14%) was observed. Mean (SD) LD of 43,616 adjacent loci was 0.22 (0.28) with an average distance of 59,710 bases (b); corresponding values for 386,657 loci within 1 Mb intervals were 0.13 (0.18) and 284,639 b. These levels of LD are somewhat lower than those from a study of North American Holstein bulls, where the average LD of adjacent SNP loci was 0.31 (Sargolzaei *et al.*, 2008). Different

methods of LD assessment and different DNA arrays used in the two studies probably explain these differences. Average LD decreased with increasing distance between SNP locus pairs, as shown in Figure 1. This phenomenon is known as LD decay and has been observed in other similar studies (Sargolzaei *et al.*, 2008). As expected, higher LD levels were observed for loci within at least 100 Kb. Table 1 shows number of SNP locus pairs with LD greater than 0.30, which is considered the minimum useful LD value for genomic prediction (Meuwissen *et al.*, 2001).



**Table 1** Analysis of SNP located in 1 Mb intervals with LD greater than 0.30

Number of SNP locus pairs	46,949
Average distance between pairs (b)	212,487
Proportion of SNP locus pairs in this interval	12.15%
Average (SD) LD	0.54 (0.21)

**Figure 1** Average LD in relation to loci distance

**Conclusions** Genotyping results of UK Holstein bulls revealed reasonable levels of polymorphism and LD in the studied loci meaning that useful haplotype information can be derived for mapping purposes, QTL detection and genomic selection. The dataset studied is deemed suitable for the calculation of genomic predictions of dairy bulls. However, use of the recently released array comprising 800,000 SNP will probably improve all parameters presented here and increase the accuracy of genomic predictions.

**Acknowledgements:** Thanks to Cogent Breeding for making the genotypes available, Marco Winters for facilitating and Ian Archibald and Ross McGinn for curation of the data.

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**A meta-analysis of public microarray datasets reveals the transcriptional immune response to multiple pathogens in the chicken**

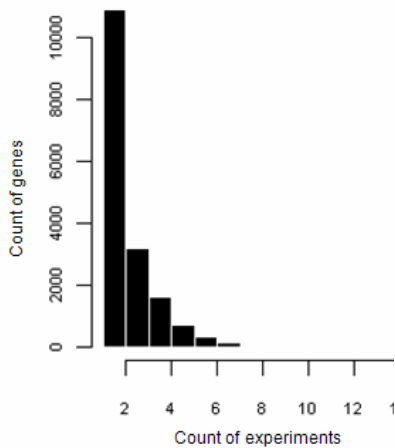
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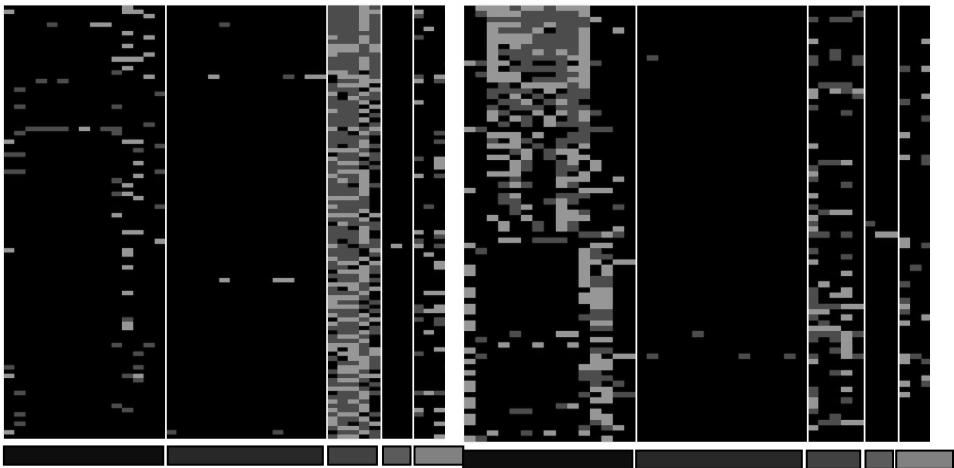
**Introduction** DNA microarrays allow researchers to profile the expression of 1000s of genes in a single assay. In tandem with the development of microarrays themselves came the development of public repositories for the data, including the Gene Expression Omnibus at NCBI and ArrayExpress at the EBI. These databases hold hugely valuable data on the expression of genes across a number of different organisms, tissues, cell types, conditions and diseases. Here we present a meta-analysis of immune challenge experiments in the chicken from GEO, and show how valuable information can be mined from these public repositories.

**Methods** Analysis of GEO identified 13 series involving an immune challenge experiment in chickens, including expression profiling of *Salmonella*, *Lactobacillus*, *IBV*, *IBDV*, and *Eimeria* infections. Many of these series have complicated experimental designs testing multiple factors (e.g. infective species and time), and these were split into smaller designs that tested only one factor. This resulted in 44 datasets to be analysed. The normalised data were downloaded from GEO and run through limma to test for differential expression (FDR <= 0.05). Probes for each of the platforms were mapped to Ensembl gene identifiers using a combination of biomaRt, NCBI BLAST and supplier annotation. Lists of genes significant in a variety of experiments were tested for enrichment of GO terms and KEGG pathways using CORNA.

**Results** A total of 17,000 transcripts are differentially expressed in at least one of the 44 comparisons. Out of 44 datasets, the maximum number of datasets that any gene was differentially expressed across was 14, with an average of 2.3 (fig .1). Over 500 genes were differentially expressed in six or more comparisons. Table 1 shows enriched KEGG pathways, and table 2 shows enriched GO terms within this dataset. Figure 2 shows immune response genes ordered according to viral pathogens, and figure 3 shows genes ordered according to *Salmonella*.



**Figure 1** Distribution of genes according to the number of experiments in which they were found to be significant



**Figure 2** Viral response genes (third group). Many differentially expressed genes (grey) are unique to the viral response.

**Figure 3** *Salmonella* response genes (first group). Many differentially expressed genes (grey) are unique to the *Salmonella* response.

**Table 1** Enriched pathways

Pathway Title	P-value
Toll-like receptor signaling pathway	6.20E-05
NOD-like receptor signaling pathway	6.20E-05
Intestinal immune network for IgA production	0.00029
PPAR signaling pathway	0.00054
Cytokine-cytokine receptor interaction	0.001
Cytosolic DNA-sensing pathway	0.025

**Conclusions**

A huge number of microarray datasets have been deposited in public databases over the last 15 years, representing a valuable source of information on the expression of 1000s of genes across many species and pathogens. We show here that there is a large universe of differentially expressed genes when taking into account all pathogens across all tissues, cell lines and chicken lines. We also show that many expected functions and pathways are differentially regulated, demonstrating that our method works and that valuable information is stored within these databases. Finally, we show that the genes that respond to the different classes of pathogen can be very different, with little overlap between those sets of genes that respond to viral pathogens versus those that respond to *Salmonella*.

**Table 2** Enriched GO terms

GO description	P-value
immune response	9.80E-07
cytokine activity	7.10E-06
regulation of apoptosis	0.00028
apoptosis	0.00028
cytoplasm	5.00E-04
chemokine activity	0.0042

## Use of non-invasive methods to collect DNA for genome wide analysis from companion animals

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**Introduction** In the United Kingdom, Home Office requirements ensure the welfare of animals used in research. In particular, invasive procedures involving companion animals are discouraged and hence phlebotomy to obtain blood for DNA extraction from healthy animals requires a home office licence. Since DNA can be isolated from any nucleated cell source, we ascertained whether there are cell types which can be obtained non-invasively that can be used for genome-wide analysis of single nucleotide polymorphisms.

**Material and methods** To compare different sources of nucleated cells, saliva (Oragene.ANIMAL/saliva kit; DNA Genotek), buccal cells (Isohelix kit; Cell Projects), freshly pulled hair bulbs and EDTA blood samples were taken from three client owned Cavalier King Charles Spaniels at the R(D)SVS, Hospital for Small Animals (HSA) that were having routine blood screens. DNA was extracted from 100 µL whole blood or 10 hair bulbs using the DNeasy blood and tissue kit (Qiagen) following the appropriate protocol. DNA was extracted from saliva (two collection sponges pooled) and buccal cells (one swab) following the manufacturers' instructions. A single sample of each tissue type was analysed from each animal. Genome wide genotyping was performed using the Infinium HD Assay Ultra Canine VI BeadChip (Illumina) according to the manufacturer's instructions, using 200 - 400 ng of DNA. Results were compared using the R statistical environment (<http://www.r-project.org/>).

**Results** We obtained cells from three dogs (two males, one female), with consent from the owners. Yields of DNA from all four sources were adequate for the analysis (Table 1), varying from 0.4µg (a buccal cell sample) to 11µg (blood and hair bulbs). Genotypes obtained using the Illumina SNP chip were compared for four DNA sources for each animal. 1722 genes failed to give a result in all three dogs and all four tissue types, a failure rate of 0.99%. In addition, a further 114 could not be scored in all four tissues in at least one dog, and three could not be scored in at least one tissue in all three dogs.

The genotypes were consistent across all four tissue types. A total of 14 SNPs (of more than 170,000 sampled) showed inconsistent genotypes. The loci involved were different for each dog. Individual 1 had seven discordant loci, with saliva discordant in six and buccal cells discordant in one. There was no pattern with respect to homozygous or heterozygous discordancy. Individual 2 had four discordant loci, two for buccal cells, one for saliva and one for blood. Individual 3 had five inconsistent genotypes, with saliva discordant in two, hair discordant in one, one locus with all three possible genotypes and one with buccal cells and saliva showing the heterozygous genotype and hair and blood showing one homozygous genotype. Cells sampled from the mouth were the most likely cell source to give a discordant result (saliva: mean of 3 (SD 2.64) discordant per sample; buccal cells: mean of 1.0 (SD 1.0) discordant per sample).

**Table 1** Total DNA yield (µg) from different tissues

ID (sex)	1 (male)	2 (male)	3 (female)	Mean (SD)
Yield from 100 µL blood	1.5 µg	2.8 µg	0.23 ug	1.51µg (1.28)
Yield from 20 hair bulbs	2.8µg	2.2 µg	0.72 µg	1.91µg (1.07)
Yield from two saliva sponges	10.6 µg	4.3 µg	8.6 µg	7.83 µg (3.22)
Yield from one buccal swab	0.80 µg	0.69 µg	0.13 µg	0.54 µg (0.40)

**Conclusions** We obtained a very high consistency in genome wide genotypes using DNA from different cell sources with only 14 discordant genotypes, a failure rate of 1 in 12,404. This shows that valid SNP-chip studies can be done with DNA from non-invasive sources such as cheek cells (from swabs), saliva or hair bulbs in addition to blood. Since swabs or saliva sponges can easily be sent to owners for sampling, and Home Office licences are not required for this form of cell collection, the study will facilitate the collection of the numbers of samples required for genetic analysis.

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## Genome wide analysis of mitral valve disease in Cavalier King Charles Spaniels

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**Introduction** Myxomatous thickening and secondary poor coaptation of the mitral valve is a major cause of morbidity and mortality in dogs, with incidence increasing with age in all dog breeds. Mitral valve disease can be detected as a murmur upon auscultation, graded from 1 to 6 with increasing severity. The Cavalier King Charles spaniel (CKCS) is particularly prone to this condition and many animals have a severe early onset form of the disease. A recent study (Lewis *et al*, 2010) indicated a high heritability of both presence/absence and grade of murmur in CKCS, when assessed between age 4 and 5 years. Because of the highly inbred nature of the breed (with a small number of popular sires since registration of the breed with the UK Kennel Club in 1944) it is likely that the genetic variants involved in determining the early onset mitral valve disease are identical by descent (IBD) in all affected CKCS today. We therefore used a genome-wide genetic analysis to assess whether there is evidence for a small number of genetic loci segregating in this breed and to determine potential inheritance patterns.

**Material and methods** All dogs were client owned purebred CKCS. Samples were obtained either at the R(D)SVS HSA or at breeding kennels. All samples were taken with owner consent. Where possible detailed pedigrees were obtained from the owners and registration records. All animals were auscultated over all valve areas by a boarded Diplomat in Veterinary Cardiology (ATF). The location of any murmurs was noted and all murmurs were graded on a 1-6 scheme. Information was obtained from the owner on age of onset of any murmurs and was verified from previous auscultation records. DNA was obtained from buccal swabs (Isohelix kit; Cell Projects) following the manufacturer's instructions. Genome wide genotyping was performed using the Infinium HD Assay Ultra Canine VI BeadChip (Illumina) according to the manufacturer's instructions, using 200 - 400 ng of DNA. Results were analysed using the R statistical environment and the program Homozygosity. Genome wide association analysis (GWAS) was performed using a number of different models.

**Results** Thirty six CKCS were evaluated for mitral valve disease. Eighteen had a detectable murmur before the age of 5 years (median murmur grade 2.5 at median age 6.5 years). The remainder had no murmur at age 7 years. The median age of these 18 at most recent examination was 11.2 years and the mean grade of murmur at that time was 2.5 (range 0 – 4). Thus the population was divided into animals with early onset of a murmur and those with late onset or no murmur at advanced age. Genotypes of these 36 animals were assessed for evidence of linkage or association with mitral valve disease. Amongst the SNPs, 68,475 (39.4%) were homozygous in all 36 animals (fixed) consistent with an estimate of 55% heterozygosity (45% homozygosity) from an analysis of genetic variation in microsatellite loci. In this series of animals, 2,110 SNPs failed to produce a result, a failure rate of 1.2%. In addition, 159 SNPs were heterozygous in every animal, and were therefore removed from the analysis. This left 102,918 (59.3%) SNPs for the analysis of linkage with mitral valve disease.

The high heritability of mitral valve disease (Lewis *et al*, 2010) suggested that there could be one or a small number of genes involved in determining mitral valve dysfunction. Initially we looked for regions which were homozygous in either the early onset or late onset animals, indicating possible recessive or dominant inheritance respectively of the early onset condition. The longest region of homozygosity in early onset animals was 33 SNPs. All but one of the late onset animals were also homozygous for this region. The longest region of homozygosity in late onset animals was 19. For this region 15 of the early onset animals were homozygous for the same allele, one was homozygous for the other allele and two were heterozygous. These results indicate that there are no regions of highly discrepant homo/heterozygosity in the two groups. We performed a genome wide linkage analysis to see whether there was any evidence for loci associated with mitral valve disease, using a number of models for analysis. There were no significant peaks of linkage. The lowest P value found was  $10^{-3.5}$ . A value of less than  $10^{-6}$  was considered significant.

**Conclusions** Although mitral valve disease has a high heritability in CKCS dogs, our analysis suggests that this is not due to a single major gene effect. This means that breeding strategies to eliminate the disease cannot be based on genotype information at this time.

**Acknowledgements** We thank the owners for allowing their dogs to be used in this study. We are grateful for funding from the Royal College of Veterinary Surgeons.

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## Characterisation of a disease resistance QTL in Atlantic salmon using next-generation sequencing

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**Introduction** The viral disease Infectious Pancreatic Necrosis (IPN) causes morbidity and mortality in Atlantic salmon (*Salmo salar* L.) aquaculture stocks. Outbreaks of IPN can affect farmed salmon during two specific windows of the life cycle; the early freshwater lifecycle stage (typically 30-80% mortality), and shortly after transfer to seawater as post-smolts (typically 5-30% mortality). We have mapped a QTL with a major effect on resistance to linkage group 21 (Houston *et al.*, 2008). The QTL has a striking effect on mortality rate at both lifecycle windows, and explains almost all the genetic variation in host resistance. The QTL effects are consistent in controlled experimental challenges (Houston *et al.*, 2010) as well as field challenge conditions, and it has been applied using linkage-based marker-assisted selection in commercial salmon breeding programmes. We are now aiming to move towards identifying the underlying causal gene using genomic and transcriptomic approaches. There is no reference genome sequence or dense SNP marker map yet available for Atlantic salmon, which hinders this process. However, next-generation (NG) sequencing technology is providing new opportunities for SNP discovery and high resolution mapping, and for detailed transcriptome profiling. The major objective of this study was to discover and verify new SNP markers in the QTL region using a novel NG sequencing approach, and to test the population-wide association of these SNPs with IPN mortality.

**Material and Methods** Families of salmon fry from the commercial broodstock of Landcatch Natural Selection Ltd were IPNV challenged under controlled conditions as described in Houston *et al.* (2010.) QTL-segregating families were identified by genotyping 3 microsatellite markers (BHMS217, Rsa476 and Alu333) and standard linkage mapping analysis using test mortality/survival as the binary phenotype. Two families where all parents were QTL-heterozygous were chosen for sequencing and SNP discovery, with all parents and a subset of QTL-homozygous offspring chosen for sequencing. A method known as restriction-site associated DNA (RAD) sequencing was applied, where genome-wide sampling can rapidly provide high-throughput SNP detection and simultaneous verification in genomes with or without a reference sequence (Baird *et al.* 2008.) Briefly, the genome was restricted using a rare-cutting restriction enzyme (*Sbf*I), tagged with an adaptor containing a nucleotide barcode, sheared and pooled samples sequenced at high depth (Illumina technology). Custom software was used to align and cluster the reads into putative loci and to call SNPs, informed by the observed Mendelian segregations. Putative SNPs matching the pattern of the QTL genotype were then converted to a high-throughput genotyping assay where possible, and screened across a larger population by Kbiosciences Ltd. The linkage and association of the new SNPs with IPN mortality was assessed.

**Results** The initial results from the sequencing of one family (parents and eight offspring) resulted in the sequencing of approximately 70k genomic sites containing approximately 11k putative SNPs. After excluding known repeat sequences, 23 putative SNPs completely matched the segregation pattern of the IPN QTL, and ten of these were successfully genotyped across a population of IPNV challenged fish (n~1400). Preliminary linkage analysis mapped these SNPs to the QTL region of LG 21 and demonstrated significant linkage to the IPN mortality phenotype. Interestingly, several of the SNPs were highly significant in an association analysis with IPN mortality across families, the most significant of which had an additive effect on mortality of ~25% per copy of the susceptibility allele ( $P \sim 10^{-74}$ ).

**Conclusions** The RAD sequencing method of genome sampling is a rapid and cost-effective route to the global and targeted discovery and verification of SNP markers in Atlantic salmon, a non-model species. Preliminary population-wide analyses of the QTL-linked SNPs suggest the discovery of SNPs in strong linkage-disequilibrium (LD) with the QTL, which suggests they have potential as LD-based genetic tests for IPN resistance. The results also provide a platform for more detailed comparative genomic and functional analyses to identify candidate genes in the QTL region.

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## Genome-wide Association using a 60k SNP chip to explore the genomic control of boar taint in pigs

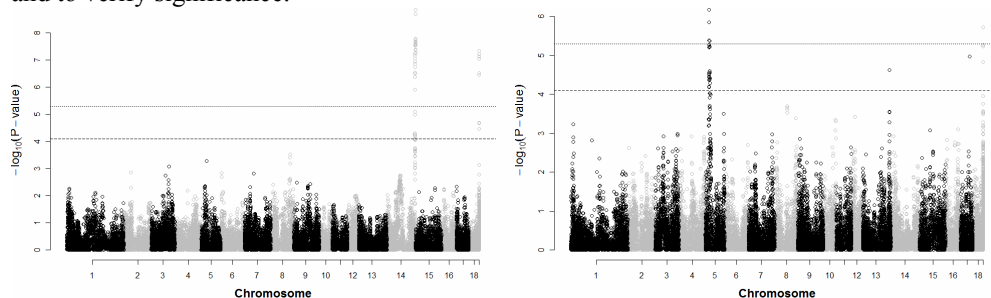
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**Introduction** Androstenone and Skatole accumulate in the fat of mature intact (i.e. non-castrated) male pigs. These compounds can be detected as boar taint - an offensive odour affecting the smell and taste of the cooked product. Genetic variation has been shown to exist for levels of these compounds and provides a more sustainable solution than castration for commercial pig production. Objectives were to investigate genomic regions associated with Androstenone and Skatole within Landrace pigs.

**Material and methods** A high density genome-wide association (GWAS) study utilising a 60K SNP chip was carried out to explore variation throughout the genome including biological pathways and interactions for Androstenone and Skatole. Data were selected from 6000 Danish landrace pigs. Five hundred individuals with high skatole ( $>0.3 \mu\text{g/g}$ ) at slaughter were selected and each matched with a low skatole litter mate. Phenotypic measurements for Androstenone were subsequently collected for the same 1000 individuals. Genotyping was done using the Illumina SNP60 porcine beadchip. Quality control (QC) was an iterative process performed using the GenABEL package (Aulchenko *et al.*, 2007) in R 2.9.1 software. Criteria for SNPs were call rate  $> 0.95$ , MAF  $> 0.01$ , and for individuals were call rate  $> 0.95$ , heterozygosity  $> 0.45$  (1% FDR) and IBS  $< 0.95$ . After QC 44,648 SNPs and 938 individuals were included in the final analysis. Trait distributions for both traits were positively skewed and were log transformed prior to analysis. Heritabilities and fixed effects of meat percentage, slaughter weight and age at slaughter were estimated using a linear mixed model in software package ASReml 2 (Gilmour *et al.*, 2006). The SNP based relationship matrix from GenABEL was fitted in the model to estimate polygenic effects. GWAS was performed using a GRAMMAR analysis in GenABEL software. GRAMMAR uses score tests to test for association between residuals and trait phenotype after fixed and polygenic effects are accounted. To account for population substructure IBS data were modelled using multidimensional scaling (mds) in the Mclust package in R software to determine the number of independent clusters. The co-efficients from the mds were subsequently fitted in the linear model as covariates. Permutation analysis (10,000) and an FDR cut off of  $<0.05$  were used to correct for multiple testing. Significant SNP genotypes were fitted individually as covariates in the linear mixed model to estimate SNP effects and to verify significance.



**Figure 1** Manhattan plots of genome wide association with Skatole (left) and Androstenone (right). Dotted line genome-wide significance, and dashed line FDR $<0.05$ .

**Results** The effect of the CYP2E1 gene on SSC14 was confirmed as genome-wide significant  $P < 1.4\text{E}-09$  explaining 6% of the phenotypic variance for Skatole (Figure 1). Interestingly, this locus did not affect levels of Androstenone. Significant SNP effects on Androstenone were seen on SSC5  $P < 6.8\text{E}-07$  explaining 4% of phenotypic variation (Figure 1). Interesting SNPs were also found on SSC2, SSC3, SSC5, SSC6, and SSC8 for Skatole and on SSC5, SSC7 and SSC13 and the X chromosome for Androstenone.

**Conclusions** We have shown evidence for the genetic control of Skatole and Androstenone. Further work will also involve analysis of the data using a genomic selection type approach to explore effects of analysing SNP's simultaneously. This has implications for breeding programs to reduce boar taint with substantial associated animal welfare, food quality and financial benefits.

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## Bayesian modelling to estimate the sensitivity of the Immuno-Magnetic Separation (IMS) method of detecting *Escherichia coli* O157 in bovine faecal samples

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**Introduction** *Escherichia coli* O157 (*E. coli* O157) is an enterohaemorrhagic bacterium that causes food-borne illness in humans; cattle and other ruminants act as a natural reservoir. Due to the significant role of the food chain in human infection, the detection of infected animals in a herd is essential to prevent contamination of food. The Immuno-Magnetic Separation (IMS) method is a relatively rapid, simple and sensitive method to detect *E. coli* O157 in bovine faeces, relative to the conventional direct plate count (PC) method. The IMS method classifies animals as positive or negative for infection status. From a statistical viewpoint, a positive test result can be thought of as an outcome of the sensitivity of the test given an underlying random variable. Hence, the objective of the present work was to use a Bayesian approach to model the relative contribution of different sources of variation and estimate the sensitivity of the IMS method for detecting *E. coli* O157 in bovine faecal samples.

**Material and methods** Faecal samples were collected from 12 different pats of housed and grazing cattle in two experimental blocks. Different faecal pats were artificially inoculated at seven different concentrations (1.00E-01, 1.00E+00, 1.00E+01, 1.00E+02, 1.00E+03, 1.00E+04 and 1.00E+05 CFU per g faeces) of six different strains of *E. coli* O157. A total of 504 samples were evaluated by the IMS method. To estimate the concentration of *E. coli* O157 in the inocula, a total of 280 samples prepared from the initial nominal concentrations of stock solutions were evaluated by the PC method at two different time points. Samples were randomly allocated to two laboratory operators for evaluation by IMS or PC methods. An integrated generalised linear mixed model was fitted to IMS and PC data in a Bayesian framework. The event of a positive test result by IMS was modelled as a Bernoulli random variable with a logit link function. The model included the fixed effects of strain, laboratory operator and log of initial nominal concentration of the bacteria and the random effect of pat. The observed counts from the PC data were modelled as Poisson random variables with a logarithmic link function. The model included strain, laboratory operator and time of inoculation as fixed effects and the logarithm of initial concentration of bacteria as an offset variable. The prior distributions for the fixed effects were non-informative normal distributions and the prior distribution for the standard deviation of pat was a uniform distribution (min 0 and max 100). Inferences about the parameters of the model were obtained within a Bayesian framework using Markov Chain Monte Carlo (MCMC) inference in the WinBUGS software (Spiegelhalter *et al.*, 2003). The output from the model was used to estimate the sensitivity of the IMS method for a single *E. coli* O157 colony forming unit (CFU) which was defined as the probability of detection of single CFU (at the concentration level 1.00E+00) by the IMS method. The single-CFU sensitivity of the IMS method was used to calculate the number of bacteria required at the inoculation (pre-enrichment) stage to achieve 50, 75, 95 and 99% sensitivity by the IMS method.

**Results** The model demonstrated that the initial nominal concentration, strain and pat were important sources of variation in the sensitivity of the IMS method. The posterior mean for the effect of the log concentration of *E. coli* O157 was 0.90 (95% credible interval, 0.72, 1.12). The pat variance was estimated as 0.27 (95% credible interval, 0.07, 0.68). The sensitivity of the IMS method was the lowest for strain 4. The results showed that the required initial number at pre-enrichment stage for strain 1, 3, 5 and 6 should be around 8-9 CFU per g faeces for a 99% chance of recovering one or more *E. coli* O157 colony by the IMS method (Table 1).

**Table** Required CFU per g faeces at the pre-enrichment stage to achieve 50%, 75%, 95% and 99% sensitivity by the IMS method

Strain	50%	75%	95%	99%
50C1496	1	3	6	9
51C2128-5	2	4	9	14
51C227-1	1	2	5	8
51C573	7	14	30	46
52C1251	1	2	5	7
57C1066	1	2	5	8

**Conclusions** By incorporating variability and uncertainty associated with different aspects of the IMS method, the integrated Bayesian model is useful in estimating sensitivity and in obtaining estimates and credible intervals for the different factors that influence this sensitivity.

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## The impacts of climate change on *Fasciola hepatica* risk in the UK

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**Introduction** *Fasciola hepatica* (liver fluke) is a physically and economically devastating parasitic trematode whose rise in recent years has been attributed to climate change. The National Animal Disease Information Service currently provide farmers with short term fascioliasis risk based on the Ollerenshaw index (Ollerenshaw & Rowlands, 1959); a model driven by the influence of rainfall and temperature on the free living stages of the parasite and its intermediate host *Lymnaea truncatula* (dwarf pond snail). Here we combine the Ollerenshaw index with long term past climate data (UKCIP) to demonstrate how climate has driven the change in fascioliasis risk to date. We then use the UK met office climate projections (UKCP09) to determine the potential impacts of climate change on future fascioliasis risk in the UK.

**Material and methods** For the calculation of past fascioliasis risk (1970-2006), the Ollerenshaw index was applied to climate data from the UKCIP gridded datasets using the following parameters: minimum, maximum and mean monthly temperature (°C), number of rain days per month (>1mm), and monthly rainfall (mm). For predicting future risk a modified Ollerenshaw index was applied to the UKCP09 climate data, at 25km square resolution, from 2020 to 2070. The parameters used for the modified index were mean, maximum and minimum temperature (°C), precipitation (mm/day), total cloud (%) and relative humidity (%). As the climate projection data does not include rain days, a modified index was used in calculating future risk. For this a generalized additive model (GAM) was used to calculate a surrogate rain days value using mean temperature, cloud cover, rainfall and humidity. The GAM was validated using past climate data, showing that there was no significant difference in fascioliasis risk calculated using the original and modified approach (Paired t-test,  $t=1.42$ ,  $df = 1052$ ,  $p\text{-value} > 0.1$ ). Summer and winter seasonal risk values were calculated by summing monthly risk values for the relevant months, where mean temperatures were above the parasites 10°C development threshold. Fine scale risk maps for past and future risk were generated using ArcGIS.

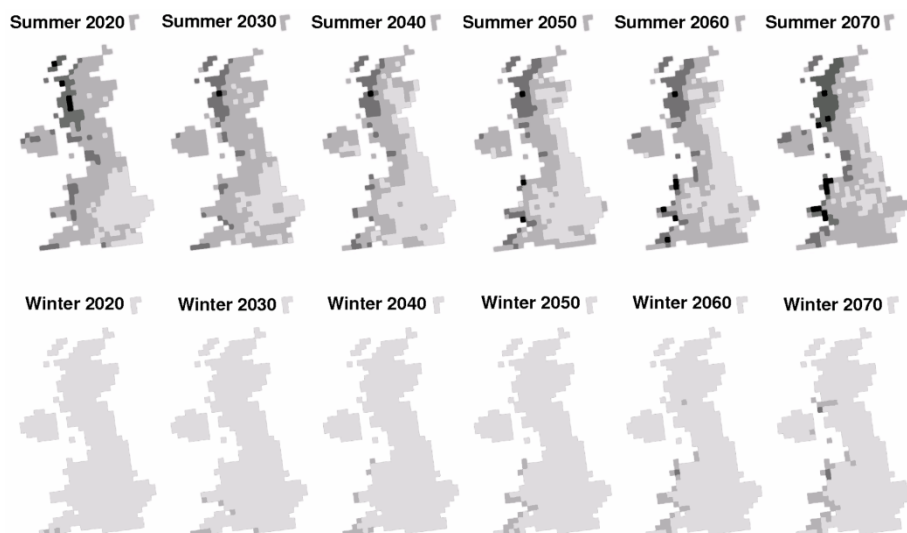
**Results** Throughout the UK the average fascioliasis risk from summer infection has increased over the past four decades, however there has been little risk from overwintering larvae. The future risk maps (figure 1) illustrate that, for the summer, risk is predicted to generally increase further, with serious epidemics predicted in Scotland by 2020 and Wales by 2050. Additionally a steady increase in risk from winter infections is forecast along the West Coast with Wales being most at risk. The mean fascioliasis risk in each season will be higher in the future than the past for all regions of the UK, with the highest overall risk being in Wales. However, there will be periods of widespread decrease in risk and localised areas of long term risk reduction.

**Conclusions** The increase shown in the 1970-2006 risk maps is consistent with empirical data (McCann *et al.*, 2010) and provides further evidence that the observed changes in fascioliasis are indeed climate-driven. It is predicted that the UK will experience unprecedented levels of infection over the next 60 years, with serious epidemics expected to be the norm by 2020 in parts of Scotland, and by 2050 in parts of Wales. With some parts of the UK experiencing risk from overwintering larvae, fascioliasis infection could extend from being a seasonal to a year-round threat. The forecast is the first approximation of the potential changes in fascioliasis risk in the UK, and indicates where active disease surveillance should be targeted.

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**Figure 1** Future summer and winter *F. hepatica* risk across the UK at a resolution of 25km squares, 2020 – 2070. Risk categories are based on those used by Ollerenshaw & Rowlands (1959): Little or no disease (■), occasional losses: (■), disease prevalent: (■), serious epidemic: (■).

## Implications of host genetic variation on the risk and prevalence of infectious diseases transmitted through the environment

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**Introduction** Infectious disease in livestock constitutes a major threat to food security. There is accumulating evidence that animals vary genetically in their response to infectious challenge. However, there is substantial uncertainty about the effect of genetic host heterogeneity on the emergence risk and the progression of diseases. Also, the relevance of results of models for diseases transmitted through direct contact between individuals for diseases transmitted through environmental reservoirs is unclear. The aim of this study was to quantify the impact of host genetic diversity, when represented in different epidemiological traits, on disease risk and severity for diseases transmitted through environmental reservoirs. For this purpose, a compartmental genetic-epidemiological model was developed, and parameterised for footrot in sheep. This bacterial disease is the primary cause of lameness in sheep in the UK, and a major threat to sheep production.

**Material and methods** The model developed in this study is a stochastic representation of a compartmental 'SLDCRS' model, where animals of a closed population of constant size (i.e. no birth, removal or death) may progress through different disease states (susceptible (*S*), latent infected (*L*), diseased (*D*), asymptomatic carrier (*C*), recovered (*R*), susceptible (*S*)) over the time course of infection. Infection occurs through environmental contamination, which is quantified by the density of infectious pathogen shed by infected animals in categories *D* and *C* into the environment. At the start of the simulations, each individual is assigned a value for the expected duration of time *T* that it may spend in any of the disease states (i.e.  $T_S$ ,  $T_L$ ,  $T_D$ ,  $T_C$ ,  $T_R$ , respectively) and for bacterial shedding rates  $k_D$  and  $k_C$  associated with disease states *D* and *C*, respectively. The epidemic process is simulated as a series of random events (e.g. transition of an animal from one disease state to the next, bacterial shedding or decay) which occur at given average rates specified by the parameters  $T_S$ ,  $T_L$ ,  $T_D$ ,  $T_C$  and  $T_R$ . For example, infection of a susceptible individual occurs at average rate  $r_1 = \sum_{i \in S} r_S(i) E$ , where *E* is the environmental bacterial load,  $r_S(i) = 1/T_S(i)$  and the sum is taken over all susceptible individuals. The average rates of the transition events corresponding to other states (i.e. *L*, *D*, *C*, and *R*) are calculated in similarly.

In the benchmark model no variation between individuals in any of the epidemiological parameters  $T_S$ ,  $T_L$ ,  $T_D$ ,  $T_C$ ,  $T_R$ ,  $k_D$  and  $k_C$  was assumed. Parameter values for this model were adopted from a recent deterministic epidemiological model of footrot (Nieuwhof *et al.* 2009). Then, variation between hosts was introduced for each host specific epidemiological trait one at a time. Host genetic variation was defined through continuous gamma distributions with varying shape and degree of dispersion for these traits (Figure 1, dispersion =  $1/\alpha$ ).

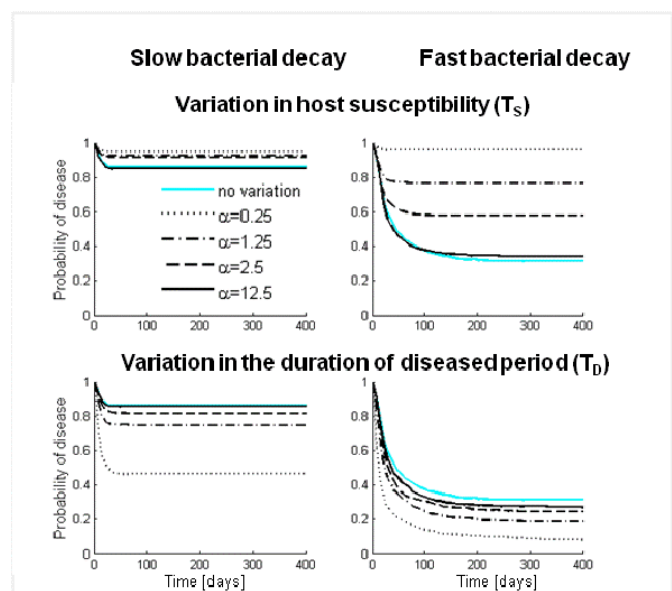
**Results** The model predicts a strong impact of genetic heterogeneity on the probability of footrot establishment and persistence (Figure 1), as well as on disease prevalence and progression, and on observable disease phenotypes such as frequency and duration of infections (results not shown), when dispersion in key epidemiological parameters is high. In contrast, moderate dispersion was found to have little influence on epidemiological characteristics (Figure 1). The impact of host variation was found to depend on the disease trait for which variation is occurring and on environmental conditions affecting pathogen survival (Figure 1). If bacterial decay is fast, variation in susceptibility was found to have the strongest impact on epidemiological characteristics, while variation in the duration of the diseased period had the strongest impact in conditions leading to slow bacterial decay.

**Conclusions** The model results suggest that accurate prediction of response to selection for disease resistance requires a thorough understanding of the type and structure of genetic variation. Also, the optimal selection strategy for reducing disease risk and severity depends on environmental conditions: if bacterial decay is fast, selection against high susceptibility would be most beneficial, whereas selection for fast recovery would be more beneficial in case of slow bacterial decay.

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**Figure 1: Impact of genetic heterogeneity in different traits ( $T_S$  and  $T_D$ ) on the probability of disease prevailing in the population over time.**

## Seeking missing genetic variance in disease resistance

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**Introduction** Reducing disease prevalence through selection for host resistance offers a viable alternative to chemical treatment, which is disliked by consumers, sometimes only offers limited protection due to pathogen resistance and is a potential food security threat due to residues in meat. Selection for host resistance has proven difficult, however, due to low heritabilities which may be caused by a failure to capture all the relevant genetic variance. For example, following an epidemiological Susceptible-Infected-Recovered (SIR) model, infectious disease prevalence in a population may be affected by host genetic variation in susceptibility, infectivity and/or recovery rate. Genetic variation in infectivity, the propensity of transmitting infection upon contact with a susceptible individual, can be considered as an associative genetic effect, as the genotype of infected individuals affects the disease status of susceptible group members. Genetic analysis, however, classically ignores information from group members and hence associative genetic effects. The objective of this study was to examine to what extent genetic variance in infectivity/susceptibility is captured by an animal model and an associative effects model applied to binary disease data and how this depends on disease and population characteristics.

**Material and methods** To avoid overburdening the results with unnecessary complexity we chose a simple stochastic SIR model to simulate disease spread. Each individual was assigned its own level of susceptibility, infectivity and recovery rate. We simulated the epidemic as a Poisson process using Gillespie's direct algorithm to calculate interevent time and choice of events (infection or recovery). Each population, which consisted of a paternal half-sib structure, was divided into 500 groups of equal size chosen at random without reference to pedigree. The breeding values for susceptibility and infectivity were allocated to the individuals in the parental generation using two genetic models (single locus and multi-loci) to account for different underlying genetic architectures. Mean susceptibility and infectivity were kept at a constant  $\mu$  for all simulations. The environmental value of susceptibility and infectivity was set at zero in order to maximise the amount of genetic variation that could be captured, and both traits were assumed to be independent. Simulations were carried out assuming variation in susceptibility only, infectivity only, in both or neither. Each simulation run was replicated ten times. The disease status (binary presence/absence of disease) of individuals was recorded at given time points and at different stages of the epidemic and then analysed using a sire model and the associative effects model developed by Muir (2005).

## Results

### a. Sire model

The estimated genetic variance in disease presence, obtained with a sire model, was only significantly different from zero if individuals vary genetically in susceptibility (Table 1). If variation was introduced in infectivity only the estimated genetic variance was not significantly different from zero. Moreover, there was no difference in estimated genetic variation between populations which had variation introduced only in susceptibility or in both, susceptibility and infectivity.

### b. Associative effects model

Analysis with an associative effects model required between group difference in prevalence. Observations at a given time  $t$  were therefore analysed. Variance in susceptibility was captured by the direct effect variance. Variance in infectivity was also captured by the associative effects variance but was smaller than expected (Table 2). Despite uncorrelated input values the associative effects model predicted a significant positive covariance between direct and associative effect when there was variation in susceptibility (Table 2).

**Table 1** Estimated sire variance. Multiple loci genetic model. <sup>#</sup>Not significantly different from zero ( $P>0.05$ ). Values  $\times 10^{-3}$ .

%	Variation introduced in:			
	Infected/Recovered	None	Infectivity	Susceptibility
25%	0.04 <sup>#</sup>	0.13 <sup>#</sup>	7.25	7.02
50%	0.15 <sup>#</sup>	0.30 <sup>#</sup>	16.71	16.18
75%	0.23 <sup>#</sup>	0.15 <sup>#</sup>	13.63	12.83

**Table 2** Estimated genetic variance using an associative effects model. Multiple loci genetic model. At time  $t=5.3$ , values  $\times 10^{-3}$ . <sup>#</sup> Not significantly different from zero ( $P>0.05$ ).

	Variation introduced in:			
	None	Infectivity	Susceptibility	Both
Direct	0.26 <sup>#</sup>	0.36 <sup>#</sup>	28.07	19.55
Associative	0.16 <sup>#</sup>	1.00	0.11 <sup>#</sup>	0.43
Covariance	0.08 <sup>#</sup>	0.13 <sup>#</sup>	1.05	0.86
Mean prevalence	0.51	0.42	0.42	0.35

**Conclusion** Our results show that a standard animal model does not capture genetic variation in infectivity. The current associative effects model does capture some variation in infectivity but this variance is underestimated presumably because the model assumes constant expression of infectivity/susceptibility. The positive bias in the covariance between direct and associative effects is probably caused by the expression of infectivity being conditional upon being infected, which in turn depends on individual susceptibility. For those reasons, a dynamic model which takes disease status into account may be more appropriate.

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## Investigating within-farm and between-farm disease transmission interactions: Implications for the control of British poultry diseases

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**Introduction** Social network data can be useful for determining optimal control strategies through the identification of “high risk” groups such as highly connected farms that exacerbate disease spread in a national livestock epidemic. Modelling work, based on between-farm associations informed by the Poultry Network Database (PND), has so far highlighted some conditions under which a large highly pathogenic avian influenza (HPAI) outbreak in Great Britain (GB) is possible. This dataset reflects a ‘static’ poultry network representation of the maximum potential for disease transmission, via fomites associated with the movements of people and vehicles on and off farms (Truscott *et al.*, 2007, Dent *et al.*, 2008, Sharkey *et al.*, 2008). However, no study has yet incorporated the temporal patterns of movements on and off farms. In order to investigate the spread of disease between farms, both the within-farm spread of disease and this pattern must be considered, as transmission risk is a combination of the potential (via infected birds) and opportunity (via off-movements) to infect other farms. So far, the relative importance of within-farm and between-farm spread has not been looked at with respect to poultry diseases in GB – this has important implications for the targeting of disease control measures. Motivated by the work on HPAI, we therefore aimed to assess the interaction between the speed of within-farm spread and the pattern of between-farm spread, in order to determine the implications of considering the combined effect of transmission potential and connectivity for disease control.

**Material and methods** An S-E-I-R compartmental model of within-farm disease spread was constructed, incorporating a range of parameter values representing different poultry pathogens of varying virulence, transmissibility and bird susceptibility. A dataset of temporally explicit movements of slaughterhouse vehicles to farms, consisting of individual records from a large catching company in England, was used to inform daily farm movements. By matching the daily movement patterns per farm to each day of a simulated epidemic, an overall ‘transmission potential’ was calculated for each combination of model parameter values. The relative importance of within- and between-farm disease spread was then investigated by comparing the sensitivity of within-farm transmissibility (as measured by the basic reproduction number,  $R_0$ ) and the ‘transmission potential’ across each model parameter value combination, by averaging these values across all farms. The impact of combining the ‘static’ farm connectivity and temporally explicit movements was investigated by cross classifying small, medium and large between-farm association frequencies, as determined by industry contacts occurring through shared slaughterhouses, catching companies and company integration (the ‘static’ representation informed by the PND, Nickbakhsh *et al.*, in review) and transmission potential (with farm movement patterns informed by the catching company data) to determine an overall ‘risk’ profile, which can be related to farms of certain demographic characteristics.

**Results** Preliminary results show that the within-farm spread of disease can affect farm-level disease transmission potential, but is dependent upon farm-level movement patterns. For example, different farm movement frequencies and times between consecutive movements were found across farms with different between-farm association frequencies (related to farm-level demographic characteristics such as the number of birds and production type, Nickbakhsh *et al.*, in review). For farms where the transmission potential was found to be more sensitive to other model parameter values than within-flock  $R_0$ , the suggestion is that the temporal pattern of between-farm spread is important for determining onward transmission risk. Preliminary results also highlight the importance of considering both the ‘static’ farm connectivity (representing the potential epidemic final size) and temporally explicit movements (representing the potential for between-farm transmission) in combination as these are important implications for disease control. For example, farms with a moderate potential for disease transmission and a relatively few associations may represent a lower ‘risk’ group, compared to farms with a low potential for disease transmission but with a relatively high number of associations.

**Conclusions** Consideration of the interaction between the spread of disease at both the within- and between-farm scale is important for improving understanding of the implications that industry contacts may have for the spread of poultry diseases within GB. Farm risk profiles combining both farm connectivity and transmission potential could have useful implications for targeting disease control; for example biosecurity for farms where within-flock spread is more important than between-farm spread, and the targeting of ‘high risk’ farm groups where between-farm spread is more important.

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# Effect of the Texel muscling quantitative trait locus (TM-QTL) on fore quarter, hind quarter and saddle weights, weight distribution and carcass composition in purebred Texel lambs

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**Introduction** TM-QTL is a QTL which increases loin muscling by 4 to 11% in Texel lambs (e.g. Macfarlane *et al.*, 2010a) and Texel-sired crossbred lambs (e.g. Macfarlane *et al.*, 2009) with a polar overdominant action (phenotype is expressed only if one copy inherited from sire) and has an additive effect on carcass weight (Macfarlane *et al.*, 2010b). This study examines TM-QTL effects on carcass composition and weight distribution in Texel lambs.

**Material and Methods** Purebred Texel lambs (n = 209) born in 2009 on two farms were reared with their dams on pasture until slaughter at ~20 weeks of age, except for 12 artificially-reared lambs, which were reared indoors until ~8 weeks then grazed and creep-fed until slaughter. After slaughter, carcasses were chilled for 7, 8 or 9 days, weighed, split and each side cut into fore-quarter (FORE ¼), saddle (SADDLE) and hind-quarter (HIND ¼). These were weighed and butchered into lean meat (LMY), fat trim and bone. Proportion of total carcass weight contained in each region and proportion of LMY (LMY/CWT), fat trim (FAT/CWT) and bone (BONE/CWT) in the carcass were calculated. Lambs were blood-sampled and TM-QTL genotypes assigned (described by Macfarlane *et al.*, 2010a). There were 40 non-carriers (+/+), 17 heterozygote carriers inheriting TM-QTL from the dam (+/TM), 53 heterozygote carriers inheriting TM-QTL from the sire (TM/+), 34 homozygote carriers (TM/TM) and 65 with unknown genotype. Data on lambs with unknown genotype were included in analyses to give more robust estimations of fixed effects, but only results for the 4 known genotypes are presented. General linear mixed models (REML) for the variables described above were run in Genstat (GenStat 11, 2008) including TM-QTL genotype, sex (entire male or female), rearing rank (single, twin or artificial), farm (SAC or IBERS) and dam age (2, 3, 4 years or older) as fixed effects, sire as a random effect (7 sires, 3 common across farms) and CWT as a linear covariate for all variables other than itself. CWT was included as a linear covariate for the proportion variables due to a strong relationship between these variables and CWT, probably due to the wide range of CWT present in the data (8 – 25kg, mean 15.2 kg).

**Results** TM-QTL effects on *M. longissimus lumborum* weight adjusted for CWT (+/+ vs. TM/+, difference = 4%, P = 0.046) and CWT (+/+ vs. TM/TM difference = 10%, P = 0.036) were verified. When not adjusted for CWT, FORE ¼, SADDLE and HIND ¼ weights were heavier for TM/TM than +/+ (by 8.7 to 13.5%) with no significant differences between other groups. When adjusted for CWT, there were no significant differences between genotypes for weights in each area or the proportion of total CWT contained in each carcass area (Table 1). LMY/CWT was 1.36 to 2.37% higher in TM/TM compared to the other groups although the difference was significant only for TM/TM vs. +/TM. FAT/CWT and BONE/CWT were not significantly different between genotypes.

**Table 1** Least squares means<sup>†</sup> for area weights (g), weight distribution and carcass composition for the 4 TM-QTL genotype groups

	HIND wt	¼ wt	SADDLE wt	FORE wt	¼ wt/CWT	HIND wt/CWT	¼ wt/CWT	SADDLE wt/CWT	FORE wt/CWT	¼ wt/CWT	LMY /CWT	FAT /CWT	BONE /CWT
+/+	2650		1678	2804	0.183	0.115	0.194	0.586 <sup>ab</sup>	0.043	0.304			
+/TM	2637		1705	2768	0.182	0.116	0.192	0.581 <sup>b</sup>	0.042	0.306			
TM/+	2666		1678	2791	0.184	0.115	0.193	0.587 <sup>ab</sup>	0.039	0.308			
TM/TM	2645		1708	2808	0.183	0.117	0.194	0.595 <sup>a</sup>	0.042	0.301			
ave s.e.d.	21.4		31.3	28.7	0.0016	0.0019	0.0019	0.0053	0.0034	0.0055			

<sup>†</sup>Within column, means with a common superscript character are not significantly different when tested with pair-wise s.e.d. (P>0.05); average s.e.d. (ave s.e.d.) shown for reference only. Least square means in table are those with CWT fitted in the model

**Conclusions** The previously reported effect of TM-QTL on carcass weight has been shown here to be reflected in increased weight of all carcass areas, with no effect of TM-QTL on weight distribution. Carcass composition was affected by TM-QTL; homozygote TM-QTL carriers had a greater proportion of LMY compared to other groups. In addition to previously reported benefits, if TM-QTL were included in breeding programmes to produce homozygote carrier lambs, meat yield could be increased in carcasses of equal weight.

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# An evaluation of the effects of maturity of maize at harvest, grass silage feed value and concentrate feed level on finishing lamb performance

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**Introduction** Maize silage, when grown under the complete cover plastic mulch system (CCPM), can be produced at a similar cost to grazed grass and is approximately 20% cheaper than grass silage (Keady *et al.* 2002). Maize silage inclusion in the diet increases the performance of dairy (Keady *et al.* 2003, 2008) and beef cattle (Keady and Gordon 2006, Keady *et al.* 2007) and can replace high feed value grass silage (FVGS) in the diet of pregnant ewes (Keady and Hanrahan 2008, 2009). When offered to beef and dairy cattle, maize silage has a concentrate sparing effect of up to 2.5 and 5.0 kg/head daily respectively (Keady 2005). Increasing the maturity of maize at harvest increases animal performance (Keady and Gordon 2006, Keady and Hanrahan 2008, 2009, Keady *et al.* 2008). The objective of this study was to evaluate the effect of maturity of maize at harvest, grass silage feed value, concentrate level and their interactions on lamb performance. The potential concentrate sparing effect of maize and high FVGS was also determined.

**Material and methods** High and low FVGS's were harvested on 11 May (24h wilt) and 8 June (3h wilt) and ensiled, precision chopped, with a bacterial inoculant. Two maize silages were produced from the variety Benica either grown in the open (sown 8 May) or under the CCPM system (sown 16 April) and ensiled with an inoculant-based additive. The four forages were offered ad-libitum with either 0.2, 0.5 or 0.8 kg concentrate/lamb daily. Lambs were also offered ad-libitum concentrate plus 0.5 kg of the high FVGS daily. The 13 treatments were offered to 260 Suffolk-X lambs (initial liveweight 36.8 kg) for 76 days. Twenty representative lambs were slaughtered to determine initial carcass weight.

**Results** The mean DM and starch concentrations of the low and high DM maize silages were 185 and 218 g/kg and 36 and 284 g/kg DM, respectively. The mean DM and ME concentrations of the low and high FVGSs were 219 and 292 g/kg ; and 11.1 and 11.7 MJ/kg DM, respectively. The effects of forage type and concentrate feed level on animal performance are presented in Table 1. High FVGS increased DM intake relative to both maize silages whilst the low FVGS resulted in the lowest DM intake. High FVGS increased lamb performance relative to low FVGS and low DM maize silage. There were significant forage type x concentrate level interactions. Regardless of concentrate level, lambs offered the early cut silage had higher performance. As concentrate increased the effect of forage type on animal performance declined. When offered 0.5 or 0.8 kg concentrate daily, performance was similar for lambs offered low DM maize, high DM maize and late cut silages.

**Table 1** Effect of forage type and concentrate level on lamb performance

	Conc (kg/d)	Grass silage (G)		Maize DM (M)		s.e.	Linear response to 0.6 kg conc			
		High (H)	Low (L)	High	Low		Forage	Response $\pm$ s.e	Contrasts	
Liveweight	0.2	150 <sup>a</sup>	46 <sup>b</sup>	111 <sup>a</sup>	72 <sup>b</sup>	9.3	GH	49.4 $\pm$ 12.5	GH v GL	***
gain (g/d)	0.5	178 <sup>a</sup>	109 <sup>a</sup>	122 <sup>a</sup>	112 <sup>b</sup>		GL	114.5 $\pm$ 12.2	ML v MH	P=0.06
	0.8	200 <sup>a</sup>	160 <sup>ab</sup>	160 <sup>ab</sup>	155 <sup>b</sup>		MH	49.7 $\pm$ 12.4	G v M	NS
	ad-lib	267					ML	82.5 $\pm$ 12.6		
	Average	175 <sup>a</sup>	104 <sup>c</sup>	128 <sup>b</sup>	112 <sup>bc</sup>	6.3				
Carcass	0.2	74 <sup>a</sup>	14 <sup>b</sup>	54 <sup>a</sup>	30 <sup>b</sup>	5.4	GH	39.7 $\pm$ 7.25	GH v GL	**
gain (g/d)	0.5	85 <sup>a</sup>	62 <sup>a</sup>	67 <sup>a</sup>	62 <sup>a</sup>		GL	71.4 $\pm$ 7.07	ML v MH	*
	0.8	114 <sup>a</sup>	86 <sup>b</sup>	90 <sup>b</sup>	86 <sup>b</sup>		MH	36.2 $\pm$ 7.20	G v M	NS
	ad-lib	157					ML	56.8 $\pm$ 7.31		
	Average	91 <sup>a</sup>	54 <sup>c</sup>	69 <sup>ab</sup>	60 <sup>bc</sup>	3.6				
Total DM	0.2	1.07 <sup>a</sup>	0.73 <sup>c</sup>	0.93 <sup>ab</sup>	0.85 <sup>bc</sup>	0.041	GH	-0.37 $\pm$ 0.056	GH v GL	NS
Intake (kg/d)	0.5	1.14 <sup>a</sup>	0.88 <sup>b</sup>	0.93 <sup>b</sup>	0.97 <sup>ab</sup>		GL	-0.25 $\pm$ 0.056	ML v MH	NS
	0.8	1.22 <sup>a</sup>	1.01 <sup>ab</sup>	1.06 <sup>b</sup>	1.08 <sup>ab</sup>		MH	-0.39 $\pm$ 0.056	G v M	NS
	ad-lib	1.43					ML	-0.29 $\pm$ 0.056		
	Average	1.14 <sup>a</sup>	0.87 <sup>c</sup>	0.97 <sup>b</sup>	0.97 <sup>b</sup>	0.024				

**Conclusions** High levels of lamb performance are achievable from offering high FVGS with 0.2 kg of concentrate. Increasing forage feed value decreased food conversion ratio (kg feed/kg carcass gain). Relative to the low FVGS, the concentrate sparing effect of the high FVGS, and the high and low DM maize silages as determined by carcass gain, was 0.44, 0.28 and 0.11 kg, respectively.

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## Genotype by environment interactions (GxE) in Scottish Blackface lambs

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**Introduction** Current breeding indexes for UK hill sheep such as the Scottish Blackface include both lamb and maternal traits, and have led to substantial improvements in economic returns. However, offspring are often expected to perform in very different environments to where their sires were evaluated. The assumption commonly made is that they will respond the same, regardless of their location, but inevitably this is not always the case, and the variation in genotype performance across different environments is known as genotype by environment interaction (GxE). The presence of GxE can lead to reductions in the efficiency of index selection, economic performance and overall genetic gain. It can also result in the re-ranking of rams which, for future breeding selection, is perhaps of most concern. The purpose of this study was therefore to identify possible GxE in lamb growth and carcass traits, using the environments of two contrasting Scottish hill farms.

**Material and Methods** The presence of GxE in lamb traits, between two SAC hill flocks, was investigated using performance data from 16867 Scottish Blackface lambs collected between 1997 and 2009. Pedigree information was available for 25969 animals and 24 of the sires used during this time period were used on both farms. Twenty of these common sires were used via artificial insemination (AI), while 4 were used naturally, spending 1-2 years at Farm A then 1-2 years at Farm B (or *vice-versa*). Unlike the AI sires, the four naturally-used rams were not used in both flocks in the same year. 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 early growth traits birth weight (kg; BWT) and 8-week weight (kg; 8WT); weaning traits weaning weight (kg; WWT), ultrasound fat depth (cm; UFD) and ultrasound muscle depth (cm; UMD); and carcass traits carcass weight (kg; CWT), Meat and Livestock Commission carcass fat grade (MLCF) and conformation grade (MLCC). To determine the level of GxE present for these traits, genetic correlations 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 both direct genetic and maternal permanent environmental ( $c^2$ ) random effects. Likelihood ratio (LR) tests were used to determine if the genetic correlations between farms, for each trait, were significantly different from 1. 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** For the traits currently included in the selection index (weaning and carcass traits), as well as 8-week weight, all genetic correlations were not significantly different to 1, although the high s.e. for the MLC traits is of note. The only significant deviation from 1 was for BWT ( $P < 0.05$ ), suggesting the presence of GxE for this trait (Table 1). Some re-ranking and scaling of sires were observed, particularly for BWT, MLCF and MLCC. Pearson's correlations between EBVs for common sires were 1.0 apart from for BWT (0.65), MLCF (0.68) and MLCC (0.04). Spearman's rank correlations were also 1.0 for all traits apart from BWT (0.48), MLCF (0.71) and MLCC (-0.002)

**Table 1** Genetic Correlations ( $r_g$ ) and Likelihood ratios (LR) between farms for each trait

	BWT	8WKT	WWT	UFD	UMD	CWT	MLCF	MLCC
$r_g$	0.403	0.997	0.997	0.999	0.995	0.99	0.378	0.022
s.e.	0.36	0.212	†	†	†	†	0.714	0.948
LR	5.7	0.14	0.14	0.08	0.13	0.42	1.12	0.59
P-value	0.02	0.71	0.71	0.78	0.72	0.52	0.29	0.44

† Standard error not estimable

**Conclusions** The lack of significant GxE observed for traits currently included in the hill sheep index suggest 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 commercial breeding programme under which the majority of these common sires were selected, have therefore chosen "robust" reference sires for the weaning and carcass traits. However, it is also possible that the flocks were not divergent enough when the study began. The GxE observed for birth weight, which is not included in the current index, could have implications for lambing associated problems if sires produce lambs with unexpectedly high or low birth weights. Further analysis will investigate if similar results are found when the number of farms and common sires is increased and the divergence of environments is higher.

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## Trans-generational effects of nutrient restriction during early and mid-pregnancy on reproductive function in Scottish Blackface and Suffolk sheep

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**Introduction** While several studies have assessed the effects of under-nutrition during pregnancy on foetal and offspring gonad development, few have addressed the reproductive performance of offspring as adults, or whether the developing reproductive axis of different genotypes responds differently to comparable nutritional stress. We have recently demonstrated that nutrient restriction during pregnancy has differential effects on lamb birth weight and faecal eggs counts at weaning in Scottish Blackface and Suffolk sheep breeds (Rooke *et al.*, 2010), suggesting that effects of nutrition on foetal development differ between breeds. Furthermore, it has been proposed that females in better body condition produce more female than male progeny (reviewed by Rosenfeld and Roberts, 2004). This study investigated the effects of under-nutrition during early and mid-pregnancy on the development of the reproductive axis in both sexes and the reproductive performance of female offspring in two contrasting commercial breeds of sheep.

**Material and methods** Following artificial insemination on Day 0, 157 Scottish Blackface (BF) and 116 (S) Suffolk primiparous ewes received, in a 2x2 factorial design, either 1.0 (control: C) or 0.75 (restricted: R) of ME requirements between Days 1 and 90 of pregnancy. Pregnancy status and anticipated litter size were determined by ultra-sound scanning between Days 50 and 70. All ewes received rations formulated to meet requirements for predicted litter size, from Day 90 to term. Pregnancy rate, litter size and sex ratios at birth were determined. More detailed investigations were conducted on a sub-set of ewes (BFC n=62, BFR n=64, SC n=42 and SR n=44) and their offspring. Testes from 16 singleton Day 125 fetuses (n=3-5 per breed/treatment combination), 32 37-week old rams (n=8 per breed/treatment combination) and ovaries from 8 singleton female Day 125 fetuses carried by 4 BF and 4 S ewes were collected at slaughter, fixed in Bouin's solution, dehydrated, embedded in paraffin wax, cut into 7µm (testes) or 5 µm (ovaries) sections and stained with haematoxylin and eosin. Seminiferous tubule diameter and area were measured and numbers of Sertoli cells and spermatogonia (lambs only) counted in 10 (foetal) or 5 (lamb) sections from each testis. Numbers of ovarian follicles and their stage of development (primordial, transitory, primary, small preantral, large preantral, small antral, as described by Lundy *et al.* (1999)) were assessed in 8 areas from 6-8 non-adjacent sections from each ovary. Ovarian weight of ten 5-month old female lambs born to ewes from each of the four treatment groups was also determined. The remaining daughters (BFC n=18, BFR n=16, SC n=22 and SR n=20) were mated, fed rations to meet requirements throughout pregnancy and pregnancy rate, litter size and offspring sex assessed. Data were analysed in a 2x2 ANOVA fitting breed, nutritional treatment and their interaction using Genstat (13<sup>th</sup> edition).

**Results** There was no effect of nutritional treatment on pregnancy rate at scanning, the proportion of ewes that lambled or litter size. Neither breed nor nutrition affected offspring sex ratios, foetal testis weight, Sertoli cell number or the dimensions of the seminiferous tubules. In lambs, both breed and maternal nutritional treatment affected the number of spermatogonia per seminiferous tubule (BFC 29.0, BFR 22.1, SC 30.44, SR 25.45, s.e.d. = 1.03; breed: P= 0.026, nutrition P<0.001). Male lambs born to R ewes had fewer Sertoli cells per seminiferous tubule (21.34 versus 13.84, s.e.d. = 0.84, P<0.001) and per area of testis tissue (0.97/nm<sup>2</sup> versus 0.60/nm<sup>2</sup>, s.e.d. = 0.003 P<0.001) than lambs carried by C ewes. In both breeds, nutrient restriction reduced the total number of foetal ovarian follicles (BFC 379/mm<sup>2</sup>, BFR 188/mm<sup>2</sup>, SC 232/mm<sup>2</sup>, SR 99/mm<sup>2</sup>; s.e.d =70, nutrition P=0.05), but, in S fetuses, increased the proportion of more developed follicles (BFC 0.04, BFR 0.04, SC 0.03, SR 0.25, s.e.d. 0.12, P=0.02). BF lamb ovaries accounted for a smaller proportion of lamb body weight than S lamb ovaries (41.6mg/kg versus 63.3mg/kg, s.e.d. = 5.9; P=0.012). There was also a breed by nutrition interaction such that the relative weight of the ovaries was higher in SR than SC lambs (72.7 mg/kg versus 38.8 mg/kg, respectively, s.e.d. = 8.7; P=0.05). In the daughters, pregnancy rate at scanning or birth, litter size and offspring sex ratios were unaffected by the pregnancy diets consumed by their mothers.

**Conclusions** These results show that modest under-nutrition during early and mid-pregnancy in sheep disrupts gonad development in both sexes, although the stage of development when these effects are evident varies. Effects of maternal under-nutrition on the developing testes were only evident post-natally, and these occurred in the absence of changes in testicular size or testosterone concentrations (Rooke *et al.*, 2008). In contrast, maternal under-nutrition affected the distribution of foetal ovarian follicles and was associated with alterations in ovarian weight. Our data suggest that fetuses carried by S ewes appear more susceptible to the effects of maternal nutrient restriction. Although nutrient restriction appeared to accelerate foetal and lamb ovarian development, particularly in S ewes, there was no indication of altered reproductive performance when these females themselves became mothers. These data provide no evidence that nutrient-restriction alters offspring sex ratios.

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## Effects of the Texel muscling quantitative trait locus (TM-QTL) on carcass and VIA traits in purebred Texel lambs

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**Introduction** The Texel muscling quantitative trait locus (TM-QTL) increases loin muscle measurements and weight (by ~4 to 14%) in crossbred and purebred lambs as measured by ultrasound (Walling *et al.*, 2004), computed tomography (CT) or dissection (Macfarlane *et al.*, 2009; 2010). TM-QTL was mapped in UK Texel sheep on ovine chromosome 18 (Walling *et al.*, 2004). TM-QTL mode of inheritance is polar over-dominant: only animals receiving a single copy of the QTL from the sire demonstrate the phenotypic effect (Macfarlane *et al.*, 2010). This study investigates the effects of 0, 1 and 2 copies and the effects of parental origin of TM-QTL on post-mortem carcass classification (Meat and Livestock Commission, MLC) and video image analysis (VIA) traits.

**Material and methods** Purebred Texel lambs (95 entire males, 114 females) sired by seven TM-QTL carrier sires and raised on two different farms, were used in this study. Due to the nature of the haplotype genotyping test, assigned genotypic classes were available only for 144 lambs: 40 non-carriers (+/+), 17 heterozygotes inheriting TM-QTL from the dam (+/TM), 53 heterozygotes inheriting TM-QTL from the sire (TM/+) and 34 homozygous carriers (TM/TM), although lambs with undetermined genotypes were still included in the statistical analysis. Lambs were slaughtered at an average age of 144 days and carcasses were weighed (average cold carcass weight (CCWT) 15.2 kg), classified using the MLC scheme for fat cover (MLC-F) and conformation (MLC-C) and then VIA scanned. The VIA traits obtained were predicted weights of primal cuts and trimmed primal cuts (TP) (shoulder, breast, leg, chump, loin) and predicted weights of carcass saleable meat yield and trimmed saleable meat yield. VIA also predicted some muscularity traits in the hind leg (muscle volume, HLMV) and the loin region (cross-sectional loin muscle area (LMA), width (LMW) and depth, and muscle volume (LRMV)). VIA provided also some carcass dimensions: widths, lengths and areas. Data were analysed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of TM-QTL on the measured traits. Fixed effects fitted were TM-QTL carrier status and sex in all models and dam age, rearing rank and farm when appropriate. Sire was fitted as a random effect and CCWT as a covariate for MLC classes and VIA traits, but not for CCWT and carcass dimension traits.

**Results** The traits significantly affected by TM-QTL carrier status are shown in Table 1. CCWT was significantly higher in homozygous carriers compared to non-carriers, but not when compared to both heterozygous groups. TM/TM lambs had significantly higher MLC-C scores than both heterozygous groups, but TM-QTL did not affect MLC-F. VIA-predicted TP-LEG and TP-LOIN weights were similarly affected by TM-QTL, TM/+ was significantly greater when compared only to +/+. TM/+ had the heaviest TP-CHUMP, but was significantly different only to +/TM. TM/+ also had the highest HLMV and the widest loin muscle with the greatest area, but when compared to the other groups, differences were only partially significant. +/+ lambs had the least predicted LRMV amongst the genotypic classes, however it was only significantly different from TM/+ and TM/TM. VIA-predicted carcass saleable meat yield (adjusted for carcass weight) was not affected by TM-QTL. TM/TM lambs also had greater carcass dimensions measured by VIA (longer, wider and larger area).

**Table1** Effects of TM-QTL genotype on carcass and VIA traits<sup>†</sup>

	CCWT (kg)	MLC-C	TP- LEG (kg)	TP- CHUMP (kg)	TP-LOIN (kg)	HLMV (cm <sup>3</sup> )	LMW (mm)	LMA (cm <sup>2</sup> )	LRMV (cm <sup>3</sup> )
+/+	13.88 <sup>b</sup>	2.82 <sup>a,b</sup>	2.988 <sup>b</sup>	0.715 <sup>a,b</sup>	1.125 <sup>b</sup>	3171 <sup>a,b</sup>	72.5 <sup>b</sup>	17.6 <sup>b</sup>	441.1 <sup>b</sup>
+/TM	14.10 <sup>a,b</sup>	2.56 <sup>b</sup>	2.976 <sup>a,b</sup>	0.702 <sup>b</sup>	1.117 <sup>a,b</sup>	3064 <sup>b</sup>	71.6 <sup>b</sup>	16.9 <sup>b</sup>	457.4 <sup>a,b</sup>
TM/+	14.41 <sup>a,b</sup>	2.74 <sup>b</sup>	3.065 <sup>a</sup>	0.728 <sup>a</sup>	1.191 <sup>a</sup>	3270 <sup>a</sup>	74.9 <sup>a</sup>	18.7 <sup>a</sup>	492.0 <sup>a</sup>
TM/TM	15.48 <sup>a</sup>	2.98 <sup>a</sup>	3.051 <sup>a,b</sup>	0.722 <sup>a,b</sup>	1.145 <sup>a,b</sup>	3219 <sup>a</sup>	73.4 <sup>a,b</sup>	18.0 <sup>a,b</sup>	496.6 <sup>a</sup>
Av. s.e.d.	1.085	0.224	0.053	0.011	0.042	60.91	1.15	0.58	23.80

<sup>†</sup>Within columns, means sharing a common character in their superscripts are not significantly different when tested with pair-wise s.e.d. ( $P > 0.05$ ).

**Conclusions** TM-QTL significantly affected CCWT and MLC-C. TM/TM carriers were 1.6 kg heavier than non-carriers and were awarded the highest MLC-C scores. These results are of economic value based on the current payment system. VIA results confirmed its ability to detect differences between genotypic groups in loin muscle characteristics and support previous reports on the effects of TM-QTL on loin muscling and the TM-QTL mode of action on loin traits.

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## Effect of the Texel muscling quantitative trait locus (TM-QTL) and sex on meat quality parameters of the semimembranosus muscle of purebred Texel lambs

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**Introduction** The TM-QTL located on chromosome 18 (Walling *et al.* 2004) increases loin muscle dimensions and weights (by ~4 to 14%) in purebred and crossbred carrier Texel lambs but other muscles seem to be unaffected (Macfarlane *et al.* 2009). Lambe *et al.* (2010) reported that TM-QTL had no detrimental effects on tenderness of *Longissimus lumborum* or *vastus lateralis* muscles of purebred Texel lambs, but before the TM-QTL can be recommended to industry it is important to assess its effects on other meat quality (MQ) traits. Therefore results are reported here for the *semimembranosus* muscle including additional MQ traits.

**Material and methods** Purebred Texel lambs born in 2009 and reared with their dams on pasture (n = 197) on two farms or indoors (n = 12) were slaughtered at mean age of 144d (range 126 to 155d) and mean hot carcass weight of 15.2 kg (range 8 to 25 kg). After electrical stimulation and kept at ~2°C for either 7 or 9 days, the topside cut (containing the *semimembranosus* (SM), *adductor* and *gracilis* muscles) was taken from the right side of each carcass. A cut surface of SM was allowed to bloom for 1 hour before colour measurements (Minolta CR-410) and pH (Testo 205) were recorded. Samples were stored in sealed plastic bags and frozen (-30°C), were later defrosted in 17 batches for 24 hours at 3°C. A ~150g cut of the SM was weighed prior to cooking in a polythene bag in a 70°C water bath for 90 minutes. After cooking, fluid was drained off and samples stored overnight at 3°C. The next day, samples were weighed after drying with a paper towel, to determine cooking loss as a percentage of uncooked weight. Twelve shears, perpendicular to the fibre axis were performed on six 13mm by 13mm by 25 mm cores using a modified Warner-Bratzler protocol, as described by Purchas & Aungsupakorn (1993). Median peak force (PF) and median work done (WD) were determined for the 12 shear force values. Colour parameters L\*, a\* and b\* were measured and used to calculate hue angle ( $\arctan[a^*/b^*]$ , increased brownness). WD and PF were transformed ( $\log_e$ ) to ensure a normal distribution. Data were analysed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Sex, farm, rearing rank, genotype and the interaction sex by genotype were included in the model as fixed effects. Hot carcass weight and aging time were included as covariates. Sire (n = 7) was fitted as a random effect and defrosting batch was included for the PF and WD traits only. No significant ( $P \leq 0.05$ ) higher order interactions were found between terms, and non-significant ( $P > 0.05$ ) fixed effects and covariates were excluded from the models. Only samples from lambs with known TM-QTL genotypes were included in the analysis (n = 143; Table 1). Pair-wise comparisons of least-squares (LS) means of the MQ traits for the four TM-QTL genotypes and two sexes (see below) were estimated using t-tests (Bonferroni-adjusted for multiple comparisons).

**Results** LS means are reported for MQ traits where S (sire) and D (dam) superscripts denote allele origin, while “TM” and “+” represent the TM-QTL and wildtype allele, respectively. PF and WD in  $+^S/TM^D$  males was significantly greater than  $+^S/+^D$  females and  $TM^S/+^D$  females for WD only ( $P \leq 0.05$ ). L\* values were significantly higher in  $TM^S/+^D$  males than females. No further differences were observed between sexes or genotype for other MQ traits, including a\*, b\* and pH (data not shown). No significant differences were found between genotypes within sex, however there were significant sex differences irrespective of genotype ( $P \leq 0.05$ ).

**Table 1** Sex by TM-QTL genotype LS means and average standard errors of difference (Ave. s.e.d.) for meat quality traits

Trait	Number		PF ( $\log_e N$ )		WD ( $\log_e N$ )		CL (%)		L*		Hue angle	
Genotype	M	F	M	F	M	F	M	F	M	F	M	F
$+^S/+^D$	14	26	3.79 <sup>ab</sup>	3.69 <sup>b</sup>	2.25 <sup>ab</sup>	2.17 <sup>b</sup>	32.53 <sup>ab</sup>	30.47 <sup>b</sup>	44.23 <sup>ab</sup>	43.06 <sup>b</sup>	0.38 <sup>a</sup>	0.37 <sup>a</sup>
$+^S/TM^D$	8	9	3.95 <sup>a</sup>	3.73 <sup>ab</sup>	2.45 <sup>a</sup>	2.22 <sup>ab</sup>	33.51 <sup>a</sup>	30.94 <sup>ab</sup>	44.57 <sup>a</sup>	43.50 <sup>ab</sup>	0.40 <sup>a</sup>	0.37 <sup>a</sup>
$TM^S/+^D$	23	30	3.78 <sup>ab</sup>	3.72 <sup>ab</sup>	2.29 <sup>ab</sup>	2.20 <sup>b</sup>	33.38 <sup>a</sup>	31.24 <sup>b</sup>	45.53 <sup>a</sup>	43.40 <sup>b</sup>	0.40 <sup>a</sup>	0.38 <sup>a</sup>
$TM^S/TM^D$	14	19	3.77 <sup>ab</sup>	3.74 <sup>ab</sup>	2.27 <sup>ab</sup>	2.22 <sup>ab</sup>	32.79 <sup>ab</sup>	30.75 <sup>b</sup>	45.47 <sup>ab</sup>	42.52 <sup>b</sup>	0.40 <sup>a</sup>	0.37 <sup>a</sup>
Ave s.e.d.			0.051		0.047		0.601		0.528		0.010	
Adj. means (sex effects)			3.82 <sup>x</sup>	3.72 <sup>y</sup>	2.31 <sup>x</sup>	2.20 <sup>y</sup>	33.05 <sup>x</sup>	30.85 <sup>y</sup>	44.95 <sup>x</sup>	43.12 <sup>y</sup>	0.40 <sup>x</sup>	0.37 <sup>y</sup>

M = Male (entire), F = Female. Means within trait sharing a common superscript are not significantly different ( $P > 0.05$ )

**Conclusions** Effects of the TM-QTL on MQ traits for the *semimembranosus* muscle in purebred Texel lamb are negligible when lamb has been aged for 7 or more days. All MQ traits were however significantly affected by sex with females having better MQ.

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## A comparison of growth and carcass characteristics of hill lambs finished on a selection of forage-based diets

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**Introduction** Finishing hill lambs on lowland farms is an important feature of the UK sheep industry and a major contributor to the rural economy. Historically, due to their low growth potential, hill lambs were finished on high grain diets in order to achieve moderate-to-high growth rates. However, concentrate feed costs have increased significantly in recent years so that there is a need now to investigate lower cost forage-based alternatives. Silage-based diets for lambs have been studied extensively and are capable of sustaining growth rates of 80-130 g/d (Carson *et al.*, 2001). Forage maize also can be a high quality feedstuff and has the potential to reduce feeding costs on mixed beef/sheep farms (Keady *et al.*, 2008) but there is only limited information on the performance of lambs fed maize silage-based diets. Grazed grass is the cheapest forage available for lambs, but the low nutritive value of late season grass swards may make grazed grass inadequate to maintain high levels of performance. The aims of the current study were to investigate the performance and carcass characteristics of lambs finished on a range of forage-based diets.

**Material and methods** In September 2009, 136 castrate male lambs were sourced from six hill farms soon after weaning. Following a 3-week acclimatisation period, the lambs (mean±SD age and live weight 164 ± 11.0 d and 33 ± 5.5 kg respectively), were allocated to 3 treatment groups (n = 38/39) balanced for live weight, genotype, farm of origin and age. Within each treatment group, lambs were randomly allocated for slaughter at 42, 46 and 50 kg live weight. Two treatment groups were housed as groups of six lambs per pen and finished on *ad libitum* grass silage (GS) or *ad libitum* maize silage (MS) and concentrates. The remaining lambs were grazed together on a mixed perennial ryegrass/Italian ryegrass sward (GG). The grass silage was predicted by Near Infrared Reflectance Spectroscopy to contain 228 g DM/kg, 692 g/kg digestible organic matter/kg DM, 11.1 MJ ME/kg DM and 134 g crude-protein/kg DM while maize silage contained 312 g DM/kg, 11.1 MJ ME/kg DM, 97 g CP/kg DM and 269 g starch/kg DM. The grass swards supplied 160 g DM/kg, 9.5 MJ ME/kg DM and 109 g CP/kg DM. All lambs were offered 0.5 kg concentrates once daily but, due to the low CP content of MS, the concentrates fed with MS were formulated to a higher level of CP (222 g/kg DM) than those fed with GG and GS (183 g/kg DM). Silages were offered fresh daily at 0930 h and DM intake was recorded daily until slaughter. Lambs were weighed fortnightly until they reached their target slaughter weight. Cold carcass weight, dressing proportion and grade were recorded at slaughter. Carcass conformation was scored on a 5-point scale using the EUROP classification system (E=5, P=1) while carcass fatness was scored on a 6-point scale using the Livestock and Meat Commission (NI) classification system (1=1, 2=2, 3=3, 4L=4, 4H=4.5 and 5=5). Data were analysed using linear models with fitted fixed effects for farm of origin, carcass weight, diet, genotype and diet x genotype (dietary effects only are presented). Means were predicted for a 20 kg cold carcass weight endpoint.

**Results** Lambs fed GG had higher live weight gains (+31 g/d;  $P<0.01$ ) and achieved 20 kg carcass weight on average 22 days earlier than those fed GS ( $P<0.05$ ). Lambs fed MS consumed 20% more silage DM ( $P<0.001$ ) and, as a result, achieved 20 g/d higher live weight gains ( $P<0.01$ ) and were slaughtered 12 days earlier than those fed GS. However MS tended to reduce carcass dressing proportion ( $P=0.06$ ) and consequently tended to increase ( $P=0.06$ ) slaughter weight compared with GS, thus reducing the benefits of their higher weight gains for age at slaughter. There was no effect of forage type on carcass conformation score. However lambs finished outdoors on GG had lower ( $P<0.001$ ) fat scores compared with those finished indoors on silage-based diets.

**Table 1** Effects of forage type on the performance and carcass characteristics of hill lambs

Forage	Grazed grass	Grass silage	Maize silage	s.e.d	Probability
Silage dry-matter intake (kg/d)	-	0.44	0.53	0.022	<0.001
Live weight gain (g/d)	116 <sup>b</sup>	85 <sup>a</sup>	105 <sup>b</sup>	7.8	0.002
Slaughter weight (kg)	44.7	44.3	45.5	0.54	0.060
Days to slaughter	116 <sup>a</sup>	138 <sup>b</sup>	126 <sup>a</sup>	6.0	0.012
Conformation score	3.11	2.93	2.95	0.100	0.216
Fat score	2.95 <sup>a</sup>	3.26 <sup>b</sup>	3.34 <sup>b</sup>	0.109	<0.001
Dressing proportion	0.448	0.452	0.440	0.0056	0.064

Means sharing the same letter in their superscript are not significantly different ( $P>0.05$ ). All lambs offered 0.5 kg/d concentrates.

**Conclusion** These results show that, under good grazing conditions, grazed grass is superior to high quality grass silage for finishing hill lambs during the autumn/winter. On farms where outdoor finishing is not possible, maize silage is ideal for achieving good growth rates indoors due to its higher intake characteristics compared with grass silage. However, lamb growth rates on forage-based diets are approximately only 50% of the growth rates reported for lambs finished on concentrates.

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## Breed and sex effects on spine characteristics in sheep

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**Introduction** The *longissimus* (loin) muscle is one of the higher priced cuts of meat and commercially one of the most important muscles. The muscle runs the length of the spinal column; hence animals with short backs (reduced number or length of vertebrae) have a reduced length of *longissimus* muscle. In the past few decades a number of studies on pigs (e.g. Fredeen *et al.* 1962) have described variation in vertebrae number in the lumbar and thoracic regions of the spine and indicate a genetic basis for this. This has led to significant attention by breeders, as a way of increasing the production of meat from these popular cuts in pigs, in particular, via a genetic change of spine characteristics. In comparison to the knowledge in pigs, there is little yet known in sheep regarding this topic; therefore this study aims to investigate variation in spine characteristics (vertebrae length and number) and to assess the differences between males and females and within and between breeds of sheep.

**Material and methods** Lambs (n = 1860) reared in different years (from 2003 – 2008) were scanned using X-ray computed tomography (CT) at ~20 weeks of age. The total sample included animals from four different breeds or crosses: Texel (TEX; n = 254), Scottish Blackface (SBF; n = 1100), Texel cross Mule (TEX x MULE; n = 328) and Poll Dorset cross Mule (PD x MULE; n = 178). Topograms generated from the CT scans were used to quantify the skeletal/spine characteristics of two regions of the spine (thoracic and lumbar). Each spine region was first treated individually and measurements taken for every animal included: counts of vertebrae number in the lumbar and thoracic regions (SPV<sub>LUM</sub> and SPV<sub>THOR</sub>, respectively); lengths (mm) of the lumbar and thoracic regions of the spine (SPL<sub>LUM</sub> and SPL<sub>THOR</sub>, respectively) and the average length (mm) of individual vertebrae in the lumbar and thoracic regions (VL<sub>LUM</sub> and VL<sub>THOR</sub>, respectively). The two results were then combined to give thoracolumbar (thoracic + lumbar) measurements, which included the total count of vertebrae (SPV<sub>T+L</sub>), total length (mm) of the thoracolumbar region (SPL<sub>T+L</sub>) and the average length (mm) of individual vertebrae across the thoracolumbar region (VL<sub>T+L</sub>). Data were analysed using the GLM procedure in SAS (SAS Institute Inc., Cary, NC, USA) to determine the effects of breed and sex on the traits measured. Fixed effects fitted in the model were breed, sex, dam age and rearing rank, with live weight fitted as a covariate.

**Results** There were no significant sex effects on the majority of the spine traits; VL<sub>LUM</sub> being the only exception (males shorter). However breed/cross appears to have significant effects on all spine characteristics (Table 1). For example, vertebral counts SPV<sub>LUM</sub> and SPV<sub>T+L</sub> were significantly lower in TEX compared to SBF and the crosses, whilst SPV<sub>THOR</sub> and SPV<sub>T+L</sub> were significantly higher in SBF than other groups. All vertebral length measurements (SPL and VL) showed significant differences across breeds/crosses, with lowest values for TEX and the majority of the higher values in the crosses, particularly the PD x MULE lambs.

**Table 1** Least-squares means (and standard errors) for spine characteristics in different breeds/crosses and sexes

Trait	Breed/Cross Effects								Sex Effects			
	TEX		SBF		TEX x MULE		PD x MULE		MALE		FEMALE	
SPV <sub>LUM</sub>	6.36 <sup>c</sup>	(0.06)	6.63 <sup>a</sup>	(0.05)	6.50 <sup>b</sup>	(0.06)	6.59 <sup>a,b</sup>	(0.07)	6.53 <sup>A</sup>	(0.06)	6.50 <sup>A</sup>	(0.05)
SPV <sub>THOR</sub>	12.9 <sup>b</sup>	(0.04)	13.0 <sup>a</sup>	(0.03)	12.9 <sup>b</sup>	(0.04)	12.9 <sup>b</sup>	(0.04)	12.9 <sup>A</sup>	(0.03)	12.9 <sup>A</sup>	(0.03)
SPV <sub>T+L</sub>	19.2 <sup>c</sup>	(0.06)	19.6 <sup>a</sup>	(0.06)	19.4 <sup>b</sup>	(0.06)	19.5 <sup>b</sup>	(0.07)	19.4 <sup>A</sup>	(0.06)	19.4 <sup>A</sup>	(0.06)
SPL <sub>LUM</sub>	181 <sup>d</sup>	(1.52)	195 <sup>b</sup>	(1.44)	192 <sup>c</sup>	(1.64)	199 <sup>a</sup>	(1.79)	192 <sup>A</sup>	(1.49)	192 <sup>A</sup>	(1.45)
SPL <sub>THOR</sub>	251 <sup>d</sup>	(1.33)	262 <sup>c</sup>	(1.25)	266 <sup>b</sup>	(1.43)	272 <sup>a</sup>	(1.56)	263 <sup>A</sup>	(1.30)	262 <sup>A</sup>	(1.27)
SPL <sub>T+L</sub>	432 <sup>c</sup>	(1.73)	457 <sup>b</sup>	(1.64)	459 <sup>b</sup>	(1.87)	471 <sup>a</sup>	(2.04)	455 <sup>A</sup>	(1.69)	454 <sup>A</sup>	(1.65)
VL <sub>LUM</sub>	28.5 <sup>c</sup>	(0.11)	29.5 <sup>b</sup>	(0.10)	29.6 <sup>b</sup>	(0.12)	30.1 <sup>a</sup>	(0.13)	29.4 <sup>B</sup>	(0.11)	29.6 <sup>A</sup>	(0.10)
VL <sub>THOR</sub>	19.5 <sup>d</sup>	(0.09)	20.2 <sup>c</sup>	(0.09)	20.6 <sup>b</sup>	(0.10)	21.0 <sup>a</sup>	(0.11)	20.4 <sup>A</sup>	(0.09)	20.3 <sup>A</sup>	(0.09)
VL <sub>T+L</sub>	22.5 <sup>d</sup>	(0.08)	23.3 <sup>c</sup>	(0.07)	23.7 <sup>b</sup>	(0.08)	24.2 <sup>a</sup>	(0.09)	23.4 <sup>A</sup>	(0.07)	23.4 <sup>A</sup>	(0.07)

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

**Conclusions** Significant breed differences give an initial indication of a genetic basis for variation in spine characteristics. In the animals studied there is evidence of a considerable amount of variation in the length of individual vertebrae. Future intrabreed estimations of genetic parameters should further evaluate this underlying variation and its genetic basis, including any possible effects on meat yield traits, revealing the potential of spine characteristics to be useful as selection traits.

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## Incorporation of fluorescent markers into lamb finishing rations to aid detection of faecal contamination in the abattoir

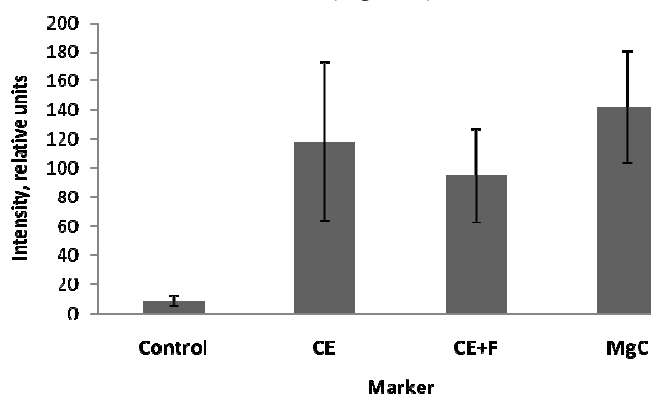
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**Introduction** Contamination of carcasses with faecal matter commonly occurs during skinning and evisceration of the animal (Natasijevec *et al.*, 2009). Faeces of ruminants can harbour pathogens like *E.coli* which may cause illness in humans (LeJeune *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 two fluorescent markers can be used to detect minute traces of contamination using on-line spectral imaging of sheep carcasses (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 everyday 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 samples were freeze dried, ground and extracted in acetone: water 50:50;v/v. Analysis was carried out using a spectrofluorometer (FP-6200, Jasco Ltd, Essex, UK) with excitation wavelength at 382nm, with the total area under the peak taken as an indication of fluorescent intensity. Data were analysed using general ANOVA with concentrate as the fixed effect and blocking according to Animal + Period.

**Results** Faeces from animals fed CE, CE+F and MgC had significantly ( $P<0.008$ ) higher fluorescent intensity than animals offered the control. There was a trend for MgC to be higher than CE+F but no difference between MgC and CE and likewise between CE+F and CE (Figure 1).



**Figure 1 Faecal fluorescence after 7 days of feeding the markers**

**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 detection and thereby provide safer food to the consumer. 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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## The effects of beta-adrenergic agonist (BA) and growth hormone (GH) on factors involved in determining skeletal muscle fibre type in growing lambs

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**Introduction** The anabolic agents growth hormone (GH) and beta-adrenergic agonists (BA) have hypertrophic effects on skeletal muscle with the latter causing a switch to faster muscle fibre types (Lynch and Ryall 2008). The myogenic regulatory factors (MRFs), myoD, myf-5, myogenin and MRF4 are responsible for muscle development and differentiation, but the relative ratio of these factors may also influence fibre type (Te Kronnie and Reggiani, 2002). In addition, signalling enzymes such as calmodulin kinase II (CaMKII) and calcineurin (CaN) have been implicated in muscle glycolytic to oxidative changes in fibre type transitions (Bassel-Duby and Olson, 2006). The aim of this study was to determine whether there were early changes in these factors associated with fibre type transitions, determined by mRNA expression of MyHC isoforms, when lambs were treated with anabolic agents.

**Material and Methods** Mule × Charolais male twin lambs were weaned at  $53 \pm 5$  days of age after being given free access to a standard creep feed for approximately 4 weeks. The pairs of twins were then split into day 60 (D60) and day 120 (D120) age groups. These groups were then split into three treatment groups: group CO (n=11 per age group) was the control having creep feed *ad libitum*; group BA (n=10 per age group) were *ad-libitum* fed the creep diet containing the beta-agonist cimaterol at 10 ppm; and group GH (n=10 per age group) were administered prolonged release bovine GH (3.75mg/kg BW, Monsanto) by a single subcutaneous injection and *ad-libitum* fed the creep diet. Treatments were for 6 days starting at day  $60 \pm 5$  or  $120 \pm 4$  days of age. As these agents are not permitted in the EU the carcasses did not enter the food chain. At slaughter a whole transverse section of the *Longissimus Dorsi* (LD) muscle from the region of the 10<sup>th</sup> rib was snap frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . The frozen LDs were crushed and mixed, CaMKII and CaN activities were determined as described by the manufacturer of the assay kits (Promega and Biomol International). LD total RNA was extracted (Trizol) then first strand cDNA generated using reverse transcriptase and random primers. The relative level of mRNA expression was determined using quantitative RT-PCR analysis (Roche). Real-time PCR primers and probes specific for MyHC I, IIA and IIX isoforms were designed using published partial length ovine cDNA sequences. Porcine MyHC IIX and IIB cDNAs are identical in the region where the IIX primers and probe were designed; therefore these primers are predicted to also detect sheep MyHC IIB mRNA, if present. Real-time PCR primers and probes specific for ovine MRFs (myoD, myf-5, myogenin and MRF4) were also designed. Data were analysed by ANOVA (Genstat) and *Post-hoc* Dunnetts test.

**Results** There was a significant effect of BA treatment on the relative expression level (%) of MyHC mRNAs in both age groups with a decrease in MyHC IIA and increase in MyHC IIX/B levels, relative to control, whilst MyHC I levels decreased in the D60 group only (Table 1). BA treatment significantly decreased MyoD expression in D120 lambs and increased Myogenin expression at both ages. There were no significant effects of either treatment at either age on MRF4 and Myf5 expression. At both D60 and D120, there were no effects of either anabolic agent on CaMKII or CaN activities.

**Table 1** Effects of BA or GH treatment of lambs for a 6 day period on LD Muscle

	Age	D60					D120				
	Treatment	CO	BA	GH	SED	P	CO	BA	GH	SED	P
mRNA levels (% of total)	MyHC I (%)	7.2	3.9*	6.6	0.8	<.001	6.8	5.0	8.1	1.9	0.277
	MyHC IIA (%)	26.9	7.0*	34.1	3.4	<.001	21.1	5.2*	25.4	3.6	<.001
	MyHC IIX/B (%)	65.1	89.1*	59.3	3.6	<.001	72.1	89.8*	66.5	4.2	<.001
Enzyme activity	CaMK II (pmol/min/ug protein)	11.5	14.3	11.0	2.1	0.246	13.8	14.9	11.5	1.8	0.160
	Calcineurin (Arbitrary Units)	49.5	43.0	44.4	15.5	0.901	34.1	27.1	22.5	11.1	0.562
mRNA expression (Arbitrary units)	MyoD	2.95	2.66	2.73	0.40	0.735	3.00	2.01*	3.28	0.39	0.008
	Myogenin	3.34	4.65*	3.65	0.47	0.023	3.15	4.54*	3.23	0.40	0.002
	MRF4	3.02	4.47	3.59	1.36	0.552	4.08	4.14	5.06	0.63	0.230
	Myf5	2.71	3.69	3.22	0.40	0.059	4.33	3.71	3.71	0.57	0.436

**Discussion** There was a significant effect of BA administration on the expression of myosin heavy chain mRNAs, driving MyHC expression toward the isoforms associated with fast glycolytic muscles (MyHC IIX/IIB). The lack of changes in CaMKII and CaN activities suggests that they are not involved in transitions toward a glycolytic fibre type. It has previously been reported that myoD mRNA expression was higher in glycolytic muscle fibres, whilst myogenin expression was higher in slow oxidative muscles (Hughes *et al.*, 1993). Our observations appear to contradict this, with an increase in myogenin and decrease in myoD expression associated with an increase in fast glycolytic fibres. In contrast to BA, GH treatment had no effect on the enzyme activities or mRNA expression measured with BA acting as a more potent anabolic agent (over this short period and dose).

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## The effects of beta-adrenergic agonist (BA) and growth hormone (GH) on lamb growth characteristics and muscle proteolytic systems

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**Introduction** The anabolic agents growth hormone (GH) and beta-adrenergic agonists (BA) have hypertrophic effects on skeletal muscle, with the latter causing a switch to faster muscle fibre types (Lynch and Ryall 2008). The hypertrophic effects of BA have been reported, in part, to be mediated through reduced levels of protein degradation (Bohorov *et al.*, 1987). Multiple systems are responsible for proteolysis in muscle, but those thought to be involved in initiating this include the calpain, caspase and proteasome systems (Kandarian and Jackman, 2006). The aim of this study was to determine the early changes in muscle phenotype and associated proteolytic systems of lambs treated with anabolic agents.

**Material and Methods** Mule × Charolais male twin lambs were weaned at  $53 \pm 5$  days of age after being given free access to a standard creep feed for approximately 4 weeks. The pairs of twins were then split into day 60 (D60) and day 120 (D120) age groups. These groups were then split into three treatment groups: group CO (n=11 per age group) was the control having creep feed *ad libitum*; group BA (n=10 per age group) were *ad-libitum* fed the creep diet containing the beta-agonist cimaterol at 10 ppm; and group GH (n=10 per age group) were administered prolonged release bovine GH (3.75mg/kg BW, Monsanto) by a single subcutaneous injection and *ad-libitum* fed the creep diet. Treatments were for 6 days starting at day 60 ± 5 or 120 ± 4 days of age. As these agents are not permitted in the EU the carcasses did not enter the food chain. At slaughter plasma was collected as well as a whole transverse section of the *Longissimus Dorsi* (LD) muscle from the region of the 10<sup>th</sup> rib (*lumborum et thoracis*), which was snap frozen in liquid nitrogen and stored at -80°C. Whole LD, *Semitenosus* (ST), and *Supraspinatus* (SS) muscles were dissected from the right side of the carcass and weighed. Combined caspase 3 and 7 activities were determined in LD using a Apo-One Caspase 3/7 assay kit (Promega). In addition, LD total RNA was extracted (Trizol) then first strand cDNA generated. Expression of mRNA was determined using quantitative RT-PCR analysis (Roche). Real-time PCR primers and probes specific for ovine calpastatin, muscle RING finger 1 (MuRF1) and Atrogin-1 were designed using Primer Express (Applied Biosystems). Data were analysed by ANOVA (Genstat) and *Post-hoc* Dunnetts test.

**Results** No significant differences in weight gain or feed efficiency were observed between treatment groups (Table 1). At D60 there were no effects of either treatment on muscle weights, whereas BA treatment significantly increased the SS and ST muscle weights at D120, with a trend for an increase in LD muscle weights. The combined activity of caspase 3 and 7 in LD was significantly decreased at D120. Similarly mRNA expression of the E3 ubiquitin ligases, MuRF1 and Atrogin-1, which are part of the proteasome system, were both decreased at D120, but only MuRF1 mRNA was significantly decreased at D60. The level of calpastatin mRNA, the specific endogenous inhibitor of calpains, was not significantly affected by the treatments, although it tended to increase with BA at D60.

**Table 1** Effects of BA or GH treatment of lambs for a 6 day period

	Age Treatment	D60					D120				
		CO	BA	GH	SED	P	CO	BA	GH	SED	P
Whole body	Weight gain (kg)	2.70	2.64	3.10	0.413	0.501	1.97	2.61	2.23	0.593	0.566
	Feed efficiency (gain/feed)	0.410	0.459	0.447	0.062	0.702	0.175	0.219	0.187	0.052	0.684
Muscle wts	SS (g)	53.5	55.2	55.9	3.9	0.812	98.5	116.4*	98.0	6.3	0.009
	ST (g)	63.3	67.1	65.0	4.3	0.667	112.4	131.2*	120.4	6.6	0.023
	LD (g)	305	306	327	27	0.655	592	667	632	35	0.108
LD Enzyme activity	Caspase 3/7 (Fluorescence units/ug protein)	5.53	5.89	6.35	0.360	0.082	4.79	3.91*	4.70	0.365	0.040
LD mRNA expression (Arbitrary units)	MuRF1	1.87	1.00*	2.35	0.323	<.001	2.80	0.35*	3.68	0.467	<.001
	Atrogin-1	0.43	0.37	0.44	0.144	0.868	1.89	0.41*	2.04	0.562	0.012
	Calpastatin	0.97	1.44	1.24	0.207	0.086	1.09	1.33	1.28	0.193	0.419

**Discussion** Despite only a short-term (6 day) treatment there was a tendency for muscle weights to increase in response to BA in the older (D120) lambs, but GH treatment had little or no effect on muscle weights. These changes were reflected in body weight gain and feed efficiency, but neither was statistically significant. Although calpastatin mRNA levels tended to change in young (D60) lambs, BA decreased the activity and mRNA levels of the caspase and proteasome systems at D120. Overall BA was the more potent anabolic agent (over this short period and dose), increasing muscle weights and causing an apparent decrease in the components of proteolytic systems, particularly in older (D120) lambs.

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## Remote measurement of enteric methane from dairy cows under different activities

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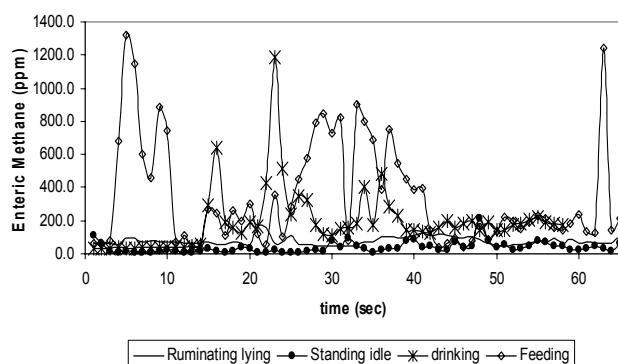
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**Introduction** The use of a recently developed, proprietary, laser methane detector (LMD) to estimate enteric methane output in dairy cows was initially suggested in an experimental study by Chagunda *et al.* (2009). The current analysis is one in a series testing the application of the LMD in livestock systems. One major advantage of the LMD is that measurements are taken without any disturbance to the normal activity of the cows. The objective of the current analysis was to determine the effect of the cow's activity on enteric methane production.

**Material and Methods** The hand-held gas detector for remote measurements of column density for methane containing gases (Tokyo Gas Engineering) was used to measure enteric methane by directing the laser beam on the nostrils of the cow for a period of up to 65 seconds. This was in order to capture complete breath cycles at every measurement window (Figure 1). The equipment is based on infrared absorption spectroscopy, using a semiconductor laser as a collimated excitation source and employing the second harmonic detection of wavelength modulation spectroscopy to establish a methane concentration measurement (Crowcon Detection Instruments, 2006). The study involved 24 cows in cubicles going about their normal activities. The cows were from the long-term SAC Systems/Genetics experiment and hence belonged to any one of four distinct groups; Select line-Low forage (LFS), Select line-High forage (HFS), Control line-Low forage (LFC), and Control line-High forage (HFC). Six cows were selected from each of the four groups. Of the six, two cows were in early lactation, two in mid lactation and two in late lactation. Hourly measurements were taken over 24 hour periods on 3 different days with the recorders taking 2 to 4 hour shifts. Cow activity at time of measurement was recorded. The activities were, standing idle, lying, walking, ruminating lying, ruminating standing, drinking, feeding and sleeping. Average peak breath cycle measurements were subjected to analysis of variance. Data were analysed using GLM procedure of SAS applying a model that included individual cow as a random variable, production system, stage of lactation, cow activity, and the recorder was nested within the recording hour as fixed effects.

## Results

Cow activity and recorder nested within the recording hour had highly significant effect ( $p < 0.001$ ) on enteric methane production from dairy cows. The individual cow also affected enteric methane production ( $p < 0.01$ ). Measurements taken when the cows were either walking or sleeping were the lowest while measurements taken when the cows were eating and drinking were the highest (Table 1).



**Figure 1** Breath cycles of cows in different activities

**Table 1** Least square means (ppm) and standard errors for enteric methane production of cows during different activities

Effect	Lsmean	s.e
Standing idle	208.2	13.8
Lying	216.0	17.5
Walking	106.8	32.0
Ruminating lying	210.2	13.9
Ruminating standing	214.7	21.4
drinking	368.7	63.3
Feeding	284.0	16.4
Sleeping	186.9	36.7

**Conclusion** LMD demonstrated the ability to segregate the concentration of enteric methane produced by dairy cows performing different physiological activities. Further work to determine the relationship between time budgets and enteric methane production is required. This will enable the estimation of daily methane output based on measurements taken at any time point.

**Acknowledgements** The assistance by Stephen Ross, Steph Birch, Jennifer Flockhart, John Dickinson is highly appreciated. SAC receives financial support from The Scottish Government.

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Crowcon Detection Instruments, 2006. <http://www.crowcon.com>.

## Variation in methane emissions measured during milking for individual dairy cows under commercial conditions

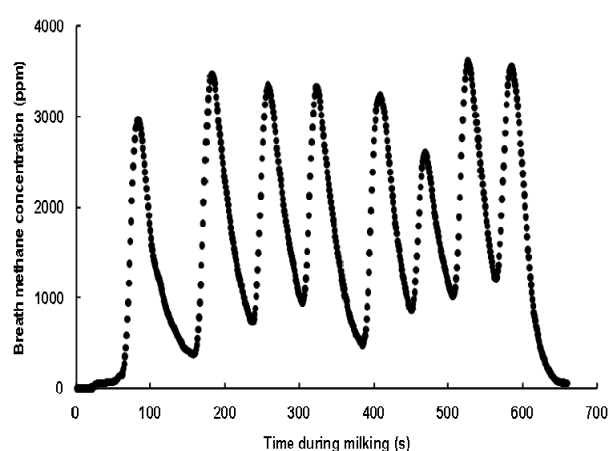
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**Introduction** Total methane emissions at farm or national level are the product of number of cows and emissions per cow. It is difficult to measure emissions by individual cows on farms using conventional methods. We have developed a technique based on continuous breath analysis during milking. The objectives of the current study were to examine sources of variation in methane emissions by individual cows and to compare breath measurements with daily methane output measured in respiration chambers.

**Material and methods** Methane concentrations in breath were measured for 219 Holstein cows (average milk yield 10,000 l/annum) during 66,734 individual robotic milkings over a 5-month period at the Nottingham University Dairy Centre (NUDC). Breath was sampled by a methane analyzer at one l/min via a PVC pipe with an inlet near the cow's nose. An example trace of methane concentration during one milking is shown in Figure 1, in which each peak corresponds to an eructation. Methane emission rate during milking (MERm; mg/min) was calculated for each milking as mean peak integral multiplied by peak frequency. Cows were fed ad libitum on a total mixed ration (TMR-LM; 0.32 Maize, 0.13 grass and 0.12 whole-crop silages; 0.05 straw, 0.1 beet pulp, 0.13 rape, 0.08 soya, 0.02 fat, 0.04 minerals), plus concentrates during milking (1.6 kg/d plus 0.16 kg/kg milk yield above 23 kg/d).



**Figure 1** Breath methane concentrations during a milking

To calibrate the technique, 12 cows were transferred to respiration chambers and fed on the same diet as at NUDC. Daily methane output was recorded for 3 to 7 days and regressed against mean MERm for the 10 days preceding transfer.

To examine dietary effects on MERm, TMR-LM was compared with a high-methane TMR (HM; 0.6 LM, 0.24 grass silage, 0.11 peas, 0.06 sugar beet pulp) (HM) in a crossover design with 42 cows per diet for a period of 14 days. Diet HM was fed also to 12 cows in respiration chambers to confirm effects on total daily methane emissions.

Daily mean MERm data were analysed using linear models to partition variation between and within cows and analysis of variance to test the significance of each source of variation.

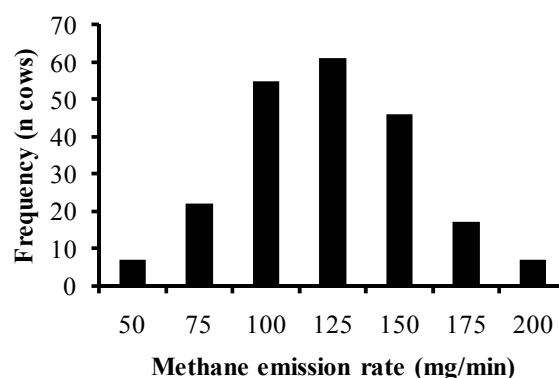
**Results** Considerable variation among cows was observed in MERm (Figure 2). Between-cow variation was 32 times greater than within-cow variation ( $P < 0.001$ ). Much of the between-cow variation could be explained by live weight ( $P < 0.001$ ) and milk yield ( $P < 0.001$ ) but, even after adjusting for these factors, a significant amount of between-cow variation was still unexplained. Changes in live weight and milk yield explained significant proportions ( $P < 0.001$ ) of the within-cow variation. It is likely that live weight and milk yield act as proxies for dry matter intake, which is the main driver of methane emissions.

The relationship between mean MERm recorded over 10 days at NUDC and subsequent methane emissions by the same cows in respiration chambers was: Chamber methane emissions (g/d) =  $278.6 (\pm 16.9) + \text{MERm} \times 0.853 (\pm 0.14)$  ( $r^2 = 0.788$ ;  $P < 0.001$ ).

When cows were fed on two diets at NUDC, mean MERm was 110 mg/min for LM and 115 mg/min for HM (s.e.d. 2.43;  $P = 0.042$ ). Dry matter intake was lower on HM than LM (20.3 versus 23.6 kg/d; s.e.d. 0.31;  $P < 0.001$ ). When adjusted for DMI, the proportional difference in MERm (0.20) was similar to the proportional difference (0.16) in methane measured in chambers (LM  $24.9 \pm 0.89$ , HM  $29.0 \pm 0.95$  g/kg DMI;  $P < 0.001$ ).

**Conclusions** The results reveal considerable variation in MERm by individual dairy cows under commercial conditions. Although much of this variation is probably due to feed intake, consistent differences are predicted between cows at equal live weights and milk yields when fed on the same diet. Comparison of breath measurements with respiration-chamber measurements confirms that MERm is a reliable indicator of total daily methane emissions and that effects of diet composition are detected. Variability among individuals has implications for methane inventories and monitoring mitigation strategies, and also raises the possibility of selecting cows for low emissions.

**Acknowledgements** The authors gratefully acknowledge funding from Defra. We also thank: Adam Garnsworthy for development of data-handling software; Morag Hunter for technical assistance; and staff of NUDC and the Biosciences Research Unit for assistance with care of the animals.



**Figure 2** Variation among cows in MERm

## Effect of feeding milled rapeseed on methane emission and milk fatty acid composition in lactating dairy cows

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**Introduction** With the aim of improving production efficiency, lipid has long been added to ruminant diets as an energy supplement, particularly for high yielding dairy cows in early or mid lactation. Manipulation of dietary fatty acid composition with a view to altering milk fatty acid profile has also received much interest, most recently in light of health issues arising from consumption of saturated fatty acids. The ameliorating effect of dietary lipid on methane emissions from ruminants has also been known for many years (Blaxter and Czerkawski, 1966). However, current global efforts to reduce anthropogenic sources of greenhouse gas emissions has led to further interest in the effects of dietary lipids on methane emissions by ruminants. Previous work has also shown that feeding frequency can affect methane emission in dairy cows (Crompton *et al.*, 2010).

**Material and Methods** Four multiparous Holstein-Friesian dairy cows, in mid-lactation were randomly allocated to one of the four treatments in a 4×4 balanced Latin Square design experiment with four 35 day periods. The control diet was formulated to provide a crude protein level of approximately 150 g/kg dry matter (DM) and was fed as a total mixed ration consisting of a 50:50 mixture (on a DM basis) of forage:concentrate, whilst the forage consisted of a 75:25 blend (DM basis) of maize:grass silage. The treatments consisted of the control diet fed twice daily (Con ×2), the control diet containing 35 g/kg DM of lipid from milled rapeseed fed twice (Rape ×2) or once (Rape ×1) daily and the milled rapeseed diet with daily fluctuations in forage:concentrate ratio from 45:55 to 55:45 (whilst keeping the rapeseed at a constant proportion) fed once daily (Rape4/5 ×1). Diets were formulated to be isoenergetic and isonitrogenous. Measurements of respiratory exchange, including methane production, in conjunction with total urine and faecal collection were obtained over four days during the final week of each period, when cows were housed in open-circuit respiration chambers. Milk fatty acid composition was measured as described by Kliem *et al.* (2008) and expressed as a weight percentage of total fatty acids. Data were analyzed statistically using the Mixed procedure of SAS® and a model testing fixed effects of treatments (3 df) and the random effects of cow (3 df) and period (3 df).

**Results** Daily methane emission was reduced by all diets containing milled rapeseed compared to the control diet (Table 1), with the largest decrease observed for the Rape ×1 treatment ( $P < 0.01$ ), which also tended to reduce DM intake. The average reduction in methane emission for the three milled rapeseed diets was 15%. Methane emissions per unit of dry matter intake was also decreased by feeding rapeseed, however, the reduction was only significant for the diets that were fed once daily (i.e. Rape ×1 and Rape4/5 ×1). Methane emissions per unit of milk yield were also reduced by feeding rapeseed, with an average decrease of 20% (Table 1). Feeding rapeseed reduced ( $P < 0.001$ ) the concentration of total saturated fatty acids in milk fat by approximately 17%. This occurred irrespective of feeding regime or oscillating nitrogen content and was due to a decrease in both C16:0 and <=C14:0 concentrations. Concurrently, total *cis*- and *trans*-mono unsaturated fatty acid concentrations in milk fat (including *cis*- and *trans*- C18:1 MUFA) were significantly increased.

**Table 1** Methane emission and milk fatty acid composition in dairy cows

	Con ×2	Rape ×2	Rape ×1	Rape4/5 ×1	sed	P =
Dry matter intake (kg/d)	21.98	20.41	19.73 <sup>†</sup>	20.77	0.684	0.076
Milk yield (kg/d)	32.4	36.4	33.9	35.0	1.52	0.166
Methane (L/d)	630	544*	517**	543*	23.6	0.014
Methane (L/kg DM Intake)	28.9	26.8	26.5 <sup>†</sup>	26.3 <sup>†</sup>	0.880	0.082
Methane (L/kg milk)	19.6	15.2**	15.6**	16.2*	0.814	0.005
Milk fatty acid composition (g/100 g fatty acids)						
Total saturates	72.64	61.01***	60.66***	60.09***	1.33	0.001
Total <i>cis</i> MUFAs	19.47	28.76***	28.80***	29.45***	1.05	0.001

Significantly different from control at <sup>†</sup> $P < 0.10$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Conclusions** Feeding milled rapeseed as a lipid and protein supplement for lactating dairy cows reduced methane production per day, per unit DM intake and per unit of milk yield. Every 1% of added lipid reduced methane emissions relative to DM intake by 2.5% and methane emission relative to milk yield by 7%. Feeding milled rapeseed decreased milk saturated fatty acids and increased *cis*-MUFA, whilst minimising milk *trans*-MUFA and could be used as part of an overall strategy to improve human health and food security.

**Acknowledgments** The financial support of UK Defra project LS3656 is gratefully acknowledged.

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## Effect of herd expansion and reduced inorganic fertiliser use on the global warming potential of four divergent dairy production systems

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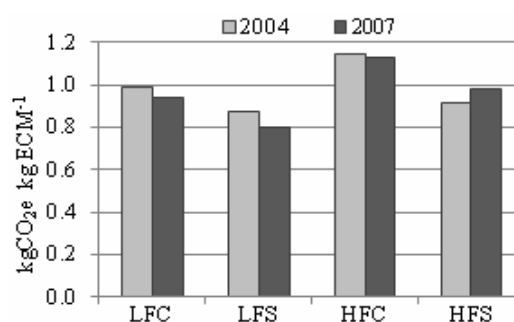
**Introduction** Dairy production is an important contributor of methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>), which are greenhouse gases identified by the IPCC (IPCC 2007). A substantial unavoidable component arises from natural biological processes; however, the high level of emissions opens up opportunities for mitigation. The majority of studies on Global Warming Potential (GWP) have examined dairy systems, or snapshots of a dairy system, at national and farm level. Analyses on the potential variation between production systems are sparse. The aim of the current study was to assess the impact of herd expansion and farm management practices on the GWP of four divergent dairy production systems using Life Cycle Analysis (LCA).

**Material and Methods** Analysis was based on four dairy systems within Scottish Agricultural College's (SAC) long-term Holstein-Friesian genetic and management systems project at Crichton Royal Farm (CRF), Dumfries. Data for two contrasting calendar years were used, on two feeding regimes of high and low forage, and two genetic lines, Select and Control. High forage (HF) group had 75% of their diet from home grown crops (grass silage, maize, alkalage) with grazing outdoors when available. Low forage (LF) group were retained indoors all year round and were fed a diet of approximately 45-50% home grown feeds and the remainder sourced from imported concentrates. Select animals (S) represent the top 5% of UK genetic merit, determined by fat and protein content of milk production, while control animals (C) are of UK average genetic merit. This provided four divergent systems; LFS, LFC, HFS, and HFC and the two contrasting years were 2004 and 2007. The year 2004 was taken as a baseline because this was the full first calendar year after the herd had been established at CRF. In 2007 milking herd numbers had increased by 25% and non-milking stock increased by 39%, while grazing for high forage groups increased from 125 to 160 days. In addition, a 40% reduction in inorganic fertiliser application was achieved with the introduction of slurry injection. Implementation of LCA enabled accounting for the environmental impacts of the whole farm systems and their production of milk from 'cradle to the gate'. Inventory analysis was conducted analysing data on herd dynamics, milk yield and composition, feed intake, crop and land requirements, fertiliser and fuel use. System-specific coefficients were calculated for enteric CH<sub>4</sub>, excreted nitrogen and storage of animal wastes. Impact assessment was conducted using SAC Carbon Calculator, developed in line with IPCC and UK National Inventory guidelines (RBU 2009).

**Results** Between the two years, LFC, LFS and HFC displayed a significant increase in GWP ( $P < 0.05$ ), HFS was noted to increase 1% (Table 1). However, GWP per unit energy corrected milk (ECM) reduced in LFC, LFS and HFC but increased in HFS (Figure 1). Gross CO<sub>2</sub> emissions increased 49% and 46% for LFC and LFS respectively, while gross CH<sub>4</sub> emissions increased for all groups. Emissions of N<sub>2</sub>O increased 11% in LFC with no change for LFS, while HFC and HFS reduced 8% and 16% respectively. The results of the study showed that forage and genotype influenced the GWP of different dairy systems and that CH<sub>4</sub> emissions and farm productivity were key factors. Even with a 46-49% increase in CO<sub>2</sub> due to feed imports, the LF groups were more efficient in 2007. Despite an increase in outdoor grazing and lower N<sub>2</sub>O emissions due to reduced inorganic fertiliser use, the HF groups were still less efficient per unit milk than LF groups. Although gross emissions increased in all four systems, increased milk production in LF groups resulted in reduced GWP per unit energy corrected milk from 2004 to 2007 (Table 1).

**Table 1** Systems total GWP and annual milk yield

	System	2004	s.d	2007	s.d	Δ%
Milk yield kg cow <sup>-1</sup> year <sup>-1</sup>	LFC	8385	2463	8976	2601	7%
	LFS	10271	2910	10406	2741	1%
	HFC	7375	2124	7299	1872	-1%
	HFS	8165	2345	7809	1944	-4%
GWP t CO <sub>2</sub> e	LFC	342.0		417.6		22%
	LFS	334.4		384.1		15%
	HFC	378.2		416.5		10%
	HFS	363.8		367.6		1%



**Figure 1** Systems GWP per unit energy corrected milk

**Conclusions** As the productivity of intensive LF systems increased through herd expansion and milk yield per cow, their GWP per unit of product improved. The potential benefits of reduced inorganic fertiliser use to GWP were offset by an increase in enteric CH<sub>4</sub> and waste nitrogen due to increased herd numbers. This study is part of a long term analysis into the effect of forage type and genetic merit on the balance of GWP within dairy production systems.

**Acknowledgements** The authors gratefully acknowledge the work by staff at the SAC Dairy Research Centre, Crichton Royal Farm, Dumfries and funding from the Scottish Government.

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## Effect of ranking on phenotypic residual feed intake (RFI) and diet type on ruminal methanogenic populations in beef heifers

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**Introduction** Production of enteric methane from ruminant livestock is one of the main contributors to greenhouse gas emissions both in Ireland and globally. Selection for improved feed efficiency in beef cattle has been shown to reduce total and feed intake corrected methane emissions without compromising animal production (Hegarty *et al.*, 2007) however, data to date is inconclusive (McDonnell *et al.*, 2009). As such, few published studies are available on the biological mechanisms controlling this effect and in particular the methanogen species involved. Traditional analyses of rumen microflora have focused on culture based techniques however it is now known that this approach has largely underestimated microbial diversity in the rumen. The objective of this research was to (i) characterise and (ii) quantify the predominant ruminal methanogenic populations in cattle divergent for feed efficiency across two contrasting diets using two culture-independent methods, *viz* molecular sub-cloning and sequencing; and qPCR technology, respectively.

**Material and methods** Limousin x Friesian beef heifers (n = 86), initially selected on the basis of sire EBV for RFI, were ranked on the basis of phenotypic RFI (Kelly *et al.*, 2010). The 14 highest (HRFI; least efficient) and 14 lowest (LRFI; most efficient) ranking animals were selected for use in this study. Both groups had similar mean bodyweight and ADG at ranking but HRFI had, on average, 20% higher DMI. All animals were allocated to a grass silage diet for six weeks (GS). Three months later all animals were offered a 30:70 maize silage:concentrate TMR, again over a six week period (TMR). Both GS and TMR diets were offered *ad libitum*. Ruminal fluid was sampled at the end of each period using a specialised trans-oesophageal sampling device. Total microbial DNA was isolated from the rumen fluid using a repeated bead beating method. DNA was amplified using PCR primers targeting methanogen specific regions (~200bp and ~800bp) of the partial 16S rRNA gene. Four methanogen specific clone libraries (HRFI GS, HRFI TMR, LRFI GS, LRFI TMR) were constructed and selected clones sequenced. Sequences were aligned and analysed using the online Basic-Local Alignment Search Tool (BLAST) and clustered for classification using a minimum 97% identity cut-off as calculated by the Linux software program MeanID. Total microbial DNA was subsequently used as a template in the development of four qPCR assays designed to quantify total methanogens and specific methanogenic species, identified as the most abundant in the sub-cloning experiment, *viz* *M.stadtmanae*, *M. smithii* and *M. ruminantium*. Abundance of these microbes was expressed as a proportion of total estimated rumen bacterial 16S rDNA according to the equation: relative quantification =  $2^{-(Ct_{\text{target}} - Ct_{\text{total bacteria}})}$ , where *Ct* represents threshold cycle. Data were analysed using mixed models ANOVA (PROC MIXED, SAS 2006). The final model included the fixed effects of RFI group, diet and their interaction with differences between means denoted as statistically significant at  $P < 0.05$ . Animal was included as a random effect.

**Results** BLAST analysis of sequences obtained from 100 randomly selected clones from each of the four libraries revealed *M.smithii*, *M.ruminantium* and *M. stadtmanae* as the most predominant methanogens in the animals sampled. Following qPCR analysis, an RFI phenotype x diet interaction was observed for the *M. smithii* species. There was no effect ( $P > 0.05$ ) of RFI phenotype on the quantity of any methanogen groups measured. However, dietary period affected ( $P < 0.05$ ) ruminal methanogen populations manifested as an increase in total methanogens, *M.stadtmanae*, *M.ruminantium* and *M.smithii* proportions between GS and TMR diets.

**Table 1** Effect of phenotypic RFI and diet on ruminal methanogen populations\*

	RFI (R)			Diet (D)			Significance		
	H	L	SED	GS	TMR	SED	RFI	Diet	RxD
Total Methanogens	7.2	7.9	1.20	6.6	8.5	0.60	0.565	0.004	NS
<i>Methanosphaera stadtmanae</i>	4.4	3.0	1.35	0.5	6.9	1.35	0.306	<0.0001	NS
<i>Methanobrevibacter ruminantium</i>	1.4	1.5	0.50	0.2	2.7	0.48	0.877	<0.0001	NS
<i>Methanobrevibacter smithii</i>	2.5	1.7	0.41	0.7	3.5	0.38	0.061	<0.0001	0.01

NS=Non-significant ( $P > 0.05$ ). \*Microbes measured as a proportion of total estimated rumen bacterial 16S rDNA, relative quantification =  $2^{-(Ct_{\text{target}} - Ct_{\text{total bacteria}})} \times 10^2$ .

**Conclusions** An RFI phenotype x diet interaction ( $P = 0.01$ ) was evident for the abundance of *M. smithii* species, manifested as greater abundance of this species detected in the HRFI (0.04) compared to the LRFI (0.02) phenotype during the TMR period. There was no effect of phenotypic RFI on any of the ruminal methanogen populations quantified in this study. However, diet was found to influence all of the methanogenic species analysed with increases observed when animals were offered the high starch, high energy TMR diet. These results are consistent with increased ruminal CH<sub>4</sub> emissions when animals were offered this diet (McDonnell *et al.*, 2009).

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## Will extended lactations in dairy systems result in a reduction in greenhouse gas emissions?

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**Introduction** Under its Climate Change Act of 2008, the UK Government is committed by 2025 to reducing national greenhouse gas emissions (GHGE) to 80% of 1990 levels. In the agricultural sector, livestock systems are an important source of GHGE, particularly methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). This work follows up the suggestion by Hopkins and Lobley (2009) that the adoption of extended lactations in dairy systems could lead to reduced national GHGE. The assumption underlying this work is that the use of extended lactations could lead to more efficient production and less wastage by both reducing the number of stock required in the national dairy herd and improving the efficiency of the remaining cows. A modelling approach was adopted based on relationships between key variables derived from an analysis of national recorded data.

**Material and methods** The approach taken in this work was to firstly to analyse data from UK dairy herds to define the characteristics of lactations of different lengths. These results were then used to model new herd scenarios based on extended lactations, and the GHGE calculated for each scenario. This was then translated into results at the national level using the national inventory. The characteristics of the lactations of different lengths have been reported elsewhere at this meeting (Pollott *et al.*, 2011). In this study, lactations of 305, 370 and 440d were compared to the model parameters used by Stott *et al.* (PLI; 2005). IPCC Tier II/III methodologies (IPCC, 2006) were used to model the CH<sub>4</sub> (enteric fermentation and manure management) and N<sub>2</sub>O (manure management) emissions from the whole farm system (youngstock and milking herd) and all scenarios were expressed on an annualised basis to allow comparisons between lactations of different lengths. The model was set up to keep the current total annual herd milk output constant, rather than individual cow lactation output. Although total lactation output may be higher with longer lactations it may not always hold that annual outputs of alternative lactation lengths are constant. A number of assumptions were made for the model and the sensitivity of the model to a range of parameter changes were tested; these included replacement rate (20, 22 and 25%), persistency (-10, +10 and +20%) and milk yield (bottom 10% and top 10% for each lactation length).

**Results** The final herd structure of each scenario is summarised in Table 1. As lactation length increased the number of

**Table 1** Herd structure results assuming a replacement rate of 25.8%.

	Base	305-d	370-d	440-d
Milking herd size	125	129	129	127
Non-pregnant followers	17	17	15	12
Pregnant followers	39	40	34	29
Female calves	56	58	49	42

**Table 2** Overall system GHGE for herds with different lactation length and different % replacement rates expressed in t of CO<sub>2</sub>e.

Lact. Lngth (d)	20	22	25	Base (25.8%)
Base	1188.59	1191.05	1194.88	1195.90
305	1211.96	1211.96	1211.96	1214.25
370	1294.66	1297.44	1301.76	1285.54
440	1380.74	1383.68	1388.26	1371.02

calves and followers declined. The effect of this on GHGE can be seen in the 1<sup>st</sup> column of Table 2; herd emissions increased as lactation length increased. Lower replacement rates resulted in lower emissions but the relative decline in GHGE as replacement rates reduced was low. The sensitivity analysis of lactation

persistency (not shown) demonstrated that more persistent lactations resulted in lower GHGE for all lactation length scenarios. However, the best lactation length/persistency combination (440d and 20% more persistent lactations) only resulted in a 7% reduction in emissions. In contrast, modelling different total milk yields resulted in large changes in GHGE. The use of top 10% lactations resulted in a reduction in herd size from 127 to 97 for 440-d lactations and a reduction in emissions of 13%. This effect was even greater for shorter lactations with herd size being reduced from

129 to 78 cows and a 25% reduction in GHGE for 305-d lactations. The opposite effect was found with the bottom 10% of lactations for milk yield i.e. larger herds and higher GHGE.

**Conclusions** The modelling work described here, based on current national herd performance, did not support the assertion that the use of extended lactations in the UK dairy herd will result in reduced GHGE. The effect on GHGE of improved milk yield was far greater than that of lactation length. The combination of higher milk yield and longer lactations may be suitable to reduce GHGE without the concomitant decline in fertility becoming a problem.

**Acknowledgements** This work was funded by Defra project AC0223.

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## Predicting methane emissions from beef cattle on different grasslands – does the prediction equation matter?

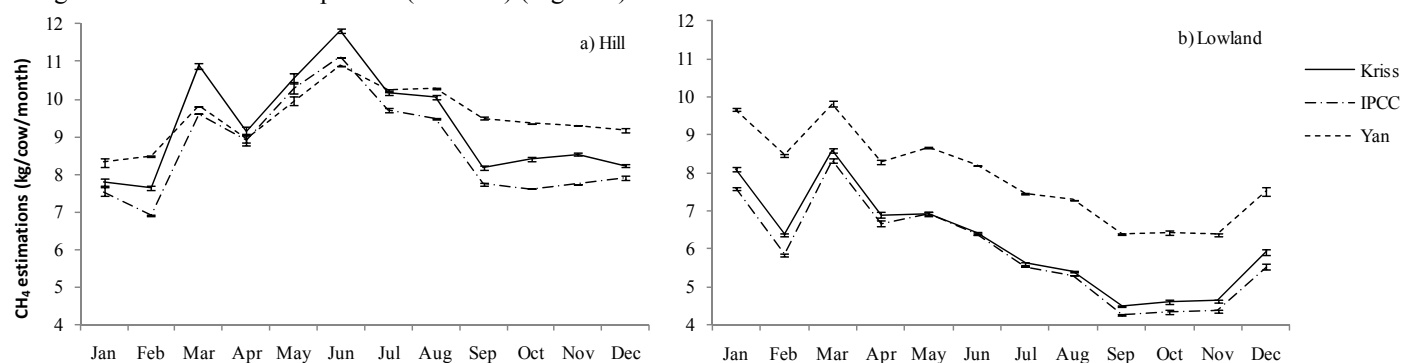
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**Introduction** Semi-natural grasslands cover significant areas of the world, many of them unsuitable for arable cropping. Ruminants can convert grasslands into food for humans but produce methane (CH<sub>4</sub>), as an inevitable output. Measurements of CH<sub>4</sub> from grazing animals are difficult to carry out; hence mathematical models are useful to predict the outcome of different management and mitigation options. Although several approaches to estimate CH<sub>4</sub> emissions have been reported, there is still a lack of information about possible impacts of these different approaches within the context of farming systems. This study aims to compare different equations to estimate CH<sub>4</sub> emissions from suckler cows in a simplified scenario with two contrasting grassland characteristics.

**Material and methods** A simulation model (Armstrong *et al.*, 1997) based on baseline grassland digestibility from the literature was used to estimate monthly digestibility of the diet of suckler cows (DIG) on typical lowland (LG) and hill (HG) grasslands in the UK. A 100 years' Monte Carlo simulation was then applied to generate grassland DIG replications, using mean values of DIG and standard deviations from the literature (MAFF 1990). Energy requirements and dry matter intakes (DMI) of spring calving cows on grazed grass as sole diet were predicted based upon AFRC (1993) using equal levels of annual cow performance and body weight, and body weight change, for all years and treatments. Monthly CH<sub>4</sub> emissions from cows were predicted by three equations 1. Kriss (1930) based on DMI; 2. IPCC Tier 2 (2006) based on energy intake; 3. Yan *et al.* (2009) based on feed energy and energy requirements. Both annually and monthly CH<sub>4</sub> estimations were analysed in a completely randomised design with grassland type, equation and month (when appropriate) as factors. Results were tested with SAS using General Linear Models.

**Results** Feed energy for LG was higher ( $P < 0.001$ ) than HG ( $10.9 \pm 0.04^a$  vs.  $8.3 \pm 0.03^b$  ME MJ/kgDM, Mean  $\pm$  SEM with different superscripts being significantly different). DMI had the opposite response being lower ( $P < 0.001$ ) for LG than HG ( $8.3 \pm 0.04^b$  vs.  $12.9 \pm 0.06^a$  kg/cow/day). There was a significant interaction ( $P < 0.001$ ) between grassland type and equations used to predict annual CH<sub>4</sub> (HG:  $104.6 \pm 0.24^a$ ,  $111.4 \pm 0.26^b$ ,  $114.2 \pm 0.21^c$ ; LG:  $71.1 \pm 0.16^d$ ,  $74.0 \pm 0.18^e$ ,  $94.6 \pm 0.17^f$  kg CH<sub>4</sub>/cow/annum for IPCC, Kriss and Yan, respectively). On average, all estimates of CH<sub>4</sub> emissions from HG were higher than LG ( $P < 0.005$ ) due to the lower DIG of HG and higher DMI of these simulated cows with similar level of performance. Differences were smaller during the winter, when DIG of grasslands and animal requirements are both lower. Monthly CH<sub>4</sub> estimations were affected by all factor interactions ( $P < 0.001$ ). Within HG simulations, monthly CH<sub>4</sub> estimations from Kriss were different ( $P < 0.0001$ ) from the others except in April, July and August. Estimations from Yan were higher than those from IPCC ( $P < 0.0001$ ) except during spring and summer periods. For LG simulations, CH<sub>4</sub> estimations from Yan were the highest and different from the other two during the year ( $P < 0.0001$ ). Estimations from Kriss were only higher than IPCC during the winter and autumn periods ( $P < 0.005$ ) (Figure 1).



**Figure 1** Means and standard errors of CH<sub>4</sub> estimations made by three equations (Kriss, 1930; IPCC, 2006; Yan *et al.*, 2009) for spring calving cows grazing a) hill (HG) and b) lowland (LG) grasslands.

**Conclusions** The annual pattern of CH<sub>4</sub> emissions fluctuated over the year following animal requirements and feed quality variations. Based on the same input values, equations behave differently indicating that the relative importance of each parameter differs between equations. Although it is not typical for cattle in the UK to rely only on grazing during the whole year, this study illustrates that predicted CH<sub>4</sub> production not only differs as a result of diverse system inputs, but also as a result of different equations used to perform the estimations.

**Acknowledgements** authors acknowledge funding from the Scottish Government RERAD and Scottish Natural Heritage.

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## Effect of spice supplementation on *in vitro* degradability, gas and methane production from two forages

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**Introduction** Individual ruminant livestock such as cattle, can produce up to about 500 litres of methane (CH<sub>4</sub>) per day. This represents a significant implication for the environment. Reducing CH<sub>4</sub> production can also be of direct economic benefit because it coincides with the greater energy-use efficiency of the feed by the ruminant animals. Forages are the main ruminant feeds which are blamed for their greater contribution to produce CH<sub>4</sub> from ruminant livestock. It has been shown that tannins, saponins, essential oils and unsaturated fatty acids (UFA) can play important roles in the reduction of CH<sub>4</sub> production in ruminants. Some spices are high in tannins, saponins, essential oils and others are high in fatty acids. It may help if the effect of these spices on CH<sub>4</sub> production can be monitored. Researchers observed that most of the feed additives those are used in reducing methane had negative effect on degradability of forages. Therefore, this study considered the effect of spices on the *in vitro* dry matter degradability (IVD), gas and CH<sub>4</sub> production from rice straw (RS) and ryegrass hay (hay) as two fibrous substrates.

**Material and methods** Two completely randomised design experiments, in duplicate, were conducted to compare the effects of adding one of three spices (Coriander=COR, Cumin=CUM or Turmeric=TUR) each at a fixed level of 40 mg/g to either RS or hay with their relevant Controls (CTR with no spice) on the IVD, gas and CH<sub>4</sub> production after a 120-h incubation period. Total gas volume was measured at intervals throughout the 0-120h incubation period but IVD and CH<sub>4</sub> production were measured after 120h only. Rumen fluid (RF) was obtained from two fistulated sheep just before feeding of fixed amounts of hay plus concentrate at a 2:1 ratio. RF was mixed with the pre-warmed buffer (1:4 v/v, respectively) under CO<sub>2</sub> to prepare the inoculum. Samples of 0.4g of hay or RS and a spice were separately weighed into test tubes to which 40 ml of the inoculum were added under CO<sub>2</sub>. The tubes were incubated at 39°C for the pre-determined times. Syringes were attached to these tubes to collect the gas produced. Stopcocks were used to control the gas entry. Total gas volume was estimated by the displacement of the syringe piston during each incubation time. The gas collected in each syringe was then analysed for CH<sub>4</sub> by using a gas chromatograph equipped with a flame ionisation detector. The residues were separated by centrifuging the tubes (3000 rpm for 10 min), washed with distilled water and dried at 60°C to determine IVD. Data were analysed by using General Linear Model of Minitab to compare spices for their effect on RS and hay at P<0.05.

**Table 1** Effect of adding spices to rice straw or hay, on IVD, gas and methane production .

Spices	IVD (g/kg)		Total gas (ml/g DM)		Methane (ml/g DM)		CH <sub>4</sub> concentration (ml/l gas)	
	Rice straw	Hay	Rice straw	Hay	Rice straw	Hay	Rice straw	Hay
Control	517 <sup>a</sup>	648 <sup>a</sup>	9.1	15.0 <sup>b</sup>	0.55 <sup>ab</sup>	0.72 <sup>b</sup>	60 <sup>b</sup>	48 <sup>b</sup>
Coriander	595 <sup>b</sup>	678 <sup>ab</sup>	9.5	13.6 <sup>ab</sup>	0.45 <sup>a</sup>	0.50 <sup>a</sup>	47 <sup>ab</sup>	37 <sup>a</sup>
Cumin	559 <sup>b</sup>	706 <sup>b</sup>	12.8	10.8 <sup>a</sup>	0.64 <sup>b</sup>	0.43 <sup>a</sup>	50 <sup>ab</sup>	40 <sup>ab</sup>
Turmeric	571 <sup>b</sup>	709 <sup>b</sup>	12.0	13.8 <sup>ab</sup>	0.55 <sup>ab</sup>	0.48 <sup>a</sup>	46 <sup>a</sup>	35 <sup>a</sup>
SEM	10.9	10.3	0.74	0.87	0.027	0.043	2.27	2.00
P	0.006	0.03	0.06	0.05	0.02	0.008	0.05	0.03

<sup>a,b</sup> Means with different superscripts in the same column are different (P<0.05)

**Results** The addition of spices to each forage significantly increased IVD (at 120h) compared with CTR (P<0.03). The effects of spices were not significant for total gas production for RS whereas the concentration of CH<sub>4</sub> and CH<sub>4</sub> production were significantly (P<0.05) lower in the presence of spices than the CTR. The spices also reduced total gas and CH<sub>4</sub> production and concentration of CH<sub>4</sub> in hay (P<0.05). The contribution of nutrients from the spices helped to increase the IVD of rice straw and hay. The higher ether extract and UFA in COR and CUM (Khan and Chaudhry, 2009) might have caused lower CH<sub>4</sub> production by capturing the free hydrogen during their potential bio-hydrogenation in the *in vitro* system. UFA also have direct toxic effects on ruminant microorganisms especially protozoa and gram positive bacteria, which are mainly responsible for CH<sub>4</sub> production. TUR also had higher percentage of UFA; moreover, it contained higher amounts of total phenolics and tannins (Khan and Chaudhry, 2009). These components are known to reduce CH<sub>4</sub> production in ruminants. Therefore, the concentration of CH<sub>4</sub> was lowest in the presence of turmeric for both forages. For both rice straw and hay, spice addition reduced total methane production and its concentration in fermentation gas. However, hay contained more fermentable components than rice straw, which may explain the higher gas production for hay than RS, but in the presence of spices, gas production decreased without affecting the degradability of hay. Essential oils and other components present in spices might have manipulated the fermentation profiles and increased the production of other fermentable components, such as volatile fatty acids or ammonia (not shown here) and reduced gas production from hay. In the present study, the effect of these essential oils on methane production was not studied but this aspect can be considered for the future research.

**Conclusions** As supplementation of hay and rice straw with spices reduced the concentration of CH<sub>4</sub> in fermentation gas and total CH<sub>4</sub> production without showing any detrimental effect on their IVD, this study suggests that these spices can be effectively used to reduce CH<sub>4</sub> production from forages when fed to ruminants.

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## An analysis of BCMS data to determine the breed composition of the UK beef herd

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**Introduction** An accurate profile of a livestock breeding sector is important in informing decision making regarding the potential for adoption of new genetic technologies and the monitoring of genetic resources. This study provides previously unknown data for use in modelling the effects of, for example, the implementation of genomic selection in the UK beef breeding industry. Knowledge of the target population will be a fundamental step in selecting the makeup of genomic training populations. This paper also suggests a protocol for interpretation of existing cattle information sources and proposes adjustments to the national cattle recording database to further increase its value as a research and monitoring tool.

**Material and Methods** The Scottish Agricultural College's copy (BCMS/SAC) of British Cattle Movement Service (BCMS) records, which is restricted to include GB cattle deaths from 1996 to present, was used to estimate the breed composition of the 2008 UK prime slaughter generation and the 2007 UK beef suckler cow population. Breed codes were interpreted as relating to the breed of sire of the animal rather than the breed of the animal itself. Prime slaughter animal records were adjusted to account for; the lack of NI data in BCMS, and the overrepresentation of the progeny of dairy dams in raw BCMS/SAC database queries. Breed composition was determined from sires and maternal grand sires (MGS), with the maternal grand dam (MGD) component estimated. BCMS/SAC therefore directly provided 75% of the breed makeup for each animal. The remaining 25%, the MGD breed composition, was estimated by calculating the proportion of dairy genes in the prime and suckler populations. This was achieved by estimating the numbers of replacement suckler females sourced from the dairy herd (the difference between >31 month beef sired male and female culls), and thus calculating the proportion of dairy genes in the beef herd. The Holstein/Friesian MGD proportion was then determined according to the fact that this breed accounted for 95% of dairy breed codes. The remaining MGD proportion was assumed to be composed of the other beef breeds in similar proportions to their MGS contribution.

**Results** The breed composition of the 2008 UK prime slaughter generation and the 2007 suckler cow population are shown in Table 1, with 85% and 94% respectively of these cattle being cross-bred. In total, 64% of the genetics in the prime population was from beef breeds and 36% came from dairy breeds, 95% of which was Holstein-Friesian. Of the beef genetics in the prime slaughter population, 92% was accounted for by the top seven breeds, and 80% came from non-native breeds. 42% of replacement commercial beef breeding females were sourced from beef sired crosses in the dairy herd, and the average suckler cow had 28% Holstein/Friesian genetics.

**Table 1.** Breed composition (%) of the 2008 UK prime slaughter generation and the 2007 UK suckler cow population. Sire and MGS were calculated and MGD was estimated from 2007/2008 BCMS/SAC.

Breed	Prime deaths >9 <31 months of age				Beef sired female deaths >30 months of age			
	Sire	MGS	MGD	Total	Sire	MGS	MGD	Total
Holstein/Friesian <sup>a</sup>	4.5	9.7	20.0	34.2	-	10.3	18.0	28.2
Limousin	15.5	4.6	0.8	20.8	14.2	3.0	0.8	18.0
Charolais	9.3	1.3	0.2	10.9	4.5	1.4	0.3	6.2
Simmental	5.2	2.6	0.4	8.2	7.1	3.4	0.9	11.4
Aberdeen Angus	4.4	1.9	0.3	6.6	6.3	1.6	0.4	8.3
Belgian Blue	4.5	1.0	0.2	5.7	4.7	0.7	0.2	5.5
Hereford	1.9	1.3	0.2	3.4	3.7	1.1	0.3	5.2
Blonde	1.8	0.4	0.1	2.3	1.5	0.3	0.1	1.9
Others	3.0	2.0	2.9	8.0	8.0	3.3	4.2	15.5
Total	50.0	25.0	25.0	100.0	50.0	25.0	25.0	100.0

<sup>a</sup> It was assumed that there were no suckler cows with Holstein/Friesian sires

**Conclusion** This study suggests that across-breed Genomic Selection will be most appropriate for UK beef breeding. In light of the high proportions of Holstein/Friesian genes in prime and suckler animals, this influence should be taken into account in studies on UK beef cattle. Given the MGD assumption, for which there is considerable evidence, this work shows that it is possible to categorise the UK beef population from just two generations of animals. To simplify the interpretation of breed codes, BCMS could adopt a sire breed coding protocol.

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## Effect of concentrate feed level on the performance of maize silage fed dairy-bred bulls

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**Introduction** Maize silage is widely regarded as an ideal forage for finishing cattle. Recent work by Keady *et al.*, (2007) found that the replacement of grass silage with maize silage with finishing beef cattle significantly improved performance. Work by Walsh *et al.*, (2005) compared the performance of steers finished on *ad lib* cereals or maize silage plus 3kg cereals per head per day and found no significant differences in performance. There is a paucity of information on the effect feeding different levels of concentrates on the performance of bulls offered maize silage. The objective of this experiment was therefore to evaluate rations with low (25% of DM) or high (50% of DM) levels of concentrate supplementation on the performance of intensively finished dairy-bred bulls fed *ad lib* maize silage.

**Material and Methods** Thirty six Holstein bulls weighing 224kg at 6 months of age were fed the following diets formulated to contain 140g CP/kg DM *ad libitum* through to slaughter: 75:25 containing 750kg/t DM good quality maize silage (DM 339g/kg, 305g starch/kg DM) and 250kg/t DM concentrates; 50:50 containing 500 kg/t DM and 500kg/t DM maize silage and concentrates. The concentrates contained (kg/t) 470 and 775 rolled barley, 470 and 200 rapeseed meal, 60 and 25 minerals for the 75:25 and 50:50 treatments respectively which equated to a daily concentrate feed rate of a mean 2.5kg and 5.4kg per head per day. Prior to the experiment the bulls were initially reared to 6 months old on a cereal beef system. The cattle were housed in straw-bedded pens and were selected for slaughter at EUROP fat class 3. The data was analysed using ANOVA.

**Results** The bulls were 15.2 months old at slaughter (s.e.d = 0.21).

**Table 1** Animal Performance

	75:25	50:50	s.e.d	Sig
Slaughter weight (kg)	587	585	9.3	NS
Days to slaughter	276	272	9.4	NS
DLWG (kg)	1.32	1.33	0.036	NS
Carcase wt (kg)	295	296	6.4	NS
Kill out (g/kg)	503	506	4.6	NS
Carcase daily gain (kg)	0.69	0.71	0.023	NS
Conformation class <sup>1</sup>	2.6	2.4	0.17	NS
Fat class <sup>1</sup>	2.9	3.1	0.17	NS

<sup>1</sup> EUROP carcass classification: Conformation: P+=1 and E=7, Fat class: 1=1 and 5H=7.

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

	75:25	50:50
Maize silage (kg DM)	1,705	1,303
Concentrates (kg)	691	1,480
FCR (kg DM: kg LWG)	6.39	7.20
Feed cost (p/kg LWG)	50	64

Overall the bulls met recognised targets for silage beef production with slaughter weights of 575-600kg at 15-15.5 months of age. There were no significant differences between the effects of the treatments. The bulls finished on the 75:25 treatment recorded an increased margin over feed worth £52 per head with reduced feed costs per kg gain worth 14p/kg based on the costs prevailing at the time of the study.

**Conclusions** Increasing the concentrate feed ratio from 25% to 50% of dietary dry matter intake with bulls fed good quality maize silage had no significant effect on animal performance and increased feed costs. Prior to the experiment the bulls were initially reared to 6 months old on a cereal beef system since bulls at Harper Adams from 3 to 6 months old will typically record a DLWG of 1.55kg with an FCR of 3.4:1 and get them to a size to utilise and cope with a forage based diet, despite at the time a relatively high price for barley. The bulls met recognised targets for silage beef production with slaughter weights of 575-600kg at 15-15.5 months of age whereas cereal fed dairy-bred bulls typically reach slaughter weights of 525-550kg at 13-13.5 months old. With the recent fluctuations in the price of cereals, good quality maize silage offers beef finishers the potential to reduce feed costs and increase margins.

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## Protein availability of different co-products from bio-ethanol processing to dairy cattle

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**Introduction** Use of bio-ethanol as fuel in North America has resulted in the production of millions of tonnes of new co-products such as wheat dried distiller's grains with solubles (DDGS), corn DDGS and blend DDGS (e.g. wheat/corn ratio of 70:30, 50:50 etc.) Producing such huge amounts of DDGS has urged the need for a systematic evaluation of these co-products in terms of nutrient availability and nutrient variation among different types of DDGS. With these data one can formulate diets with accuracy. Information on metabolic characteristics of DDGS protein affected by DDGS type is limited and is mainly based on the old protein evaluation systems, particularly for wheat DDGS and blend DDGS. The objective of this study was to evaluate wheat DDGS and blend DDGS for protein availability to dairy cattle using updated the DVE/OEB protein evaluation system (Tamminga *et al.*, 2007).

**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 DVE/OEB system is outlined in detail by Tamminga *et al.* (2007). Protein value of DDGS samples for dairy cattle were determined as true protein digested and absorbed in the small intestine:

$$\text{DVE} = \text{DMCP} + \text{DRUP} - \text{DMFP}$$

where DVE is true protein digested and absorbed in the small intestine, DMCP is intestinally available microbial crude protein, DRUP is intestinally available rumen undegraded protein and DMFP is endogenous protein loss.

**Results** In the DVE/OEB 1991 (Tamminga *et al.*, 1994), a constant efficiency of 0.64 is assumed for conversion of feed DVE to milk protein, while in the DVE/OEB 2007 it is assumed that the efficiency varies by the ratio between available DVE for milk production to  $\text{NE}_L$  as well as the protein and fat corrected milk production level (FPCM; 3.3 %CP and 4% fat). Taking these into consideration the efficiency was calculated and it was 58.7, 51.7 and 66.2 % for the wheat DDGS, blend1 and blend2, respectively. Therefore,  $\text{DVE}_{\text{milk yield}}$  were 51, 58 and 45 g/kg DM for the wheat DDGS, blend1 and blend2. According to Tamminga *et al.* (2007), a 650 kg lactating dairy cow producing 30 kg FPCM requires 1754 g/day of truly digested and absorbed protein in the small intestine, implying that 4.9 kg of wheat DDGS or 4.7 kg of blend1 or 5.1 kg of blend2 in the ration (on DM basis) would cover 50% of these requirements. The results indicated that the DVE of the DDGS samples was on average two and half times higher than those reported for the alfalfa (178 vs. 70 g/kg DM). Canola meal had a lower (135 vs. 178 g/kg DM) and soybean meal (221 vs. 178 g/kg DM) contained a higher DVE compared to the DDGS samples.

**Conclusions** All types of DDGS are good sources of truly digested and absorbed protein in the small intestine. According to the DVE/OEB 2007, the predicted truly digested and absorbed protein supply to dairy cattle did not differ among the DDGS samples.

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## An evaluation of crimped maize grain for finishing beef cattle

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**Introduction** There is growing interest in feeding grain maize to cattle due to its high energy (14.5 ME MJ/kg DM) and starch (710g/kg DM) content. A relatively high proportion (350g/kg) of the starch is rumen undegradable compared to 150g/kg for rolled barley which should help minimise problems with rumen acidosis. There is a paucity of information on feeding grain maize to beef cattle in the UK. The objective of this experiment was to evaluate the effect of feeding either crimped maize grain or barley to intensively finished dairy-bred bulls.

**Material and Methods** Twenty six Holstein and ten Beef cross Holstein bulls weighing 359kg at 8.5 months of age 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 845kg/t rolled barley, 40kg/t soyabean meal, 40kg/t rapeseed meal, 50kg/t molasses, 25kg/t minerals; Maize Grain Mix containing 860kg/t crimped maize grain, 60kg/t soyabean meal, 60kg/t rapeseed meal, 20kg/t minerals. The Barley and Maize Grain Mixes were analysed to contain 841 and 660g DM/kg and 142 and 141g CP/kg DM respectively. The maize grain (var: *Benicia*) was grown under plastic mulch, harvested on the 19<sup>th</sup> of October 2009 with a DM content of 620g/kg and crimped and ensiled with 3l/t inoculant (Pioneer 11A44) mixed at double strength. The bulls were initially reared to 8.5 months old on a 140g/kg CP cereal based ration fed *ad libitum*. The cattle were housed in straw-bedded pens and were selected for slaughter at EUROP fat class 3. The data was analysed using ANOVA.

**Results** Bulls fed Maize Grain had higher daily live weight gain (DLWG) and carcass gain (both  $P < 0.01$ ).

**Table 1** Animal Performance

	Barley	Maize Grain	s.e.d	Sig
Slaughter weight (kg)	565	571	15.9	NS
Days to slaughter	154	141	9.9	NS
DLWG (kg)	1.34	1.51	0.048	**
Carcass wt (kg)	287	295	6.2	NS
Kill out (g/kg)	508	516	5.0	NS
Carcass daily gain (kg)	0.78	0.91	0.040	**
Conformation class <sup>1</sup>	3.1	2.9	0.22	NS
Liver score <sup>2</sup>	2.06	1.22	0.428	=0.06

<sup>1</sup> EUROP carcass classification: Conformation: P+=1 and E=7. <sup>2</sup> Liver assessment: 1 = Healthy liver and 5 = Severe abscesses.

In the experiment the Maize Grain fed bulls recorded higher carcass weights (+8kg) and were slaughtered 13 days earlier.

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

	Barley	Maize Grain
Total dry matter intakes (kg)	1,362	1,053
FCR (kg DM: kg LWG)	6.59	4.98
Feed cost (p/kg LWG)	96	86

The bulls fed crimped Maize Grain recorded improved FCR's and lower ( $P = 0.060$ ) liver damage scores. Liver abscesses are associated with mild acidosis from feeding high starch based diets. It could be assumed that the reduced incidence of liver abscesses was due the higher proportion of by-pass starch in crimped maize. The improved performance with the crimped Maize Grain fed bulls is likely to be due to improved efficiency of energy utilisation together with a reduced incidence of rumen acidosis. The bulls finished on the Maize Grain treatment recorded a reduced feed cost per kg gain of 10p/kg 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. Replacing barley with crimped maize will result in increased DLWG and carcass gains with an improved FCR. The FCR's appear relatively high for the Barley fed bulls (7.84:1 fresh feed wt) compared to the EBLEX target of 5.4:1 but it must be taken into consideration that the trial did not include the period of growth from 110kg to 360kg. During this rearing phase dairy-bred bulls at Harper Adams typically record an FCR of 3.5:1. Feeding crimped Maize Grain increased margin over feed costs with a 10.4% reduction in feed costs per kg gain. Crimped maize offers significant potential to improve intensive finishing cattle performance, reduce feed costs and increase margins provided good (10t/ha @ 65% DM with 710g starch/kg DM) crops can be grown. If crimped maize had been grown without the use of plastic mulch with an identical yield and feed quality the feed cost per kg gain would be reduced by 24%.

## Effect of early weaning concentrate pellet size on the performance of artificially reared dairy-bred bull calves

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**Introduction** In pursuit of early dry feed intakes with artificially reared calves it has been traditional in the UK to manufacture a pelleted calf starter feed using a small extrusion die (2.5–3.5 mm). This is significantly smaller than the pellet size used for adult ruminants (6–8mm). There are economic consequences for both the farmer and the feed producer. For the farmer, achieving a higher concentrate intake could enhance rumen maturity and minimises growth retardation at and around weaning. For the feed manufacturer the time lost in the feed mill due to extrusion press die changes increases production costs. The objective of this experiment was to compare the effect of feeding either a 3mm or 6mm early weaning concentrate on the performance of artificially reared dairy-bred bull calves to 12 weeks.

**Material and Methods** Forty Holstein and Beef cross Holstein artificially reared bull calves were assigned in a randomised block designed experiment and offered either a 3mm or 6mm concentrate. The calves were individually housed and fed warm (37°C) milk replacer (Wynngold Bloom, Wynnstay Group Plc) mixed at 125g per litre of water twice per day at 4 litres per day. At 8 days of age the milk was increased to 5 litres per day and the early weaning concentrates (Start 'n' Wean pellets, Wynnstay Group Plc) were offered *ad libitum*. The 3mm and 6mm concentrates contained 863 and 856g DM/kg, 205 and 203g crude protein/kg respectively and were manufactured with an identical formulation containing wheat, wheatfeed, barley, sunflower, rapeseed, molasses, distillers, Hipro soya, citrus, full fat soya and minerals. The calves were gradually weaned at 42 days of age and individually penned on straw and offered *ad libitum* straw and water. The calves were moved into group pens at weaning. The data was analysed by ANOVA with calves blocked according to weight and breed.

**Results** The calves fed the 6mm pellets recorded a significantly higher (<0.05) DLWG from weaning to 12 weeks.

**Table 1** Effect of pellet size on live weight (kg)

	3mm	6mm	s.e.d	Sig
Start weight	51.6	51.4	1.70	NS
Weaning weight	68.0	68.4	3.06	NS
12 week weight	110.9	117.4	5.61	NS

**Table 2** Effect of pellet size on DLWG (g) and last rib girth measurement at 12 weeks

	3mm	6mm	s.e.d	Sig
Start – 3 weeks DLWG	219	243	43.1	NS
3 weeks – weaning DLWG	562	567	76.2	NS
Weaning - 12 weeks DLWG	1,021	1,167	71.4	*
Start - 12 weeks DLWG	706	786	53.7	NS
Last rib girth (cm)	131.1	134.4	4.78	NS

**Table 3** Feed intakes (kg/head) and Feed Conversion Ratio (FCR)

	3mm	6mm	s.e.d	Sig
Conc intake (start - weaning)	22.3	31.2	3.40	**
Conc intake (wean - 12 weeks)	125.0	138.0		
Milk replacer	21.4	21.4		
FCR (kg feed: kg gain)	2.84	2.89		

Concentrate intakes from start to weaning were significantly higher ( $P<0.01$ ) for the calves fed the 6mm pellet and overall consumed an extra 21.9kg more pellets per head.

**Conclusions** Overall performance was good and the calves fed the 6mm pellet exceeded the MLC (1999) target for rearing calves to 12 weeks of 115kg and recorded a significantly higher ( $P<0.05$ ) DLWG from weaning to 12 weeks old gaining an extra 6.7kg in live weight from start to 12 weeks. Concentrate intakes from start to weaning were significantly higher ( $P<0.01$ ) with the 6mm pellet and overall the calves consumed an extra 21.9kg more per head. It could be assumed that the increased concentrate intake resulted in the improved DLWG with the calves. The improved intake with the 6mm pellet would minimise growth check at weaning and enhance rumen development. As shown in table 2 the 6mm pellet fed calves had a higher but non significant 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 a 6mm pellet and thus reduce calf rearing costs.

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## The relationship between physical and chemical characteristics of maize silage and its whole tract apparent organic matter digestibility and *in situ* degradability

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**Introduction** Maize silage is an important component of dairy cows diets in the UK, and may constitute as much as 75% of the forage fed. As it is such a major component of the diet, it is particularly important that its nutritive value is known, so that a balanced diet can be formulated. The *Feed into Milk* system for formulating dairy cow diets (Thomas, 2004) requires an estimate of whole tract organic matter digestibility, and the rate and extent of dry matter and nitrogen degradability *in situ*. These techniques are expensive and, in the case of rumen degradability, surgically modified animals are required. There have been many attempts to develop less invasive methods of predicting the degradability kinetics of maize silage, for example by using the *in vitro* gas production technique (de Boever *et al.*, 2005). However, the gas production technique is itself rather complex and still requires the use of rumen fluid as an inoculum. The purpose of this study was to determine what relationship there was between the whole tract organic matter digestibility, the *in situ* dry matter degradability of maize silage and various physical and chemical characteristics of the silage.

**Material and methods** A total of 20 samples (1 t each) of maize silage were collected from sites across southern England in 2010. On reception, each sample was thoroughly mixed. The geometric mean particle size (GMPS) of the silage was determined using a Penn State Particle Size Separator before being bagged and stored (-20°C). Samples of silage were analysed for overall apparent organic matter digestibility (OMD) in blocks of 3x3 latin squares using a total of 12 sheep (three sheep were used in the analysis of each silage sample). Complete collections of faeces and urine were made for five days following a 16 d adaptation period. *In situ* degradability was determined in three cows fitted with a rumen cannula. Samples were thoroughly mixed but otherwise unprocessed. A sample size of 10 g dry matter was used, and dry matter degradability was determined with duplicate bags for each cow and each time period. Incubation times were 0, 3, 6, 12, 24, 48 and 72 h. The modified Mitscherlich model ( $\text{Degradability} = a + b(1 - e^{-ct})$ ) was fitted to the degradability data, where *a* is the fraction of immediately degraded material, *b* the potentially degradable fraction, *c* is a rate constant and *t* is time. The effective degradability was calculated assuming a rumen outflow rate of 0.08/h (ED08). Samples of dried (100°C), milled (1 mm screen) silage were analysed for crude protein, starch and neutral detergent fibre (NDF). Correlation coefficients between each pair of parameters were determined. The two parameters with the highest correlation coefficients were then selected to predict OMD and ED08 using stepwise multiple regression (*P* to enter: 0.05).

**Results** The characteristics of the 20 maize silages are summarised in Table 1. The only parameters that were significantly correlated with OMD were starch content ( $r=0.654$ ,  $P=0.002$ ) and pH ( $r=-0.462$ ,  $P=0.040$ ). For ED08, significant correlations were observed with GMPS ( $r=-0.506$ ,  $P=0.023$ ) and dry matter content ( $r=0.533$ ,  $P=0.015$ ). There was no correlation between OMD and ED08 ( $r=0.141$ ,  $P=0.554$ ). Stepwise regression selected both starch content and pH for the prediction of OMD ( $\text{OMD} = 0.931 + (0.0003 \times \text{starch content}) - (0.093 \times \text{pH})$ ,  $P < 0.001$ ),  $R^2 = 0.595$ ,  $s = 0.018$ , but only dry matter content was selected for the prediction of ED08 ( $\text{ED08} = 52.1 + (0.41 \times \text{dry matter content})$ ,  $P = 0.015$ ),  $R^2 = 0.285$ ,  $s = 3.15$ .

**Table 1** Summary of maize silage characteristics

Parameter	Mean	SEM	Min.	Max
Geometric mean particle size (mm)	11.3	0.34	9.9	16.2
Organic matter digestibility	0.673	0.0058	0.620	0.715
Dry matter (g/kg)	308	10.6	251	418
Crude protein (g/kg DM)	82	2.7	66	107
Starch (g/kg DM)	257	12.0	151	339
NDF (g/kg DM)	477	9.0	402	547
pH	3.62	0.026	3.40	3.90
<i>a</i> (%)	37.9	1.54	27.7	48.6
<i>b</i> (%)	43.7	1.16	35.7	53.1
<i>a</i> + <i>b</i> (%)	81.6	0.87	75.6	92.5
Effective degradability (%; 0.08/h)	64.7	0.81	57.9	70.4

**Conclusion** Whole tract OMD is not related to rumen degradability of dry matter. There is a weak relationship between the OMD of maize silage and its starch content and pH, but ED08 cannot be predicted from simple determinations of chemical analysis.

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## Near infrared reflectance spectroscopy (NIRS) as a tool for understanding the differences in molecular structure of dry matter: dried distiller's grains with solubles (DDGS) as a feed model

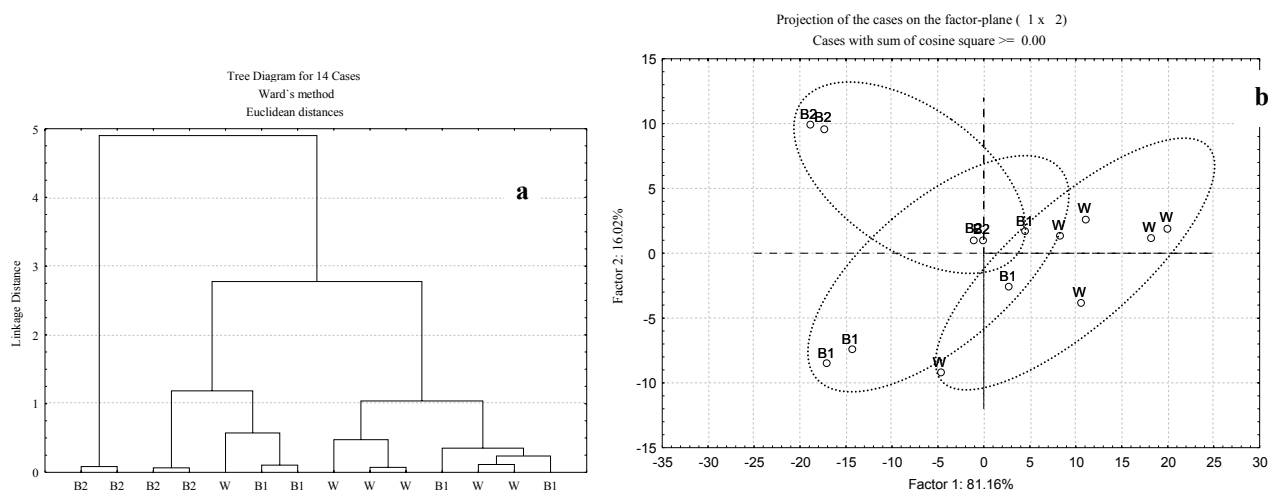
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**Introduction** The conventional method to determine the nutrient make-up of feeds is the traditional 'wet' chemical analysis. The so-called 'wet' chemical analysis is a destructive and time consuming method. This method heavily relies on the use of harsh chemicals and derivatives which alter the native structures and generate artefacts. As opposed to 'wet' chemical analysis, advanced spectroscopic techniques, such as NIRS is a rapid, labour-efficient method that can potentially represent a reasonable alternative to conventional techniques to determine the nutrient make-up of feeds. More importantly when using along with the advanced multivariate statistical methods such as cluster (CLA) and principal component analysis (PCA), NIRS is capable of exploring the differences in structure and molecular chemistry of food and feed samples. In the current study we aimed to determine the magnitude of differences in molecular structure of dry matter among the different types of the DDGS using NIRS.

**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). Near-infrared spectra were collected in a NIR scanning monochromator (FOSS, InfraAct™, Model 1001001/Rev 3.0, Höganäs, Sweden) equipped with spinning module. The instrument works in reflectance mode in the spectral range 570–1850 nm, taking readings every 2 nm. Samples were analyzed ground (roller mill, gap 0.203 mm; Apollo Machine and Products Ltd, Saskatoon, SK, Canada), using small sample cups (diameter of 60 mm). Spectral absorbance values were obtained as  $\log(1/R)$ , where  $R$  is sample reflectance. Data were collected using ISI scan software. The spectra in the range of 570–1850 nm were converted to the codes which were used for the multivariate analysis using Win ISI software. Agglomerated hierarchical cluster analysis (CLA) and principal component analysis (PCA) were performed using Statistica software 9.0 (StatSoft Inc., Tulsa, OK, USA) to classify and distinguish the inherent differences of dry matter molecular structures among the DDGS sample. Spectral region  $1719\text{--}1485\text{ cm}^{-1}$  was used for CLA and PCA. For the CLA, Ward's algorithm method was used without any prior parameterization of spectral data. For the PCA, the first two principal components were plotted.

**Results** Cluster analysis and PCA showed that differences existed in the molecular structure of wheat DDGS and blend2, but no differences were observed in the molecular structure of dry matter between wheat DDGS and blend1 and between blend1 and blend2 (**Figures 1a** and **1b**). Indeed Azarfar and Yu found that (unpublished data) the nutrient profiles, protein and carbohydrate sub-fraction, estimated energy values for dairy and beef cattle and *in situ* degradability differed among the wheat based and blend2 DDGS.



**Figure 1** Cluster (a) and principal component (b) analysis of NIRS: Dry matter molecular structure of wheat DDGS (W) and two blend DDGS (B1, 30% corn and 70% wheat; B2, 50% corn and 50% wheat).

**Conclusions** The results of the current study revealed that there were distinguishable differences in the molecular structural makeup of dry matter among the different types of the DDGS samples. More importantly we demonstrated that when using along with the advanced multivariate statistical methods such as cluster (CLA) and principal component analysis, NIRS is capable of exploring the differences in molecular structural chemical makeup of feed samples.

## Effects of maturity stage at harvest and dietary inclusion rate of whole-crop maize silage on intake, feed utilization and performance of growing dairy bulls

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**Introduction** The whole-crop maize (WCM) cultivation has increased rapidly in southern part of Scandinavia and the increasing trend is likely to continue as new, earlier maturing varieties, are developed continuously. The stage of maturity at harvest can affect both the nutrient composition of the whole-crop maize silage (WCMS) and the feeding value (Browne *et al.*, 2005). However, limited research has been conducted on maize as a forage crop in ruminant livestock production systems in Scandinavia. This study aims to investigate whether feed intake, feed utilisation and carcass quality are sensitive to the maturity stage at harvest and dietary inclusion rate of WCMS when fed to growing dairy bulls.

**Material and Methods** Whole-crop maize of the early maturing cultivar Avenir and grass forages (timothy, meadow fescue, perennial ryegrass) were harvested for silage. The crop was harvested and chopped at two different stages of maturity: dough stage (25% DM, 23% starch and 38% NDF) (E) and dent stage (34% DM, 36% starch and 38% NDF) (L). At both occasions the WCM was treated with the chemical additive KOFASIL MAIZE® (Addcon Europe, GmbH, Bonn Germany) according to manufacture recommendations and was pressed into round bales. The grass ley was harvested as a third cut in 2008, wilted to 35% DM, and ensiled with the acidic additive PROMYR® (Perstorp Inc., Perstorp, Sweden) in a bunker silo.

Sixty-four growing dairy bulls (Swedish Holstein n=49; Swedish Red, n=15) were used in the experiment. The animals were brought indoors approximately 10 weeks prior to the start of the experiment (week 50, 2009) in order to adapt to experimental conditions and diets. The bulls were assigned to 16 groups with 4 animals per group, on the basis of their initial body weight (BW; week 47, 2009), resulting in 8 groups of light and 8 groups of heavy bulls. Two groups of light and two groups of heavy bulls were then randomly allocated to one of the four feeding treatments, resulting in a total of 16 bulls per treatment. The dietary treatments formed a 2×2 experimental design with two maturity stages at harvest (E or L) of WCM for silage and two proportions of WCMS in the diet (i.e. 100% WCMS) (100%) or 50% WCMS plus 50% grass silage) (50%). The diets were formulated as total mixed rations (TMRs) on an average daily BW gain basis of 1.5kg, and were balanced to the same content of NDF, starch, metabolizable energy and crude protein. The concentrates (rolled barley, dried distillers' grain and cold-pressed rapeseed cake) were included in the TMRs in a proportion of around 40% of DM. The animals were fed *ad libitum* on a pen level at amounts corresponding to 105-110% of the average intake of the three previous days. All animals were slaughtered when they reached a target bodyweight of 630kg.

**Results** Daily feed intake (DFI) and daily BW gain (DBWG) were not affected by the maturity stage at harvest of the WCM (Table 1). The inclusion rate of the WCMS at 100% tended to result in higher daily bodyweight gain in both harvest stages (Table 1); the animals reached the target bodyweight for slaughter, on average, two weeks earlier than those fed on the 50% inclusion rate did. The feed conversion ratio (FCR) was not affected by the inclusion rate but tended to be affected by the maturity stage at harvest, with the animals fed on the dough stage of the WCMS showing better conversion ratio (Table 1). However, this effect was highly significant ( $P=0.01$ ) and at the same direction when the feed conversion ratio was calculated on the silage intake basis (data not shown). Carcass conformation was not affected by any treatment.

**Table 1** Harvest stage and inclusion rate effects on daily feed intake, daily BW gain and on feed conversion ratio

	Dough Stage		Dent Stage		SEM	Effects ( $P=$ )		
	100% WCMS	50% WCMS	100% WCMS	50% WCMS		Harvest Time (HT)	Inclusion Rate (IR)	HT× IR
DFI (kg DM/ animal)	10.99	10.38	10.97	10.85	0.24	0.382	0.159	0.323
DBWG (kg)	1.75	1.59	1.63	1.54	0.07	0.253	0.100	0.618
FCR (kg DMI per kg BWG)	6.31	6.56	6.81	7.07	0.26	0.074	0.338	0.970

**Conclusions** The results suggest that WCMS as forage in diets of growing bulls can be an important parameter towards the economics of the producer as the animals offered diets at 100% WCMS reached the target slaughter weight two weeks earlier, than those at the 50% WCMS diets did. However, this finding, merits further investigation. Interestingly, the results also show that harvest at the dough stage of maturity can be a potential farming practice as it tended to result in better FCR in growing bulls.

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## Carcass pH, temperature and colour changes during the first 48 hours post slaughter in Aberdeen Angus x Limousin and Limousin x Aberdeen Angus steers and heifers

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**Introduction** Post slaughter changes in carcass slaughter and chilling conditions can have a significant influence on perceptions of meat quality. As part of a wide ranging study to examine animal performance and quality attributes of finishing beef cattle, the objective of the current experiment was to quantify the differences in carcass pH, temperature and colour measurements within beef carcasses from both Aberdeen Angus cross Limousin (AAx) and Limousin cross Aberdeen Angus (LIMx) steers and heifers slaughtered through a commercial abattoir.

**Material and methods** 100 beef cattle were finished using a commercial diet comprising grass silage and a cereal-based concentrate offered *ad libitum* during the final 2-4 months prior to slaughter. Animal performance characteristics of these animals during this finishing phase have been given previously (Hyslop *et al*, 2009). These animals were either AAx (from 3 sires) or LIMx (from 4 sires) and both steers and heifers were studied (27 AAx and 31 LIMx steers, 21 AAx and 21 LIMx heifers). Age at the start of the finishing period ranged from 415 to 489 d (average 460 d) and live weight ranged from 379 to 574 kg (average 479 kg). Animals were slaughtered in 5 batches within a 56 d period. Age at slaughter ranged from 485 to 590 d (average 544 d) and live weights ranged from 512 to 680 kg (average 596 kg). All animals remained on the diet for a minimum of eight weeks after which they were selected for slaughter according to standard commercial practice (target grades R4L or better). Following slaughter, carcass pH, temperature and colour measurements were made at 1, 5 and 48 hours post mortem. pH and temperature was determined at the 12<sup>th</sup>/13<sup>th</sup> rib using a Testo 205 pH and temperature meter. Meat colour as L\* (lightness), a\* (red-green) and b\* (yellow-blue) was measured after blooming for 45 minutes, with a portable Minolta® colorimeter (CM-2002, D45 illuminant and 10 ° observer; Konica-Minolta Sensing, Inc., Germany). Data was analysed for the fixed breed (B), sex (S) and BxS effects and the random within S pen effect using the REML facility in Genstat 11.

**Results** At 1 hour post mortem, pH was significantly lower ( $P<0.001$ ) in the LIMx compared to the AAx animals and also significantly lower ( $P<0.05$ ) in heifer compared to steer animals. Carcass temperature was also significantly lower in AAx steers and LIMx heifers at 1 hour post mortem compared with AAx heifers or LIMx steers. No other significant effects on either pH or temperature were seen at either 5 or 48 hours post mortem. Animal sex had the most pronounced effect on carcass colour measurements with heifer carcasses generally having lower L\* values, higher a\* and lower b\* values compared to steer carcasses. LIMx carcasses also had significantly higher ( $P<0.001$ ) L\* values compared with AAx carcasses.

**Table 1** Carcass pH, temperature and colour measurements at 1, 5 and 48 hours post slaughter in AAx and LIMx steers and heifers

	AAx		LIMx		Breed (B)		Sex (S)		s.e.d.		Sig. of effects		
	Steer	Heifer	Steer	Heifer	AAx	LIMx	Steer	Heifer	B & S	BxS	B	S	BxS
pH at 1 hour	6.15 <sup>a</sup>	6.17 <sup>a</sup>	5.96 <sup>b</sup>	6.06 <sup>b</sup>	6.16	6.01	6.12	6.05	0.041	0.058	***	*	
pH at 5 hours	5.54	5.61	5.49	5.51	5.53	5.50	5.51	5.56	0.034	0.047			
pH at 48 hours	5.54	5.51	5.52	5.52	5.53	5.52	5.53	5.52	0.010	0.014			
Temperature at 1 hour	36.7 <sup>a</sup>	37.4 <sup>b</sup>	37.2 <sup>b</sup>	36.6 <sup>a</sup>	37.1	36.9	37.0	37.0	0.19	0.27			*
Temperature at 5 hours	26.3	26.7	26.4	26.2	26.5	26.3	26.4	26.4	0.20	0.29			
Temperature at 48 hours	2.5	2.5	2.4	2.4	2.5	2.4	2.4	2.5	0.03	0.05			
Colour at quartering (48 hours)													
L*	40.8 <sup>a</sup>	40.2 <sup>a</sup>	43.3 <sup>b</sup>	41.3 <sup>a</sup>	40.5	42.3	42.1	40.7	0.50	0.71	***	**	**
a*	26.0	25.4	26.1	25.4	25.7	25.7	25.4	26.1	0.25	0.35		**	
b*	10.1 <sup>ab</sup>	9.6 <sup>a</sup>	10.5 <sup>b</sup>	9.7 <sup>a</sup>	9.9	10.1	10.3	9.7	0.24	0.34		*	

Within the BxS interaction, values not sharing common superscripts differ significantly ( $P<0.05$ ).

**Conclusions** The results indicate that whilst breed and sex can affect carcass pH, temperature and colour measurements immediately post slaughter, the effects are much less pronounced by 5 or 48 hours post mortem in a well run commercial abattoir. However, sex can still have an influence on carcass colour up to 48 hours after slaughter.

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## Effect of feeding plant oil rich in stearidonic acid on growth and meat quality of Charolais crossbred steers

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**Introduction** The first step in the conversion of 18:3*n*-3 to its longer chain derivatives in muscle is elongation to 18:4*n*-3 (stearidonic acid). This first step is considered to be rate-limiting and entry to the pathway at stearidonic acid is likely to result in increased conversion to the long chain C20 polyunsaturated fatty acid (PUFA). Stearidonic acid exists in high concentrations in the oil seeds of *Echium* spp. and is available commercially. Although the benefits of this oil have been examined in studies with monogastrics (i.e. Yang and O'Shea, 2009), studies with ruminants have yet to be conducted. The objective of this study was to assess effect of feeding plant oil rich in 18:4*n*-3 PUFA (from *Echium* spp.) on the fatty acid composition of beef and meat quality in steers.

**Material and methods** Thirty-two Charolais cross-bred steers (~600 kg liveweight) were allocated to one of four dietary treatments: 1) grass silage *ad libitum* (GS), 2) grass silage *ad libitum* plus 1.5% Echium oil (Northstar Lipids Ltd., UK) / silage dry matter intake (DMI; GS-LEO), 3) grass silage *ad libitum* plus 3.0% Echium oil / silage DMI (GS-HEO) and 4) grass silage *ad libitum* plus 3.0% linseed oil / silage DMI (GS-LO). Liveweight was monitored every 14 days. Animals were slaughtered when they achieved fat class 3 and samples of *longissimus thoracis et lumborum* were taken at 48 h post-mortem for fatty acid analysis, and 10-day aged samples for shelf life studies in modified atmosphere packs. Eating quality was determined on meat conditioned for 10 days by a 10-member trained sensory panel. An analysis of variance was conducted with diet as the main factor using GenStat (13th edition) statistical software.

**Results** Carcass weights were similar across treatments. Total fatty acids, amounts of the major saturated and polyunsaturated fatty acids were not different (Table 1). Supplementing grass silage with 3% echium oil and linseed oil increased deposition of total *trans* 18:1 and CLA (*cis*-9, *trans*-11) in muscle. However, incremental echium oil on grass silage did not impact on additional synthesis and deposition of longer chain derivatives EPA and DHA. Effects of additional oils on the eating quality of grilled beef loin were not different (data not shown).

**Table 1** Animal performance, fatty acid composition (mg/100 g muscle) of *longissimus thoracis et lumborum*, and TBARS of muscle in Charolais crossbred steers given experimental diets

	GS	GS-LEO	GS-HEO	GS-LO	s.e.d.	P
Left-side cold carcass (kg)	194	195	197	193	7.8	NS
Total fatty acids	3179	4090	4075	3385	601.7	NS
16:0	845	1127	1108	890	179.4	NS
18:0	425	576	541	474	82.6	NS
18:1 <i>n</i> -9	1123	1378	1358	1117	220.3	NS
18:1 <i>trans</i> , total	42.0 <sup>a</sup>	77.1 <sup>b</sup>	122.6 <sup>c</sup>	79.1 <sup>b</sup>	13.85	<0.001
CLA ( <i>cis</i> -9, <i>trans</i> -11)	9.7 <sup>a</sup>	15.5 <sup>a</sup>	25.0 <sup>b</sup>	15.2 <sup>a</sup>	3.01	<0.001
18:2 <i>n</i> -6	47.1	52.4	54.2	50.0	3.32	NS
18:3 <i>n</i> -3	28.6	31.3	32.1	30.6	2.28	NS
20:5 <i>n</i> -3 (EPA)	16.7	15.5	14.5	17.0	0.96	0.06
22:6 <i>n</i> -3 (DHA)	3.3	3.3	2.7	3.4	0.27	0.06
P:S ratio	0.06	0.05	0.05	0.06	0.008	NS
n-6:n-3 ratio	0.93	0.99	1.00	0.94	0.030	0.06
TBARS, day 10 (mg/kg muscle)	0.66	0.55	0.46	0.49	0.093	NS

TBARS=Thiobarbituric acid reactive substances; Means within a row with different superscripts differ (P<0.05).

**Conclusions** Supplementing grass silage with incremental levels of echium oil rich in 18:4*n*-3 and linseed oil rich in 18:3*n*-3 did not affect PUFA contents in muscle, and these results are not in agreement with those in monogastric animal (Yang and O'Shea, 2009). With the results of higher levels of 18:1 *trans* and CLA in muscle, it is postulated that both echium and linseed oils, which are unprotected, are heavily biohydrogenated in the rumen.

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## **Achieving animal welfare and sustainability benefits by implementing existing knowledge: case examples in pigs and dairy cattle**

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**Introduction** The Farm Animal Welfare Council (2009) has highlighted the need to promote the uptake of existing knowledge in order to promote welfare. Similarly the need to reduce wastage from endemic disease by promoting good practice has been highlighted as an important contributor to the reduction of Greenhouse Gas Emissions in ruminant production. Fortunately there has been extensive research in other disciplines aimed at promoting the uptake of behaviours that have a public good. Using two examples from recent animal welfare intervention studies (pig tail biting and dairy cattle lameness) the effectiveness of traditional advisory and more novel facilitation and social marketing- type approaches have been investigated

**Material and methods** In the first study a husbandry advisory tool (HAT) was designed to reduce tail biting in finishing pigs was constructed by review of the literature and consultation with experts. The HAT was used to highlight farm specific risks and recommend specific changes to the husbandry system on 65 farms. The farms were visited and an overall farm risk score for tail biting and prevalence of tail biting lesions were assessed at the start and end of the study. In another example novel motivational strategies were developed in order to reduce lameness in dairy cattle. The following key concepts were included : recognizing the benefits and barriers to change, facilitating farmers to plan their own changes, establishing lameness prevention activities as a normal behaviour or “norm”, encouraging commitment to the project and providing prompts as reminders to implement new activities. The effectiveness of these approaches on reducing lameness were evaluated on a three year intervention project involving 189 dairy farms.

**Results** For the pig tail biting study, provision of technical information on the presence and significance of farm level risk factors for tail biting did not result in a reduction in tail biting risk factors observed at the end of the study even though the risk factor scores were significantly associated with levels of tail biting on the farms. However, for the dairy cattle lameness study the novel motivational approaches did result in a greater level of lameness related husbandry changes and did result in reduced lameness especially in those farms which had an initial higher levels of lameness.

**Conclusions** Maximising animal health and welfare has benefits for farmers, animals and society. The “public goods” associated with animal welfare and environmental benefits arising from avoiding wastage (i.e. morbidity and mortality) in animal husbandry systems mean that promoting uptake of best practice on livestock farms is an important goal for society. The traditional model of providing technical information based on scientific knowledge and established experience is, however, unlikely to motivate changes to husbandry practices alone. Using these two case examples, the potential value of facilitation and social marketing type approaches has been explored. Both pig tail biting and dairy cattle lameness are potential sources of significant economic loss and significant scientific knowledge is available on how to limit their impact. However, these studies suggest that lack of knowledge in itself is not necessarily a major barrier to improvement. Further work is needed to develop alternate motivational strategies. A key consideration is the cost-effectiveness of these interventions at an industry level. Both the pig and dairy industry recognise the need to control the level of these conditions, however, there will need to be further debate about the level of resource government or industry bodies should invest to bring about additional improvements.

**Acknowledgements** We are grateful for the farmers that participated in the original studies and for the following individuals involved in each study : Nina Taylor, Mike Mendl , Sandra Edwards, Richard Parker, Zoe Barker, Katharine Leach, Claire Maggs and Anouska Bell and Nick Bell. This abstract includes results from studies supported by BPEX, Tubney Charitable Trust and in partnership with Milk Link, OMSCO, Freedom Food, Soil Association and Long Clawson Dairies

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## The early detection of acute and chronic health challenges in beef cattle from changes in behaviour

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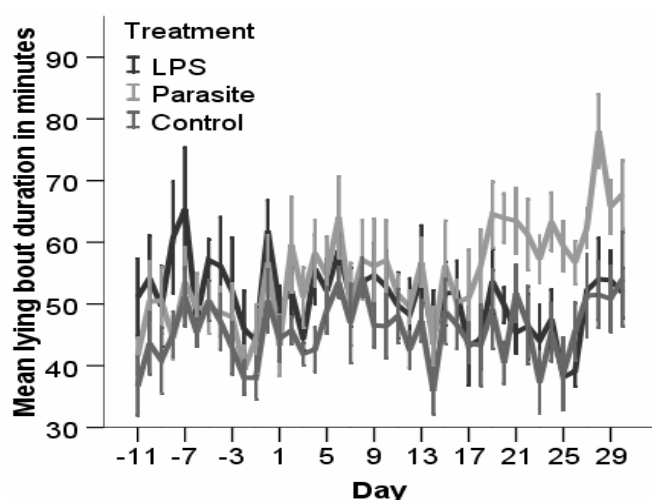
**Introduction** Health challenges that lead to sub-clinical or clinical diseases increase treatment costs and reduce performance and animal welfare. Disease impact and associated costs can be reduced by starting treatment early. Early diagnosis can be difficult, however, especially in animals suffering from a sub-clinical disease because these do not display any clinical signs of illness. Early detection is complicated further by reduction in contact between animals and their carers, as a result of changes in beef management systems. As health challenges can lead to considerable modifications of behaviour, it might be possible to achieve an early diagnosis by monitoring changes in animal behaviour to indicate the early onset of (sub-clinical) disease (González *et al.*, 2008, Quimby *et al.*, 2001). In this study, an attempt was made to quantify the changes in behaviour that could be used as health challenge indicators in beef cattle. The effects of an acute health challenge, in the form of a repeated Lipopolysaccharide (LPS) injection, were compared with that of a chronic health challenge, represented by a gastrointestinal parasite infection with *Ostertagia ostertagi*. Both challenges were sub-clinical, a quick but transient change in behaviours was expected for the LPS challenge, but a slow onset with a prolonged response was anticipated for the chronic health challenge.

**Material and Methods** Seventeen Holstein-Friesian cross beef bulls aged between 5 and 11 months were randomly allocated to one of three groups: one control group of 5 and two treatment groups of 6 animals each. The *O. ostertagi* L3 larvae were given by gavage in a single dose of 200,000 on day 0 to the first treatment group: such larvae take at least two weeks to develop into adult worms. The second treatment group received LPS that was administered in increasing dosages (to prevent accustomization) of 0.2 ng/kg on day 13, 0.225 ng/kg on day 15 and 0.25 ng/kg on day 17. Both the control and the parasite treatment group were given a saline injection on the same days as the LPS group and the control and LPS treatment group were presented with a water gavage on day 0. This allowed for one control group to cover both treatments. Bulls were weighed weekly and faecal egg counts (FEC) were taken every week from day 0. A sensor (Icetag) was fitted to the front leg of each bull to record activity and posture and lying bouts were identified according to Tolkamp *et al.*, (2010). Video recordings were taken to monitor drinking behaviour. Observations lasted from days -11 to +30. 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's from day 21 onwards in parasitized bulls (average 126 eggs/g on day 21). Average daily weight gain differed ( $P=0.012$ ) between treatments, with the parasitized group showing the lowest gain (0.90 kg/day, SE 0.23) compared to the control group (1.52 kg/day, SE 0.10). Gains did not differ between LPS exposed (1.63 kg/day, SE 0.12) and control bulls. A tendency ( $0.1 > p > 0.05$ ) towards a decreased number of steps taken during the hours following the LPS challenge was observed when compared to the control group after the first, but not after subsequent challenges. Neither treatment demonstrated an effect on drinking behaviour as measured by frequency and duration of drinking bouts.

From day 19 until trial end, the daily time spent lying ( $P=0.038$ ) and the length of the lying bouts ( $P=0.003$ ; Figure 1) were longer in parasitized than in control bulls. However, there was no overall change in activity as measured by the number of steps taken between treatments.

**Conclusion** The chronic health challenge induced by parasite infection showed the most persistent behavioural changes, whereas the effects of the acute health challenge (LPS) were only transient. The results suggest that the parasitic infection is a good model to study behavioural changes during the course of a subclinical infection and can be used to further develop a system for the early detection of health problems in beef cattle.



**Figure 1** Lying bout duration in minutes for the three treatments

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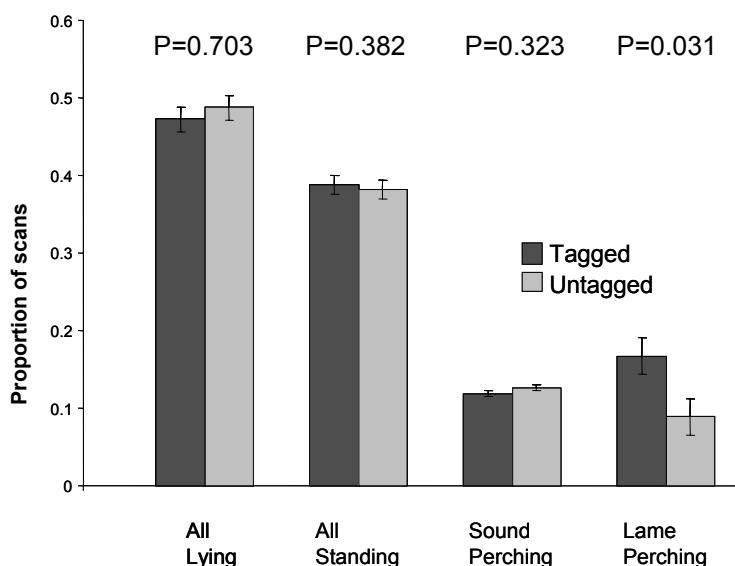
## Establishing the extent of adverse behavioural reactions in dairy cattle to a leg mounted activity monitor

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**Introduction** Accelerometer based activity monitors (often called pedometers) have been used commercially in cattle as a highly accurate form of oestrus detection (Firk *et al*, 2002). They have the potential to be used in studies to characterise individual behaviour, as they are an objective method of quantifying lying and standing bout patterns. Bout patterns can be used to identify onset of illness (Gonzalez *et al*, 2008) and as a behavioural measure of welfare. Before such studies can become widely applied, it is important to establish whether such devices affect the behaviour of the animals they are monitoring. Despite anecdotal evidence of kicking and irritation, there is no consensus on whether or not this is indicative of a greater change in time spent in each posture when an animal is fitted with an accelerometer. The aim of this study was to establish whether these responses are indicative of a greater behavioural change in the first few days of a leg mounted activity monitor being applied to dairy cattle.

**Material & Methods** In this study 28 lactating dairy cows (lactation number  $3.26 \pm 1.87$ ) were fitted with IceTag 3D accelerometers (IceRobotics Ltd. South Queensferry, Scotland). The cows were housed in a free stall barn at the SAC Dairy Research Centre (Dumfries, Scotland). There was a cow:cubicle ration of 1:1 and a feeder:cow ratio of 2:1. Feeding consisted of TMR *ad libitum* using automated feeders which recorded total daily intake for each animal. The animals were allocated to one of two replicates which were balanced for lactation number, locomotion score, days in milk and number of animals naïve to the IceTag device. Both treatment groups were housed together for the duration of the experiment. A habituation phase lasted for 5 days (days -4 to 0). Throughout this phase, the animals were subject to dummy observations for all recorded variables. The subsequent experimental phase lasted 12 days. The baseline period was days 1 to 4. Treatment Group 1 was then tagged on days 5 to 8 and untagged on days 9 to 12 and vice versa for Treatment Group 2. Behavioural data was recorded at ten minute intervals for 6 hours and 40 minutes each day, by one of two experimenters who were familiar to the animals. At each interval the posture of each animal was recorded (lying, standing and perching (standing with front feet only in cubicle)). Data were then analysed by averaging the response variable (feed intake and proportion of observations) over each experimental period (tagged or untagged). Variation in the response from baseline for each period was compared using a general ANOVA in GenStat (11<sup>th</sup> Edition, VSN International, 2010). The treatment structure was 'Experimental Period' and the block was 'Cow'.



**Figure 1** Raw means of most common postures in tagged and untagged periods .P values & standard error bars are shown.

**Results.** The variation in feed intake during the tagged period was not significantly different from the variation from untagged days ( $P=0.438$ ). This was also true for the postures: Lying ( $P=0.758$ ), Standing ( $P=0.397$ ) and Perching ( $P=0.084$ ). When analysing the subgroups, these results remained insignificant for naïve and experienced animals and sound animals. However, lame animals ( $n = 6$ ) perched significantly more in their tagged periods than in their untagged periods ( $P=0.031$ ; See Figure 1).

**Conclusions** Overall, it appears that IceTags are a suitable method of recording behaviour and have no adverse effects on the behaviour of cows. Naïve animals were as capable of adapting as experienced animals. There is a possibility that lame animals, when fitted with an activity monitor around their legs, will change their perching behaviours. For studies on lame animals using activity monitors, effort should be made to record the cause of lameness and

attention paid to the leg the activity monitor is attached to as the possibility of lame animals being affected by the devices cannot be completely discounted.

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## The influence of housing dairy heifers with multiparous cows prior to calving on welfare and productivity during the post calving period

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**Introduction** The post calving period can be a difficult time for first lactations heifers when they are subjected to a number of different stressors simultaneously. These include separation from the calf, entering a new environment and joining a new social group. This latter aspect can be particularly stressful as first lactation heifers often attain low social status and can receive high levels of aggression and bullying from mature cows (Wierenga, 1990; Neisen *et al.*, 2009). The aim of this study was to assess if housing heifers with mature cows before calving influenced their welfare and performance during the post calving period.

**Material and methods** Twenty Holstein Friesian (HF) heifers were assigned to one of two treatments: (1) Mixed with multiparous cows prior to calving ('Mixed'), or (2) not mixed with multiparous cows prior to calving ('Unmixed'). Heifers in Treatment 1 were mixed with non-lactating cows for 3 weeks prior to calving (group contained 3 heifers and 7 mature cows), whereas those in Treatment 2 were kept in an all-heifer group. All heifers received uniform treatment prior to this period, and treatments were balanced for genetic merit, body weight, condition score and sire. Heifers in both treatments were introduced to one of four resident groups containing lactating mature cows approximately 24-36 hours after calving. Each resident group containing 10 HF cows and 5 non-experimental HF heifers was established at least 7 days prior to the introduction of the first experimental heifer. In two of the resident groups, two animals from Treatment 1 and three from Treatment 2 were introduced, whereas the reverse occurred in the other two resident groups. Non-experimental heifers were removed from the group as experimental animals were added. In both treatments, experimental heifers were introduced immediately after the resident group returned from milking between the hours of 06:00 and 06:30 (day 1). The social and exploratory behaviour of each heifer was recorded directly for a continuous 2-hour period immediately after introduction to the group on Day 1. These behaviours were also observed for each experimental heifer during 4 x 5 minute continuous observations at 30-min intervals during the post-feeding period on days 2, 4, 10 and on one day during weeks 3 and 4 post introduction. The heifers' lying and standing behaviour was monitored by automated data loggers (Tinytag Plus, Re-Ed volt, Gemini Dataloggers (UK) Ltd., Chichester, UK). These were fitted for 24 hours on Day 1, and for a continuous 24 hour period each week during the first 4 weeks post-introduction. Milk yield of heifers was recorded daily from day 6 to 35 post-calving. Behavioural and production data were analysed by REML Variance Components Analysis.

**Results** On day 1 Unmixed heifers received more butts than Mixed animals ( $P<0.05$ ). Furthermore, there was a tendency for Unmixed heifers to receive more chases compared to Mixed animals ( $P<0.1$ ). Treatment also had a significant effect on the behaviour of heifers during post feeding observations with Mixed heifers performing more shoulders ( $P<0.05$ ) and butts ( $P<0.01$ ) than Unmixed animals, and Unmixed animals receiving more shoulders ( $P<0.05$ ) and butts ( $P<0.001$ ) than Mixed heifers (Table 1). Also during this period Unmixed animals were located significantly more in the cubicles compared with Mixed animals ( $P<0.05$ ). No significant treatment effect was found for total hours lying, with heifers in both treatment groups lying for an average of 8.4 hours/day ( $P>0.05$ ). Heifers on both treatments lay for less than 5 hours/24 period on Day 1. Treatment had no effect on production performance parameters (Average milk yield of both treatments was 28.8 kg/day).

**Table 1** Effect of being mixed with multiparous cows prior to calving on heifer behaviour and behaviour directed towards heifers during the post feeding period

	Mixed	Unmixed	SED	Significance
<i>Behaviour (frequency/minute)</i>				
Shoulder	0.03	0.01	0.007	<0.05
Butt	0.08	0.01	0.028	<0.01
Receive shoulder	0.01	0.06	0.013	<0.05
Receive butt	0.03	0.26	0.028	<0.001

**Conclusions** The reduction in aggression to which heifers were exposed in the post mixing and feeding periods suggests that their welfare was improved by mixing them with older cows prior to calving. In particular, during the feeding periods these animals were more willing to engage in aggressive behaviour and spent less time in cubicles which suggests an increase in levels of 'confidence'.

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## Ewes treated with lipopolysaccharide as neonates bear offspring that are more resilient to pain caused by castration and / or tail docking. Evidence for cross-generational effects of perinatal programming?

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**Introduction** The potential for early life interventions to cause long-term changes in physiology (perinatal programming) has been shown in laboratory animals, pigs and man (Shanks and Lightman 2001). We have shown that neonatal injection of bacterial lipopolysaccharide (LPS) caused acute hyperalgesia to a mechanical stimulus in lambs (Clark *et al.* 2010a) and ewes injected with LPS as neonates had long term changes in cognition compared to tail docked or handled controls (Clark *et al.* 2010b). This could be attributed to LPS induced perinatal programming; LPS exposure as neonates led to an expectation of a more negative environment and increased vigilance as adults. We hypothesized that lambs born to ewes treated with LPS as neonates wouldn't show hyperalgesia to castration and / or tail docking, because offspring born to mothers that have undergone negative neonatal experiences themselves might show greater resilience and therefore increased pain thresholds following these interventions (Painter *et al.* 2005).

**Material and methods** The study was carried out under Home Office License (PPL 30/2420). The initial study subjects were Mule x Suffolk lambs born in Spring 2007. Twenty female lambs were pseudo randomly divided into three groups; account was taken of lamb birthweight and whether they were a singleton, twin or triplet. All treatments were administered on day 4 after birth. One group (LPS) was administered bacterial LPS (*E.coli* serotype 0127: B8, Sigma-Aldrich Inc.  $1\mu\text{g ml}^{-1}$ ) intravenously  $0.2\mu\text{g kg}^{-1}$ . One group were tail docked using a rubber ring, without analgesia (TD). A third group acted as a handled control (Control). Ewe lambs underwent a number of testing procedures between birth and 17 months age when they were crossed with a Hampshire ram. The 20 ewes born in 2007 lambed in Spring 2009, producing 28 lambs (14 females [F] and 14 males [M]) whose mechanical nociceptive thresholds (MNTs) were tested in the morning and evening of day 2 and the morning of day 3 after birth (before intervention) (time points 1-3 respectively), and in the afternoon of day 3, 3 hours after both castration and tail docking or tail docking (rubber ring without analgesia) (time point 4). MNTs were measured using a pressure-driven analgesimeter (Topcat Metrology Ltd., Ely, Cambs, UK), consisting of a lightweight pneumatic actuator that pushed three rounded metal rods against the limb of the subject. The cuff was fixed securely around the hindlimb of the lamb below the hock joint. The actuator was inflated manually from a syringe. A handset button was pressed at threshold to record the force and the pressure released instantly. The left hind leg was tested first, followed by the right hind leg. Behavioural end-points that signalled threshold responses included a leg lift or movement away from the stimulus. Mean MNT was calculated using data from both legs for each lamb at each test point and data tested for normality of distribution and variance. Male and female lamb data were analysed separately using repeated-measures-ANOVA, with factors of time and ewe treatment group ( $p < 0.05$  was considered statistically significant).

**Results** Mean (SD) MNTs for M and F lambs born to ewes that underwent different treatments as neonates are shown in the table 1 for the 4 time points. Lambs born to LPS ewes had higher MNTs, indicating increased pain thresholds at all measurement time points compared to lambs born to TD and Control ewes (effect of maternal treatment on male lambs  $p = 0.009$  males, females not significant). Post hoc tests indicated that male lambs born to LPS-treated ewes had higher baseline pain thresholds than those born to TD ewes and tended to have higher thresholds after TD & CAST ( $p = 0.07$ ). The effect of time was not significant and there were no significant time x treatment interactions.

**Table 1** Mechanical nociceptive thresholds in lambs born to ewes that underwent different treatments as neonates

Ewe treatment & lamb sex	MNT T1 (N) Mean (SD)	MNT T2 (N) Mean (SD)	MNT T3 (N) Mean (SD)	MNT T4 (N) Mean (SD)
F LPS (n=4)	8.09 (1.1)	7.85 (1.7)	7.80 (2.3)	8.22 (3.0)
M LPS (n=4)	9.01 (2.8)	7.66 (1.6)	8.89 (1.4)	8.17 (1.1)
F TD (n=4)	7.29 (2.5)	6.15 (1.1)	7.80 (1.4)	6.46 (1.5)
M TD (n=4)	5.93 (1.2)	6.30 (0.7)	6.60(1.2)	5.58 (1.7)
F Control (n=6)	6.54 (0.7)	6.54 (1.9)	7.23(1.6)	6.17 (1.5)
M Control (n=6)	7.43 (1.3)	7.43 (0.7)	7.47 (1.3)	6.41 (2.4)

**Conclusions** Lambs born to LPS treated ewes had higher pain thresholds before and after castration and / or tail docking compared to lambs born to TD and Control ewes. This suggests that the effects of perinatal programming following LPS persist through to the next generation of offspring, a result not previously documented in non-laboratory animals.

**Acknowledgements** The authors gratefully acknowledge the BBSRC for funding for the study

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## The effect of two dietary regimes on ruminating and lying behaviour of Belgium-Blue cross heifers housed over winter

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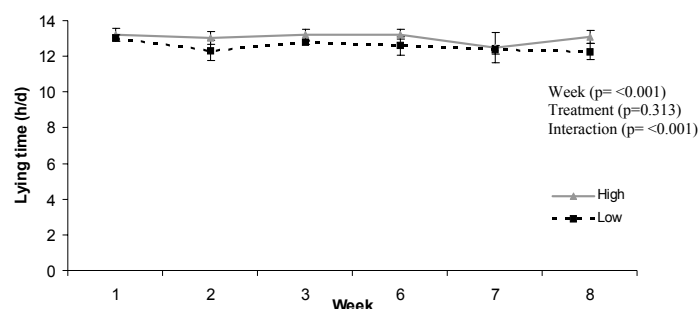
**Introduction** Fibre induces satiety which increases natural behaviours such as ruminating in cattle (Yang and Beauchemin, 2009). Ruminal pH is dependant on the amount of chewing and saliva produced which neutralises the pH. The more cattle chew, the more saliva is produced and therefore an increased chewing time will positively affect rumen pH and reduce the risk of subacute ruminal acidosis (SARA) (Yang *et al.*, 2001). Limited data are available on the lying behaviour of beef heifers over winter (Lawrence *et al.*, 2008). The aim of the present study was to assess the effect of forage only, and forage and concentrate diets on the rumination and lying behaviour of Belgium Blue cross beef heifers.

**Material and Methods** Twenty Belgian Blue cross-Holstein heifers were recruited onto the study. The heifers were  $340 \pm 64$  days old at the beginning of the study. The cows were housed in straw-bedded pens in 2 groups. The Low group were fed *ad libitum* barley straw (38 g/kg CP) and grass silage (125 g/kg CP). The High group were fed *ad libitum* grass silage plus 2 kg concentrate/head/day (16% CP). Rumination behaviour was observed (High (n=10), Low (n=10)) and consisted of counting the number of chews per bolus and timing total bolus time (s) starting from regurgitation to swallowing. IceTag monitors (IceRobotics Ltd) were attached to the right hind leg above the fetlock of the heifers from week 1 to 3 and 6 to 8 of the study (removed and downloaded in week 4). Data from the IceTags were used to determine the percentage of time that the animal spent standing, lying or active; the data were converted to hours/day (time spent lying only presented). Ruminating behaviour (time chewing and chews per bolus) were analysed in using one-way ANOVA and time spent lying was assessed using repeated measured ANOVA using Genstat (12<sup>th</sup> edition).

### Results

**Table 1** Time spent chewing (s) and number of chews per bolus in High and Low group in AM and PM

	AM		PM	
	Time (s)	Chews (no/bolus)	Time (s)	Chews (no/bolus)
High	40.8	53.6	44.6	57.9
Low	38.4	53.9	39.3	54.3
P-value	0.29	0.93	<0.001	0.06



**Figure 1** The time spent lying (h/d) between High and Low group

The High group spent similar time (s) chewing in the AM (0900 to 1200 hours) and the PM (1300 hours to 1700 hours) compared to the Low group. However, the number of chews remained similar between the two treatment groups in AM but tended to be higher in the High group in PM compared to the Low group (Table 1). The High group chewed significantly slower than the Low group in the PM ( $p < 0.001$ ). There were no differences in time spent lying between the high and low groups over the course of the study.

**Conclusion** There were no effects of adding concentrate to the diets of beef heifers on time spent lying down nor time spent chewing in the AM. Similar lying times could mean neither group were satiated and were not restless. Increased chewing time in PM compared to AM could be attributed to the farm being busier in the AM where the cattle may have been disturbed. In the afternoon the High group had a tendency to have increased chews/bolus and chewed significantly slower in comparison with the Low group due to possibly having a reduced proportion of fibre to break down in the diet.

**Acknowledgements** The authors would like to thank Adrian Brown and Claire Dudden and the farm staff for their help with this project

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## The effect of a two step weaning method on the behaviour of six-month old Belgian Blue crossbred beef calves

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**Introduction** Attempts have been made to reduce the acute stress of weaning on beef calves by divorcing the two stressors of a change in diet and separation from the dam (Haley *et al.*, 2005). The use of anti-suckling nose flaps that allow continued maternal contact may provide a solution; however, there is equivocal evidence for benefits in stress reduction. Haley *et al.* (2005) reported significant weight gains and decreased signs of stress using nose flaps compared with traditional weaning, but these positive results have been disputed (Enriquez *et al.*, 2010). The aim of this study was to clarify the effectiveness of this two step weaning method on reducing stress-related behaviour in beef calves.

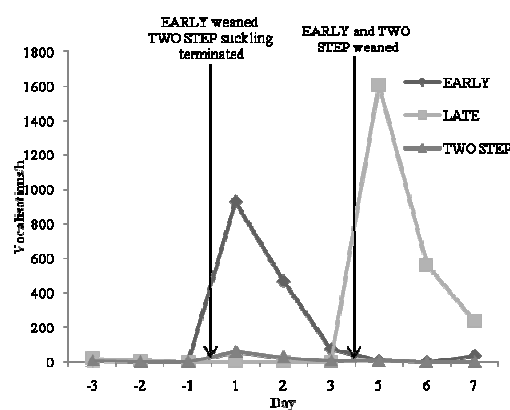
**Material and Methods** 18 Belgium Blue crossbred beef calves aged  $189 \pm 2.9$  days old (mean  $\pm$  SEM) kept on pasture with their mothers with access to a concentrate feeder, were assigned to three treatment groups (TWOSTEP, EARLY and LATE), balanced for gender and age. After 3 days of behavioural observations, the TWOSTEP group were stopped from suckling by a Quietwean nose flap (JDA Livestock Innovations, Saskatoon, Canada – courtesy of Dr Joseph Stookey). Also on day 4 the EARLY group were transferred to another site and housed in a 200m<sup>2</sup> covered strawyard. On day 8, the TWOSTEP and LATE groups were also transferred to the yard. On days 1-3, 5-7 and 9-11 (periods 1, 2 and 3) each group were observed for one hour and time spent lying, walking, grazing, ruminating and any suckling attempts were recorded every 3 minutes as well as total group vocalisations. Lying time, number of lying bouts and number of steps were measured for each calf using 2D accelerometers (IceTag™, Ice Robotics Ltd, Roslin, UK). Ictag data were averaged for each calf for the 3 days in each period and these values analysed using a General Linear Model (Genstat 12<sup>th</sup> edition) using treatment, period and their interaction as main effects. Group observations of behaviour and vocalisations are presented without further inferential analysis. One calf from the TWOSTEP treatment lost its nose flap and so its data were discarded. Accelerometer problems meant that some data were discarded (leaving EARLY n=4, LATE n=4 and TWOSTEP n=3). Low sample sizes mean that results approaching  $P < 0.05$  should be treated with caution.

**Results** Prevention of suckling, whether it be by use of the nose flap or maternal separation, was associated with significant increases in lying bout length in the EARLY and TWOSTEP treatments (but not for the LATE calves) without any real differences in overall lying time (table 1). After weaning both EARLY and LATE groups showed a large increase in their number of vocalisations made by the group from a very low baseline, but the TWOSTEP calves only showed a slight increase following prevention of suckling and no vocalisations after subsequent separation from their dams (figure 1). The number of steps was reduced for all groups moving from the pasture to the strawyard, but the TWOSTEP calves took less than half the steps of the EARLY and LATE groups following separation from their mother (table 1). On day 5 following the application of the nose flaps to the TWOSTEP calves there were 6 attempted sucklings, with 2 on day 6 and 1 on day 7 with no associated vocalisations. Ruminating behaviour was observed in 27 % of preweaning observations for the EARLY and LATE groups and dropped to 10 % and 16 %, respectively. The opposite was seen in the TWOSTEP group that was 14 % pre-nose flap, 13 % with nose flap and maternal contact and 31 % post-separation. Other behaviours did not show any particular pattern (data not shown).

**Table 1** Accelerometer data from 11 beef calves pre- and post- weaning (EARLY separated/ TWOSTEP prevented suckling after period 1, LATE & TWOSTEP separated after period 2)

Variables	Period	Treatment			Standard Errors of Difference		
		EARLY n=4	LATE n=4	TWOSTEP n=3	Treat.	Period	T x P
Time lying (%)	1	58.3	55.4	60.0	1.81**	1.75 <sup>NS</sup>	3.35***
	2	54.7	58.9	56.4			
	3	66.1	45.1	61.3			
Length of lying bouts (min)	1	48.2	47.6	51.7	5.78*	5.23 <sup>NS</sup>	10.02 <sup>NS</sup>
	2	46.1	40.1	66.5			
	3	59.7	40.9	54.8			
Number of steps taken	1	5259	6232	5094	336.7***	304.6***	583.2**
	2	3528	6001	5820			
	3	1514	3654	1245			

n.b. <sup>NS</sup> \* \*\* \*\*\* are not significant,  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively



**Figure 1** Vocalisations made by EARLY, LATE and TWO STEP weaned beef calves pre- and post-weaning

**Conclusion** The absence of vocalisations, reduction in number of steps post-separation and the increase in rumination behaviour all indicate that the TWOSTEP method using anti-suckling nose flaps at weaning confers a welfare advantage compared with traditional weaning in beef calves, supporting the findings of Haley *et al.*, 2005.

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## The Green Pig survey: constraints of using peas and faba beans in growing and finishing pig diets

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**Introduction** Europe is deficient in the amount of protein required for its livestock, importing over 70% of the protein used in animal feed (Crepon, 2006). The UK pig industry relies heavily on soyabean meal (SBM) imported from North and South America, using over 300,000 tonnes a year in grower and finisher diets alone. There are increasing concerns about the sustainability and security of UK pig production, and the environmental impact arising from this reliance on SBM. 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. In the UK, peas and faba beans are such potential home-grown protein sources. Data on current practice relating to dietary inclusion levels of peas and faba beans in UK pig feed are scarce. The Green Pig Survey aims to quantify the use (inclusion levels) of home-grown protein sources in the feeds of UK growing and finisher pigs, and the constraints (real or perceived) associated with them.

**Material and Methods** Data were collected from compound feed companies that produce grower and finisher feed, and pig producers that prepare their feeds on farm (home-mixers) via postal survey, email survey or telephone interviews. Compounder responses covered 52% and 76% of the grower and finisher feeds sold in the UK, respectively. The Home-mixer responses covered 14% of the UK home-mixer herd. The questionnaire used for data collection gathered information on current protein sources used in pig feeds, current inclusion levels used, and the participants' attitudes to using alternative protein sources in pig feed. Attitudes were measured on either a categorical scale ("yes", "no" or "don't know"), or on a 5-point interval rating scale ("strongly agree" to "strongly disagree"). Since individual participants varied in feed sold and farm size, a weighted analysis was performed on data returns to ensure appropriate representativeness of the data. To this effect, home-mixer weights were calculated using sow numbers, whilst compounder weights were calculated using tonnes of grower and finisher compound feed sold.

**Results.** The most common protein used in pig diets was SBM; over 94% of both the compounder and home-mixer samples included SBM in their diets. In contrast less than 1% of the compounder sample used peas and faba beans in their diets. None of the home-mixer responders used peas, whilst less than 2% of the home-mixer sample used faba beans in their pig diets. The two main constraints to increasing the use of peas and faba beans in pig diets identified by the survey were their availability and relative cost compared to SBM. However there was good confidence in nutritional value of peas and faba beans for pig feed. Over 90% of compounders and home-mixers felt that peas provided adequate nutritional value for grower and finisher pig feed, and over 70% felt that faba beans provided adequate nutritional value. Additionally, participants were open to increasing the use of peas and faba beans in the future; approximately 70% of compounders and 80% of home-mixers would increase their use of peas and faba beans in the future, if economic conditions allow and it can be demonstrated that they would not penalize productivity.

**Table 1** The maximum inclusion levels (% of diet) of soyabean meal (SBM), peas and faba beans currently used by compounders and home-mixers in grower and finisher pig feed, and the perceived absolute maximum inclusion levels of peas and faba beans for pig feed

	Weighted mean maximum inclusion level (%) with range			
	Compounder Grower	Compounder Finisher	Home-mixer grower	Home-mixer finisher
SBM (current)	20.1 (15-25)	19.4 (12-25)	19.3 (15-25)	16.3 (9.5-25)
Peas (current)	8 (-)	-	-	-
Peas (perceived max)	15.0 (5-20)	19.5 (5-25)	12.7 (5-30)	13.2 (5-30)
Faba beans (current)	5.8 (3.8-6)	9.5 (5-10)	11.0 (8-30)	10.0 (8-15)
Faba beans (perceived max)	11.4 (2.5-15)	13.1 (5-20)	9.3 (5-40)	14.9 (5-40)

**Conclusions** Both the maximum inclusion levels of peas and faba beans currently used and the perceived maximum inclusion levels for compound feeds (Table 1) are considerably lower than reported from other countries, e.g. France and Canada have reported up to 30% inclusion of peas and faba beans in pig diets (Jezierny *et al.*, 2010). Although home-grown legumes are currently rarely used in UK pig diets and current inclusion levels are low, the results from the survey indicate that there is opportunity to increase confidence in these home-grown protein sources as pig feedstuffs if we can identify ways to overcome the main two constraints of availability and cost of peas and faba beans, and if it can be demonstrated that replacing SBM for peas and/or faba beans does not have detrimental consequences on pig performance.

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## Nitrogen excretion during the growing-finishing period and its relationships with daily feed intake, feed conversion ratio and body composition in commercial pigs

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**Introduction** Environmental impacts from pig production are widespread, affecting soil, water, air, and fauna. The primary nutrient of concern in excreta is nitrogen. Several mitigation methods for reducing the environmental pollution of animals have been studied. Proposed methods include improvement of animal nutrition, manure management, as well as genetic improvement of production, as a complementary approach to the first two methods. The objectives of this study were to determine nitrogen loss at different stages of growth and during the entire growing-finishing period in commercial pigs, and to investigate the relationship between nitrogen excretion and daily feed intake (DFI), feed conversion ratio (FCR) or body composition.

**Material and methods** Data from 315 *ad libitum* fed pigs of a F2 population were used which originated from crosses of several commercial breeds. Protein retention was measured using the deuterium dilution technique during the following growth stages; 60-90 kg (GSI), 90-120 kg (GSII), and 120-140 kg (GSIII). Forty-eight 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 provided two different pelleted diets according to the growth stages containing 17% (GSI), and 16.5% (GSII and GSIII) crude protein. Nitrogen excretion within each stage of growth was calculated using mass balance equation which is the accumulated difference between average daily nitrogen intake (ADNI) and average daily nitrogen retention (ADNR) (British Society of Animal Science, 2003). ADNR was determined as protein deposition in body divided by 6.25. Average daily nitrogen excretion (ADNE), total nitrogen excretion (TNE), and nitrogen excretion per kg weight gain (NEWG) were calculated as nitrogen excretion traits. The GLM procedure of SAS (SAS Institute, 2006) was used to estimate least squares means for excretion traits for each stage of growth and the entire growing-finishing period using a model including sex, housing type, *ryanodine receptor 1* (*RYR1*) genotype, birth farm, and batch as fixed effects, and start and end weights as covariates. The relationship between nitrogen excretion and production traits was estimated as Pearson correlation coefficients between residuals using the above model.

**Results** Lowest ADNI and ADNE (67.38 g/d and 45.80 g/d, respectively), and highest ADNR (21.58 g/d) were achieved in GSI, whilst ADNI and ADNE were highest (75.58 g/d and 56.92 g/d, respectively), and ADNR was lowest (18.66 g/d) in GSIII. Sex, housing type, and batch effects significantly influenced nitrogen excretion, but the degree and direction of influences changed over the stages of growth. Sex differences illustrated that castrated males have higher ADNE, NEWG and TNE than gilts (Table 1). Group housed pigs had higher NEWG and TNE than individually housed pigs except for GSI. Pigs carrying genotype *NN* (homozygous normal) at the *RYR1* locus had the lowest NEWG and TNE at all stages of growth except for GSI. In each stage of growth, *Nn* pigs had higher ADNE, NEWG, and TNE compared to the other two genotypes. High significant ( $P < 0.001$ ) correlations were observed between ADNE and DFI ( $r = 0.96$ ), and between NEWG, TNE and FCR ( $r = 0.99$ , and  $0.91$ , respectively). In the growing-finishing period NEWG and TNE were moderately, negatively correlated with weight gain ( $r = -0.53$  and  $-0.48$ , respectively) and protein deposition ( $r = -0.55$  and  $-0.49$ , respectively), and low negatively correlated with lipid deposition ( $r = -0.25$  and  $-0.17$ , respectively), whereby all correlations were significant different from zero ( $P < 0.001$ ).

**Table 1** Least square means (LSM) of ADNE, NEWG, and TNE over the entire growing-finishing period (60-140 kg)

	Sex		Housing type		<i>RYR1</i>		
	Castrated male	Female	Single	Group	<i>NN</i>	<i>Nn</i>	<i>nn</i>
ADNE (g/d)	55.08 <sup>a</sup>	50.09 <sup>b</sup>	52.30 <sup>a</sup>	52.87 <sup>a</sup>	52.37 <sup>a</sup>	53.23 <sup>a</sup>	52.15 <sup>a</sup>
NEWG (kg/kg)	0.070 <sup>a</sup>	0.065 <sup>b</sup>	0.065 <sup>a</sup>	0.070 <sup>b</sup>	0.065 <sup>a</sup>	0.069 <sup>b</sup>	0.068 <sup>b</sup>
TNE (kg/pig)	5.38 <sup>a</sup>	5.08 <sup>b</sup>	5.08 <sup>a</sup>	5.38 <sup>b</sup>	5.08 <sup>a</sup>	5.36 <sup>b</sup>	5.24 <sup>a,b</sup>

LSM with different superscripts within each fixed effect and trait are significantly ( $P < 0.05$ ) different from each other

**Conclusions** Higher nitrogen retention and lower excretion were associated with higher nitrogen efficiency in GSI. Gilts showed lower NEWG and TNE than barrows, due to lower FCR and lipid:protein gain ratio. Individually housed pigs had lower ADNE, NEWG, and TNE than group housed pigs due to lower FCR, lipid:protein gain ratio and higher average daily gain (ADG). Improvement of growth and FCR on nitrogen excretions was highly important, so that increase in ADG by 100 g/d and improvement of FCR by 0.1 reduced TNE by 310 g/pig, and 173 g/pig, respectively. Since deposition of lipid required more energy than depositing protein, leaner animals required less energy intake, had lower DFI and thus excreted less nitrogen. This study shows that nitrogen excretion can be reduced effectively by improvement of production efficiency through enhancing FCR, weight gain and leanness.

**Acknowledgements** The authors acknowledge funding from British Pig Executive (BPEX) and Scottish Agricultural College (SAC).

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## Efficacy of $\beta$ -glucanase and xylanase blend in mixed grains and grain co-products-based diets for growing/finishing pigs

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**Introduction** Continued pressure on global feed grain markets has resulted in trends for the swine industry to seek alternative cost-effective ingredient options such as co-products from the biofuel and milling industries in addition to mixed grains formulation strategies. One of the major limitations of alternative ingredients is their high content of non-starch polysaccharides (NSP), which limits their use in growing swine rations. However, supplemental carbohydrase enzymes such as xylanase, long recognized to be effective in hydrolyzing NSP, have the potential of overcoming the limitation of using fibrous ingredients in swine diets. In the past, development of these enzymes focused on specific NSP/ingredients for example, xylanase for wheat-based diets. However, there is a realisation that a large array of chemical characteristics exists among NSP fractions in feed ingredients and a more complex cellulytic enzyme mixture may be more effective. When formulating diets, it is also clear that different sexes have different nutrient requirements and release of nutrients from enzymes might have different performance responses when different sexes are fed a common diet. We hypothesized that addition of a blend of cellulases and xylanase (BGXY) will improve growth performance in female and castrated male growing-finishing pigs fed mixed grains and grain co-products-based diets.

**Material and methods** Experimental diets were: 1) Basal control diet, a mixed grains and grain co-products-based diet formulated to meet or exceed the NRC (1998) nutrient requirements for grower and finisher pigs (except for DE, which was 8% lower), 2) control plus 50 g BGXY/MT and 3) control plus 200 g BGXY/MT. The target enzyme activities in diet 2 were 76 and 610 U/kg feed for  $\beta$ -glucanase and xylanase, respectively and corresponding activities for diet 3 were 304 and 2440 U/kg feed. Major ingredients in the basal diet were: corn, barley, wheat, rye, wheat middlings, corn DDGS, peas, soybean meal and canola meal. Diets were formulated for a two-phase feeding program corresponding to grower phase (~25 to ~55 kg BW) and finisher phase (~55 to ~90 kg BW) pigs. All diets were prepared in mash form and contained acid insoluble ash (celite) as a digestibility marker. Thirty-two growing pigs (~22 kg BW) were obtained from The University of Manitoba Glenlea Swine Research Unit and upon arrival pigs were weighed and randomly assigned within gender to pens (n = 2 pigs per pen) containing either 2 castrated males or gilts to give 8 pens per diet (4 pens for each gender). The pigs had free access to feed and water during the two phases. Feed intake and BW were measured bi-weekly to determine average daily feed intake (ADFI), average daily gain (ADG) and gain efficiency (G:F). When pen average BW was ~50 kg, faecal samples were collected for three days consecutively for determination of digestibility of dry matter, energy and nitrogen. Data were analyzed using GLM procedure of SAS with significance level claimed at  $P < 0.05$ .

## Results

**Table 1** Effects B-glucanase and xylanase supplementation on growth performance and total tract digestibility in growing pigs (~25 to ~55 kg BW) fed wheat-barley based diets

Item	Gilts			Castrates			SEM	Effects		
	0	50	200	0	50	200		Sex	Diet	Interaction
BGL/Y5: g/ton										
ADG, kg/d*	0.77	0.77	0.86	0.82	0.86	0.82	0.02	0.05	0.11	0.01
ADFI, kg/d	1.73	1.82	1.84	1.78	1.91	1.80	0.04	0.27	0.05	0.34
GF, kg/kg	0.45	0.43	0.47	0.46	0.45	0.45	0.01	0.36	0.09	0.17
Dry matter, %*	78.3	80.3	83.6	78.8	79.3	85.1	0.83	0.65	<0.01	0.35
Energy, %*	79.1	80.7	84.6	79.6	79.0	85.6	0.72	0.90	<0.01	0.17
Nitrogen, %*	78.9	79.0	85.2	79.5	77.2	85.8	0.99	0.81	<0.01	0.39

\*Linear effect,  $P < 0.05$ .

The castrated males had higher feed intake and growth ( $P < 0.05$ ) than the females as exhibited by the differences between the control and treatments (Table 1), which was also the case in the finishing phase (not shown). Supplemental BGXY increased ADG in gilts (Table 1); an effect that was reflected in a better overall (25 to 90 kg BW) ADG for the gilts despite lack of diet effect ( $P > 0.05$ ) on growth performance responses in the finishing phase (not shown). BGXY linearly increased ( $P < 0.05$ ) faecal digestibility of dry matter energy and nitrogen in energy-limiting mixed grains and grains co-products diet in both gilts and barrows.

**Conclusions** The castrated males had higher feed intake than the females, which was not unexpected. However, it is noteworthy that the better digestibility from the enzyme combination supplementation observed in castrates was not reflected in better growth performance perhaps suggesting that the control diet might have been over-formulated for castrates, given their higher initial feed intake. The current study demonstrates that supplemental cellulase and xylanase blend in an energy-deficient, mixed grains and grains co-products-based diet linearly improved growth performance in a diet balanced for growing gilts through improved feed intake linked with increased dry matter, energy and nitrogen digestibility.

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## Trial site influences optimal soyabean inclusion level in piglet starter diets

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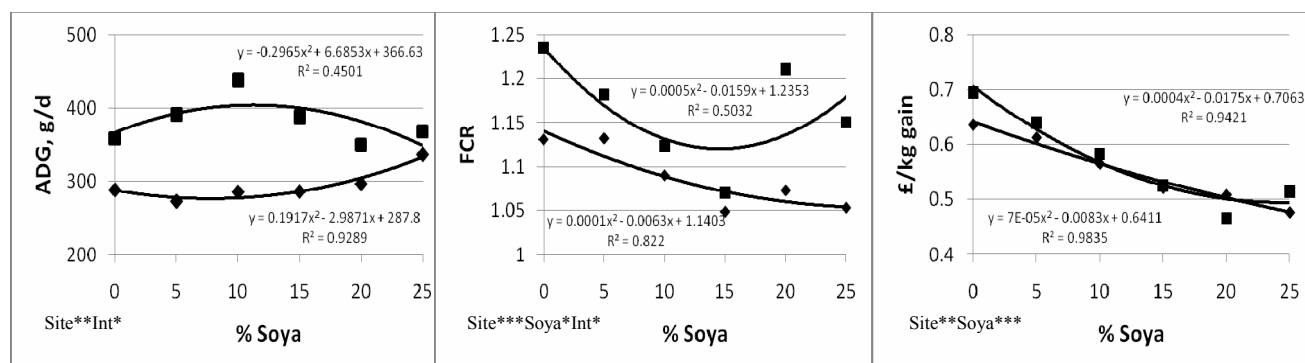
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**Introduction** Piglet production differs substantially from farm to farm within and between countries. Formulating piglet diets and setting maximum raw material inclusions which safeguard performance and health without adding intolerable cost in such diverse circumstances is a challenge. An understanding of how different raw material inclusion levels impact performance at different sites is necessary and a pre-requisite of balancing any added cost to a given diet and minimising the risk to performance and health. The aim of the experiment was to test the response of piglets at 2 different UK research centres to an increased soyabean inclusion level. It was hypothesised that the optimal soyabean inclusion level would differ between experimental sites due to such differences as housing, management practices and infection challenge.

**Materials and methods** A 2 x 6 factorial design experiment compared experimental site (S1; typical UK health vs S2; high health) and soyabean inclusion (0, vs 5, vs 10, vs 15, vs 20 vs 25%). At S1, 240 pigs (LW/L x LW) of mixed sex were weaned at  $26.8 \pm 0.18$  days ( $\pm$  S.D.) of age and  $8.0 \pm 0.94$  kg body weight were put on trial in groups of 10, resulting in 4 replicates per treatment. At S2, 180 pigs (LW x L x Duroc) x (Pietrain) of mixed sex were weaned at  $27.06 \pm 2.27$  days of age and  $8.9 \pm 0.11$  kg body weight were put on trial in groups of 5, resulting in 6 replicates per treatment. Within site piglets were balanced for initial weight, sex and litter and offered *ad libitum* access to one of the six dietary treatments for 20 days post weaning. The same diet was fed for 20 days post-weaning and were balanced for digestible energy, amino acid profile relative to digestible lysine, mineral and lactose content. Soyabean meal was added predominantly at the expense of fishmeal and cooked wheat. Individual weight gain and pen feed intake were recorded. Data were analysed by regression analysis with pen as the experimental unit, trial site as a fixed effect, soya bean inclusion level as the explanatory variable and mean pig weaning weight as a covariate.

**Results** There was a significant difference between sites in piglet performance over the 20 day trial period. Overall, average daily gain (ADG; S1 = 292 g/d vs S2 = 384 g/d,  $P < 0.001$ ), daily feed intake (ADFI; S1 = 321 g/d vs S2 = 443 g/d,  $P < 0.001$ ) and feed conversion ratio (FCR; S1 = 1.10 vs S2 = 1.16,  $P = 0.112$ ). There was no significant effect of increasing soyabean level on overall ADG (Fig 1). There was however a significant soya x site interaction, whereby optimal growth was achieved at the maximum soyabean inclusion level tested in this trial, 25% at S1 compared to 12% at S2. Increasing soyabean inclusion level significantly improved FCR (Fig 2), with an optimal inclusion level of 25 and 15% at S1 and S2 respectively. Increasing soyabean levels led to a decrease in cost per kg piglet body weight at both sites (Fig 3).



**Figures 1 to 3** The effect of soyabean inclusion level at 2 different sites ( $\diamond$  = S1,  $\blacksquare$  = S2) on (i) **Figure 1** Average daily gain (ADG); (ii) **Figure 2** Feed conversion ratio (FCR); (iii) **Figure 3** Cost per kg gain (£/kg gain)

**Conclusion** Important site differences were detected in response to increasing soyabean level in piglet starter diets. The inability to trial diet raw material constraints in a sufficient number of circumstances to model all eventualities requires the continued use of safety margins and extensive commercial evaluation to ensure the risk to health and performance is balanced against added cost of raw material inclusion constraints. This experiment is unable to elucidate the cause of the different responses at the two sites, however it is interesting to note that the higher feed intake, commonly observed at higher health status units, will have raised absolute intakes of anti-nutritive factors such as Trypsin Inhibitor and Phytate in site 2 piglets. Different amino acid ratios are required for maintenance and repair than for growth and further work might consider whether surplus amino acids created in the higher soyabean diets require denaturation in high health circumstances yet satisfy increased requirements for tissue repair in health challenged situations.

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## The effect of increasing soyabean level and phytase addition on the performance of newly weaned pigs

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**Introduction** The use of soyabean meal in piglet starter diets appears attractive because of its low cost and good nutrient profile. However, when soyabean meal, an ingredient high in phytate, replaces milk or fishmeal in isonutrient piglet diets performance is typically reduced (Wellock *et al.*, 2006). Performance reduction may be caused, at least in part, by the anti nutritive effect of phytate. It was hypothesised that the negative effect of phytate may be ameliorated by high levels of a phytase enzyme. To test this hypothesis we investigated the response of piglets to both an increased soyabean meal level and phytase addition in phosphorus adequate diets.

**Materials and methods** A 2 x 3 factorial design experiment compared soyabean meal inclusion (-, 0 g/kg vs +, 75g/kg in Diet 1, and +125g/kg in Diet 2) and phytase level (0 FTU/kg, vs 1250 FTU/kg vs 2500 FTU/kg) over 2 experimental rounds. 426 pigs (mixed genetics: LW or Hampshire or Pietran x LW/L) of mixed sex were weaned at  $27.5 \pm 0.68$  days ( $\pm$  S.D.) of age and  $8.5 \pm 0.42$  kg BW, balanced for initial weight, genotype and litter and offered *ad libitum* access to one of the six dietary treatments for 20 days post weaning. Diet 1 was fed from weaning until day 7 and diet 2 was fed from day 8 until day 20. Diet 1 was formulated to contain 16.75 MJ DE/kg and Diet 2 16.0 MJDE/kg. All diets contained 2500ppm Zinc and were balanced for essential amino acid composition as a proportion of total protein, mineral and lactose content. Nutrient levels met or exceeded BSAS standards where given. Soyabean meal was added at the expense of fishmeal and cooked dehulled oats. Pigs were housed in groups of 7 or 8. Individual weight gain, pen feed intake and faecal score (assessed on a 4 point scale, where 1 = firm and 4 = watery) were recorded. Data were analysed by REML with pen as the experimental unit, experimental round as a fixed effect and mean pig weaning weight as a covariate.

**Results** Overall performance was typical for the centre with pigs consuming  $388 \pm 47$  g/d and gaining  $329 \pm 40$  g/d, finishing the trial at  $15.1 \pm 1.03$  kg body weight. Table 1 shows the effect of soyabean inclusion and phytase level on pig performance. Soyabean inclusion had no effect on average daily gain (ADG) or feed conversion ratio (FCR). Increasing phytase level improved week 2 ADG (0 = 360, 1250 = 378, 2500 = 391 g/d;  $P = 0.080$ ) and FCR (0 = 1.15, 1250 = 1.07, 2500 = 1.06;  $P = 0.029$ ). There was no significant effect of phytase inclusion level on overall ADG but there was an improvement in FCR with increasing phytase level (0 = 1.21, 1250 = 1.17, 2500 = 1.15;  $P = 0.026$ ). The number of cases of diarrhoea decreased with an increase in phytase level (0 = 31, 1250 = 9, 2500 = 10;  $P = 0.006$ ). Margin over feed is increased with increased soyabean level (- = £2.57 vs + = £2.79,  $P = 0.010$ ) and increase in phytase level (0 = £2.50, 1250 = £2.66, 2500 = £2.88;  $P = 0.068$ )

**Table 1** The effect of soya level (H vs L) and phytase level (0 vs 1250 vs 2500 FTU/kg) on performance throughout the trial period.

Soyabean level	-	-	-	+	+	+	P-value			
Phytase level	0	1250	2500	0	1250	2500	s.e.d	Soya	Phytase	Interaction
Start weight (kg)	8.51	8.55	8.56	8.51	8.56	8.51	-	-	-	-
Wk1 ADG (g/d)	134	134	149	140	133	163	22.9	0.449	0.254	0.908
Wk 1FCR	1.39	1.62	1.31	1.43	1.36	1.19	0.19	0.335	0.207	0.522
Wk 2 ADG (g/d)	348	387	389	373	369	392	25.1	0.336	0.080	0.432
Wk 2 FCR	1.19	1.05	1.05	1.12	1.09	1.07	0.06	0.337	0.029	0.277
Wk 3 ADG (g/d)	487	481	497	487	481	507	32.7	0.781	0.505	0.944
Wk 3 FCR	1.24	1.22	1.18	1.24	1.24	1.28	0.07	0.383	0.978	0.426
Overall ADG (g/d)	314	328	342	329	317	344	19.6	0.447	0.161	0.632
Overall FCR	1.20	1.17	1.13	1.21	1.17	1.16	0.04	0.866	0.026	0.868
End weight (kg)	14.80	15.08	15.36	15.10	14.92	15.44	0.40	0.371	0.167	0.707
Faecal Score	2.10	2.09	2.15	2.15	2.05	2.08	0.07	0.820	0.532	0.403
Cases of diarrhoea	16	4	5	15	5	5	0.5	0.643	0.006	0.932
Margin Over Feed (£) <sup>1</sup>	2.28	2.59	2.83	2.73	2.73	2.93	0.23	0.010	0.068	0.423

<sup>1</sup>Calculated assuming a pig price of £1.05 per kg live-weight gain

**Conclusion** Under the conditions of the trial, soyabean inclusion failed to decrease piglet performance. Increasing phytase levels did however significantly improve feed efficiency but there is no evidence that this relates to the phytase acting on the phytate from soyabean meal. The number of cases of diarrhoea recorded was also reduced by the use of phytase. The mechanism by which phytase improves performance in phosphorus adequate diets is unknown but may be through the disruption of phytate induced protein:protein aggregation (Cowieson *et al.*, 2004). An economic analysis of the above results shows superdosing with phytase saves approximately 38p/pig worth around £3.5 million each year to the UK pig industry.

**Acknowledgements** This research was carried out at the University of Leeds for Primary Diets.

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## Post-weaning performance of pigs offered varying allowances of starter diets

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**Introduction** An analysis of benchmarking data found that, in Northern Ireland, allowances of starter diets offered to pigs varied greatly between producers. A better understanding of the relationship between starter diet allowance and subsequent pig performance might enable producers to identify the optimal allowances for their herds. Therefore, the objective of the current study was to investigate the effects of offering different allowances of starter diets to post weaned pigs on three separate herds.

**Materials and methods** Trials 1, 2 and 3 were conducted using pigs on a research herd while a further two trials used pigs on two commercial herds. The same, commercially available, pelleted starter 1 and starter 2 diets were used in all trials and were offered *ad libitum*. In descending order of inclusion, the main ingredients were cooked extruded soya, cooked wheat, cooked oats, whey powder and lactose in starter 1 diet and in starter 2 diet they were cooked extruded soya, cooked wheat, wheat, lactose and fishmeal. The starter 1 diet contained 225g/kg crude protein, 17g/kg total lysine and 16.5MJ/kg digestible energy. The starter 2 diet contained 215g/kg crude protein, 15.5g/kg total lysine and 15.5MJ/kg digestible energy. Diets offered to the pigs on the research herd also contained 3.1kg/tonne of Zincotec. The diets offered to pigs on commercial herd 1 contained 3, 3 and 3.1 kg/tonne of Maxima, Trimefiazine and Zincotec, respectively. For commercial herd 2, the starter 1 diet contained 3.1kg/tonne Zincotec while the starter 2 diet contained 1.2, 1.5 and 3 kg/tonne of Aurofac (250mg/g), Zincotec and Vevovital, respectively. Pigs on commercial herd 2 were offered 0.75kg per pig of a 'pre-starter' diet directly after weaning. When pigs had finished their starter diet allowance, they were offered a grower diet. In all trials, pigs were weaned at 28 days of age (+/- 2d). In trials 1, 2 and 3, pigs were penned in groups of 10, which were balanced for wean weight (average 9.0kg) and gender. Six replicates per treatment were conducted in each trial. In Trial 1, pens of pigs were offered 20, 40, 60, or 80kg of starter 1 diet after which each pen of pigs on each treatment received 100kg of starter 2 diet. In trial 2, each pen of pigs on each treatment was offered 40kg of starter 1 diet after which they were offered 60, 80, 100 or 120kg of starter 2 diet. In trial 3, pens of pigs were offered either 0 or 20kg of starter 1 diet, after which each pen of pigs on each treatment was offered 40kg of starter 2 diet. Pigs on commercial herd 1 (294 in total) were penned in groups of 21 with seven replicates for each treatment. Treatments were balanced for wean weight and gender. Pigs on commercial herd 2 (640 in total) were penned in groups of either 20 or 60, and eight replicates were completed per treatment. Treatments were balanced for wean weight, gender and group size. On the commercial herds, treatment 1 was designed to deliver 2kg starter 1 diet followed by 6kg of starter 2 diet per pig and treatment 2 was designed to deliver 4kg of starter 1 diet followed by 8kg of starter 2 diet per pig. Pigs were individually weighed and pen feed intakes were recorded. 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** Post-weaning pig performance (weaning to 10 wks of age) was similar for pens of pigs offered either 20, 40, 60 or 80 kg of starter 1 diet (mean ADG 512g/day, SEM 11.8; mean FCR 1.32, SEM 0.028). Pig performance from weaning to 10 wks of age was also similar for pens of pigs offered either 60, 80, 100 or 120 kg of starter 2 diet (mean ADG, 515g/day SEM 11.0; mean FCR 1.35, SEM 0.043). When 0 kg of starter 1 diet was offered, the 10 week weight of pigs and ADG (weaning to 10 wks of age) were lower (both  $P < 0.05$ , 25.7kg and 408g/day respectively) when compared with pigs offered 20 kg per pen (27.9kg and 465g/day respectively). ADFI was similar, but FCR was significantly improved, when 20kg was offered ( $P < 0.05$ , 1.34) compared with 0kg (1.41). When higher allowances of starter diets were offered to pigs on the commercial herds, there were no significant improvements either in end weight or ADG (Table 1). However, while FCR was significantly improved on commercial herd 1, it was not on commercial herd 2.

**Table 1** Effect of starter diet allowance on post weaning pig performance on the commercial herds

	Commercial herd 1				Commercial herd 2			
	Low <sup>a</sup>	High <sup>b</sup>	SEM	Sig	Low <sup>a</sup>	High <sup>b</sup>	SEM	Sig
Wean weight (kg)	7.4	7.2	0.43	NS	8.6	8.6	0.15	NS
End weight <sup>c</sup> (kg)	18.4	18.9	0.56	NS	23.5	23.8	0.46	NS
ADG <sup>d</sup> (g/day)	350	361	12.8	NS	438	439	10.5	NS
ADFI <sup>d</sup> (g/day)	455	449	9.4	NS	595	561	14.3	NS
FCR <sup>d</sup>	1.30	1.23	0.014	<0.05	1.32	1.28	0.019	NS

<sup>a</sup> Low : 2kg/pig of starter 1 diet followed by 6kg/pig of starter 2 diet, <sup>b</sup> High : 4kg/pig of starter 1 diet followed by 8kg/pig of starter 2 diet, <sup>c</sup> End weight on commercial herds 1 and 2 was measured 30 and 34 days after weaning respectively. <sup>d</sup> ADG, ADFI and FCR values relate to the period between wean and end weight.

**Conclusions** Pig performance was improved when a nutrient dense starter 1 diet was offered compared with when it was not offered. However, increasing the allowance of starter diets, did not result in improved pig performance. It should be noted that the allowance of 10kg per pig of starter 2 diet and 4kg per pig of starter 1 diet, which complemented the treatments in trials 1 and 2 respectively were also high. However, the results are in agreement with those found in previous studies (Dritz *et al* 1996). The results from the commercial herds agreed with those from the research herd, in respect of ADG and end weight. However, the effect of starter diet allowance on FCR differed between the two commercial herds which reaffirms the need for producers to assess the optimum starter diet allowance for their herd.

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## The effect of group gender and diet on finishing pig performance and carcass characteristics

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**Introduction** The growth rate of boars during finish is normally greater than that of gilts (Dunshea, 2005). Furthermore, gilts are often fatter than boars at a similar slaughter weight (Dunshea, 2005). Thus, the nutritional requirements of gilts and boars differ during finish but, in Northern Ireland, it is not common practice to group boars separately from gilts, nor to feed pigs of different genders diets specific to their requirements. This study aimed to investigate pig performance during the finishing period when boars were grouped separately from gilts and when they were not, and when these groups of pigs were offered finishing diets of either 'high' or 'normal' nutrient density.

**Materials and methods** Over eight time replicates, a total of 960 LR x LW pigs were penned in groups of 20 from 10 weeks of age. Within each replicate, groups of pigs were balanced for weight. The experimental design was a 3 (group gender: all boars, all gilts or mixed gender (50:50 boars and gilts)) x 2 (finishing diet nutrient density: high or normal) factorial. Pigs were offered a typical grower diet (DE 14MJ/kg, CP 180g/kg, total lysine 12g/kg) from 10 to 12 weeks of age, followed by the experimental diets from 12 weeks of age to slaughter (target weight of 100kg). The normal nutrient dense diet (CP 180g/kg, DE 13.5MJ/kg and total lysine of 9.5g/kg) contained (g/kg): barley 320, soya 240, maize 220, wheat 110, maize gluten 50, molaferm 20, dicalium phosphate 11, limestone 10, vegetable oil blend 8.5, Devicare pig finisher 5, salt 3.8 and lysine 0.86. The high nutrient dense diet (CP 185g/kg, DE 14.5MJ/kg and total lysine of 11.0g/kg) contained (g/kg): wheat 500, soya 214, maize 115, barley 50, maize gluten 51, molaferm 20, dicalium phosphate 4.4, limestone 10, vegetable oil blend 25, Devicare pig finisher 5, salt 2.4 and lysine 3.4. Pen feed intakes were continuously recorded and pigs were weighed at 12 and 20 weeks of age and at finish (target weight of 100kg). At slaughter, hot carcass weight was recorded and the back fat depth of pigs at P<sub>2</sub> (65mm from the midline at the level of the last rib) was measured 45 minutes after slaughter using the Ulster Probe. Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and kill out percentage (KO%) were calculated. Analysis of Variance was used to test for effects of treatments using finish weight as a covariate for data relating to pig and carcass performance at finish.

**Results** There were no significant interactions ( $P>0.05$ ) between group gender and finishing diet type on the performance of pigs during the finishing period (between 12 weeks of age and 100kg) or on carcass performance. The 20 week weight and ADG of pens of boars during the finishing period was similar to those of mixed gender pens but significantly greater than those of gilts (Table 1). Group gender had no significant effect on ADFI or FCR. The 20 week weight and ADG of pigs was not significantly affected by finishing diet type. However, pigs offered the high nutrient dense finishing diet had lower ADFI and improved FCR during the finishing period (Table 1). There was no significant effect of finishing diet type on cold weight or KO% but pigs offered a high nutrient dense diet had a higher back fat depth at P<sub>2</sub> than pigs offered the normal nutrient dense diet (Table 1). There was no significant effect of group gender on back fat depth at P<sub>2</sub> but the cold weight of the mixed gender pens of pigs was higher than that of pens of all boars and all gilts (Table 1). On the other hand, the KO% of pens of all boars was lower than both that of pens of all gilts and that of mixed gender pens (Table 1).

**Table 1** The effect of group gender and finishing diet type on pig performance (12 weeks of age to finish) and carcass performance

		Group gender					Finishing diet density			
		All boars	All gilts	50:50*	SEM	P Value	High	Normal	SEM	P Value
Weight	12wks	38.6	38.3	38.0	0.33	NS	38.5	38.2	0.27	NS
(kg)	20wks	85.5 <sup>b</sup>	82.1 <sup>a</sup>	84.4 <sup>b</sup>	0.72	<0.01	84.7	83.3	0.59	NS
	Finish	101 <sup>b</sup>	99 <sup>a</sup>	101 <sup>b</sup>	0.6	<0.05	101	100	0.46	NS
ADG (g/day)		862 <sup>b</sup>	834 <sup>a</sup>	851 <sup>b</sup>	6.9	<0.05	846	852	5.4	NS
ADFI (g/day)		2224	2238	2268	38.1	NS	2182	2306	30.1	<0.01
FCR		2.59	2.69	2.67	0.043	NS	2.59	2.71	0.034	<0.05
Cold Weight (kg)		76.3 <sup>a</sup>	76.6 <sup>a</sup>	77.7 <sup>b</sup>	0.31	<0.01	77.1	76.7	0.25	NS
KO%		73.6 <sup>a</sup>	76.5 <sup>b</sup>	76.1 <sup>b</sup>	0.47	<0.001	75.8	75.1	0.37	NS
P <sub>2</sub>		12.5	12.3	12.6	0.25	NS	12.8	12.2	0.20	<0.05

<sup>a,b,c</sup>, numbers with the same superscripts are not significantly different ( $P>0.05$ ), \* 50:50 Boars and gilts.

**Conclusions** Although back fat depth was increased by the high nutrient dense diet, it was not great enough for penalties to be incurred and effects of treatment on the other carcass quality attributes were as expected. ADG was not increased by the high nutrient dense diet but ADFI was lowered, resulting in an improved feed conversion ratio. This was mainly due to the fact that pigs received a greater amount of energy and protein per kg of feed when the high nutrient dense diet was offered. Depending on the cost of the diet, this suggests a potential for making economic savings by feeding a high nutrient dense diet during the finishing period. The daily gain of boars was greater than that of gilts and mixing the genders did not reduce growth performance. However, separate grouping of the genders may aid the use of an all in/all out policy more efficiently.

**Acknowledgement** Pig ReGen Ltd and Department of Agriculture and Rural Development for funding.

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## Can enthalpy and rate of change provide useful practical indices of the thermal loads experienced by livestock in transit?

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**Introduction** Elevated thermal loads imposed upon livestock during commercial transportation are a major cause of reduced welfare and production losses. Thermal loads experienced by animals in transit are commonly defined in terms of temperature alone and limits prescribed in legislation do not use thermal indices incorporating air temperature and water vapour content (Mitchell and Kettlewell 2008). In the present study the application of enthalpy to quantify thermal loads upon pigs in transit was investigated

**Materials and methods** Temperature-humidity sensors were installed on commercial vehicles on 7 long-distance livestock journeys from the UK to Spain using a commercial livestock transport vehicle carrying 90 pigs. These were undertaken during the summer period to impose a range of thermal loads upon the animals. The psychrometric data from the temperature-humidity measurements were used to calculate the psychrometric properties of the air during transport. The temperature derivative was computed using the Savitzky-Golay algorithm to smooth one-dimensional, tabulated data and to help compute the numerical derivatives using the Savgol routine in Matlab (version 7.0, Mathworks Inc, 2004). Thus, a polynomial was used to fit the data surrounding each data point. The smoothed points were computed by replacing each data point with the value of its fitted polynomial. Numerical derivatives were determined for each fitted polynomial at each data point. A window of 21 points was used with a fifth order polynomial. The “phase space” for temperature was determined and its corresponding gradient ( $dT/dt$ ) was calculated. The rates of change (with respect to a previous value) or gradient of temperature ( $dT/dt$ ) and enthalpy ( $dH/dt$ ) at each location were calculated. Finally, the gradients in temperature were presented as different polygons for each sensor. The areas of the polygons ( $P_{area}$ ) that included all the data points in the gradient space for each journey were calculated. Pig behaviours during a 3 hour period at the end of the journey were utilised to assess the transport stress and were correlated with the temperature and enthalpy analyses.

**Results** The average journey length was 63.3 hours. Average temperatures were highest during journeys 3 (23°C – max 40°C) and 4 (22°C – max 35°C). The gradient in temperature was higher during transport than at the loading or unloading sites. The rate of change of load temperature during transport varied between -0.025 and 0.025 °C/s during transport, 10 times higher than the range of changes at the farm (0.0008 and -0.001 °C/s) or abattoir, (0.0008 to -0.008 °C/s). Similarly, the gradient in enthalpy was much higher during transport, (0.08 to -0.08 kJ/kg dry air per second), than (0.002 to -0.002 kJ/kg dry air per second), or unloading (0.0025 to -0.0015 kJ/kg dry air per second). The areas of the polygons representing the rates of change of temperature are presented in Table 1. The area was smallest for trip 1, and highest for trip 3, which corresponds with the range of temperatures for those trips. The behavioural analysis indicated that the higher values for the relative changes in temperature and enthalpy parameters were correlated ( $p < 0.01$ ) with behavioural indicators of increased transport stress. Thus the pigs from journey 3 spent significantly more time drinking than pigs in the other journeys while pigs on journey 4 spent most time resting and drinking.

**Table 1** Summary of the areas of the polygons ( $P_{area}$ ) that included all the data points in the temperature gradient space per journey (different from Trip 1 – \*\* $p < 0.05$ , \* $p < 0.001$ )

Trip	N	$P_{area}$ (°C <sup>2</sup> /s)	$SE_{area}$
1	4	0.071	0.02
2	4	0.102	0.02
3	4	0.389*	0.02
4	4	0.120	0.02
5	4	0.151**	0.02
6	4	0.133	0.02
7	10	0.101	0.01

**Conclusions** It is proposed that calculation of enthalpy and the determination of rates of change of temperature and enthalpy in transit may represent useful practical indices of the heat load imposed upon animals in transit and thus for assessment of the degree of stress that might be imposed and the consequent welfare status of the animals. These indices may constitute more sensitive measures than absolute values of temperature or relative humidity

**Acknowledgements** Defra

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## The influence of pre-service weather conditions on farrowing rate in outdoor sows

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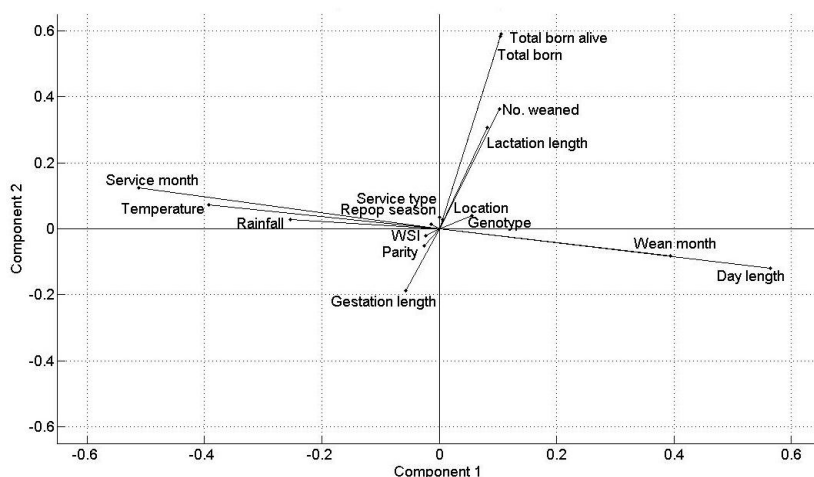
**Introduction** One major manifestation of seasonal infertility is a drop in the farrowing rate (FR) of sows served in the summer months (Hurtgen and Leman, 1980). Local environmental conditions may be attributed to this since temperature, day length and rainfall have all been found to affect sow reproductive efficiency (Auvigne *et al.*, 2010; Fernández-Illario and Mateos-quesada, 2005). It was expected that pre-service weather conditions would influence the farrowing rate of outdoor sows and so the aim of this work was to determine whether this was the case, and which were the key parameters.

**Materials and methods** Data collected from 29 commercial outdoor pig units in the UK, between 2004 and 2009, were analysed in respect to the local (within 40 km) average daily temperature and total rainfall for different lengths of time (1 – 5 days), up to 5 weeks prior to sow service date. Herds were located in Norfolk (n = 16), Suffolk (n = 7), Hampshire (n = 2) and Wiltshire (n = 4), and provided data for 44,049 sows with a total of 163,884 services. All animals were reared under similar conditions, and comprised mainly Landrace X Duroc sows served with a synthetic terminal sire (either by AI or naturally). Statistics were carried out in MATLAB® R2009b, and comprised of t-tests, 2 way analysis of variance (ANOVA) and principal component analysis (PCA). Linear mixed-effects models fit by restricted maximum likelihood (REML) were created using the lmer procedure of 'R' software 2.11.1, where FR = farrow rate, p = parity, d = duration of conditions, r = rainfall and t = temperature (models 1 and 2).

$$\text{lmer}(\text{FR} \sim r * d + (1|p), \text{dpr}) \quad (1)$$

$$\text{lmer}(\text{FR} \sim t * d + (1|p), \text{dpt}) \quad (2)$$

**Results** From (1), the effect of rain on FR was dependent on the duration of the conditions and the effect of rain was dependent on parity ( $P < 0.001$ ). No significant 3-way interactions were found. Reduced FR could be attributed to a prolonged period of dry weather around late lactation and weaning ( $P < 0.001$ ), and that this was true for all parities ( $P < 0.001$ ). Extended periods of wet weather resulted in improved FRs ( $P < 0.01$ ). From (2) FR was found to depend on all three factors. Temperatures below 19°C for up to 5 consecutive days resulted in greater FR, particularly when present during late lactation ( $P < 0.001$ ). More than 3 consecutive days at above 18°C during lactation resulted in lower FRs ( $P < 0.01$ ). Wet and warm weather resulted in more variable FRs when compared to dry and cool weather ( $P < 0.05$ ) and wet and cool weather ( $P < 0.01$ ), suggesting that temperature was the main contributor to reduced FR. The effect of the change in day length for up to 5 weeks prior to service was also assessed, showing that rapidly shortening day length in the month leading to service resulted in better FRs for gilts ( $P < 0.01$ ), multipares ( $P < 0.01$ ) and primipares ( $P < 0.05$ ). PCA (Figure 1) confirmed that service/wean month, day length and temperature were the main sources of variability within the data set as they accounted for most of the variation in the first two components.



**Figure 1** Biplot for first two principal components, accounting for a total of 30.72% of the variability within the dataset. WSI = wean to service interval

**Conclusions** These results suggest that by observing the weather conditions in the lead up to service, particularly rainfall and temperature, producers may be able to predict whether a reduced FR is expected and thus adjust the number of animals being served accordingly, in order to ensure sufficient piglets are born to maintain economic stability.

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## Effect of altering maternal feed allowance in early gestation on sow and piglet performance at farrowing

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**Introduction** Current industry recommendations are to feed pregnant sows at a constant rate throughout gestation (BSAS, 2003). However, this strategy can lead to some sows being overfed in early gestation and underfed in late gestation (Samuel *et al.*, 2007). An alternative to feeding at a single rate is to vary the feed level through gestation. However, little published information is available concerning the effects of different feed allowances during the first 85 days of gestation on sow reproductive performance and on back fat changes. The aim of this study was to investigate the effect of altering gestating feed allowance on sow reproductive performance.

**Materials and methods** A total of 106 multiparous sows were fed according to one of four feed allowance regimes during days 4 - 85 of gestation over 12 time points on a large commercial herd in Northern Ireland. The four treatments were:

- 1) 2.0kg from days 4-5, 2.7kg from days 6-28, 2.3kg from days 29-85 and 3.2 kg from days 86-108. Total feed: 262.6kg
- 2) 2.7kg from days 4-5, 2.7kg from days 6-28, 2.3kg from days 29-85 and 3.2 kg from days 86-108. Total feed: 264.0kg
- 3) 2.4kg from days 4-5, 2.4kg from days 6-28, 2.4kg from days 29-85 and 3.2 kg from days 86-108. Total feed: 262.5kg
- 4) 2.1kg from days 4-5, 2.1kg from days 6-28, 2.6kg from days 29-85 and 3.2 kg from days 86-108. Total feed: 266.4kg

Sows were kept in loose pens, in groups of four, from the time of previous weaning until three days post service and were fed *ad-libitum* with a commercial lactation diet (14.3 MJ/kg, 170 g/kg CP, 10.3 g/kg lysine). They were then transferred into large dynamic groups of approximately 80 and fed a commercial gestation diet (13.0 MJ/kg, 140 g/kg CP, 7.0 g/kg lysine) via an Osborne electronic sow feeder (Osborne Europe Ltd., Tyne and Wear, UK) at the stated rates, until seven days pre-farrowing (day 108 of gestation). At this point, they were moved into farrowing accommodation. Sows which were excessively fat or thin at service were not included in this study. From day 109, sows were fed a lactation diet at approximately 2.5 kg/day until parturition, after which, they were fed an increasing amount until they reached the point at which *ad-libitum* feeding was introduced. Sows that had not farrowed naturally by day 114 of pregnancy were induced using Planate (cloprostenol, Schering-Plough Animal Health). Back fat was measured at service, on day 28 and at parturition at the P<sub>2</sub> position using an ultrasonic back fat scanner (Lean-Meater®, Renco Corp., Minneapolis, MN). Piglets were weighed as an entire litter at birth. Data were analysed in Genstat (12<sup>th</sup> edition) using regression analysis.

**Results** Dietary treatment had a significant effect on the total weight of piglets born and on the number of piglets born mummified (Table 1) with Treatments 3 and 4 resulting in a significantly greater total weight of piglets born than treatments 1 and 2. However, sows on treatment 2 had a significantly lower number of mummified piglets at birth. Treatment did not affect the numbers of piglets born alive, average live birth weight or sow P<sub>2</sub> back fat depth at farrowing.

**Table 1** Effect of treatment on numbers and weights of piglets born and sow back fat depth at the P<sub>2</sub> position

	Treatment				s.e.m	P
	1	2	3	4		
Number born alive	13.5	13.4	14.3	13.8	0.72	n.s.
Number born dead	0.70	0.92	1.23	1.39	0.20	n.s.
Number born mummified	0.44 <sup>ab</sup>	0.19 <sup>a</sup>	0.60 <sup>b</sup>	0.65 <sup>b</sup>	0.13	0.046
Total piglets born	14.7	14.5	16.1	15.8	0.76	n.s.
Weight born live (kg)	18.85	19.17	20.53	19.93	0.647	n.s.
Weight born dead (kg)	0.96	1.05	1.63	1.71	0.320	n.s.
Weight mummified (kg)	0.20	0.07	0.29	0.42	0.107	n.s.
Total weight of piglets born (kg)	20.1 <sup>a</sup>	20.3 <sup>ab</sup>	22.4 <sup>bc</sup>	22.1 <sup>b</sup>	0.70	0.027
Average live birth weight (kg)	1.41	1.45	1.46	1.47	0.042	n.s.
P <sub>2</sub> at farrowing	18.2	18.1	18.5	17.8	0.59	n.s.

a,b,c superscripts indicate significance (P<0.05)

**Conclusions** The results show that sow feed allowance from service to day 85 of gestation affects the total weight of pigs born but not either the number or weight of pigs born alive. The results suggest that sows should be fed at either a constant rate between service and day 85 or a reduced rate from service until day 28 and then fed at a higher rate until day 85. The increased total weight of pigs born merits further research especially from day 85 until term when foetal weight gain begins to increase exponentially.

**Acknowledgements** The authors gratefully acknowledge funding from Pig Regen Ltd.

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## The effects of supplementing various levels of chitosan on performance, selected microbial populations and volatile fatty acid concentration in the weaned piglet

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**Introduction** The post-weaning period is characterised by a reduction in feed intake, poor growth rates, diarrhoea, and an increased risk of disease (Lallès *et al*, 2007). These negative effects on piglet growth during the weaning period were managed by growth promoting antibiotics. However the E.U. placed a total ban on the use of in-feed antibiotics growth promoters on the 1<sup>st</sup> January 2006 due to the public concern regarding bacterial resistant and human health issues. Chitosan may be a potential viable alternative to traditional antimicrobials in animal production. Chitosan is a natural biopolymer which has recently attracted considerable interest due to its antimicrobial and immunohancing activities (Liu *et al*, 2006). The objective of this experiment is to evaluate the effects of supplementing weaned piglets with varying molecular weights of chitosan on performance, selected microbial populations, faecal scoring and volatile fatty acid concentration.

**Materials and methods** The experiment was designed as a complete randomised design. Three hundred and ninety six weaned piglets (24 days of age, 7.3kg±1.7 live-weight) were blocked on the basis of live-weight and were assigned to one of 6 dietary treatments (n=22) for a 33 day experimental period. The dietary treatments were as follows; (1) control diet (0 ppm chitosan) (2) control diet plus < 1 Kda chitosan (3) control diet plus 3 – 5 Kda chitosan (4) control diet plus 5-10 Kda chitosan (5) control diet plus 10- 50 Kda chitosan (6) control diet plus 50- 100 Kda chitosan. Chitosan was included in dietary treatments at a rate of 250ppm. Starter diets (0-18 days) and were formulated to have identical digestible energy levels of 18 MJ/kg and link diets (18-33 days) were formulated to have identical digestible energy levels of 16 MJ/kg. Pigs were weighted at the beginning of the experiment (day 0 = day of weaning), day 7, day 18, day 25, and day 33. The pigs were housed in group of three on fully slatted floors (1.68 × 1.22m). Pigs were observed for clinical signs of diarrhoea and a scoring system applied to indicate the presence and severity of this diarrhoea. Fresh faeces samples were collected from each pen from days 10-14 and were analysed for selected microbial populations, volatile fatty acid concentration, and nutrient digestibility. Digestibility values were measured using the acid insoluble ash technique.

**Results** There was no significant effect of dietary treatment on piglet performance during the starter period (days 0-18) ( $P>0.05$ ). However, pigs offered the chitosan supplemented diets had a significantly higher average daily gain ( $P<0.05$ ) compared to pigs offered the basal diet from days 18-33 in the experiment. Furthermore, the inclusion of chitosan resulted in a higher gain to feed ratio ( $P<0.05$ ) in pigs between day 18 and 25 of the experimental period. There was no significant effect of dietary treatment on total VFA concentration ( $P>0.05$ ). The inclusion of chitosan had a significant treatment effect on the apparent digestibility of dry matter (DM), organic matter (OM), ash, nitrogen (N), gross energy (GE) ( $P<0.001$ ) and neutral detergent fibre (NDF) ( $P<0.01$ ). Pigs offered diets containing chitosan had an improved faecal score ( $P<0.001$ ) compared to the control pigs during the critical 7 to 14 day period. Faecal samples recovered from pigs receiving diets supplemented with chitosan had a reduced number of *E.coli* ( $P<0.05$ ) and *Lactobacilli* ( $P<0.05$ ) species compared with the control pigs. At the higher molecular weight inclusion of 10-50 KDa, the addition of chitosan significantly reduced *E.coli* numbers compared to the control treatment ( $P<0.05$ ).

**Table 1** Effect of dietary treatment on piglet performance during the link period (days 18-33) (L.S.M±S.E.M)

Treatments	T1	T2	T3	T4	T5	T6	s.e.	Treat	Contrast1	Contrast2	Contrast3
Daily Gain (ADG) (g/day)											
Days 18 to 25	470	486	499	491	497	489	18.4	ns	ns	ns	ns
Days 25 to 33	538	577	571	584	561	594	17.3	ns	*	ns	*
Average ADG	504	534	534	537	528	541	14.4	ns	*	ns	ns

Contrast 1 (1 vs All); Contrast 2 (1 vs 2+3); Contrast 3 (1 vs 6).

**Conclusions** In conclusion, the current results indicate that dietary supplementation of chitosan may enhance piglet performance by improving feed efficiency and decreasing *E.coli* populations.

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## Acute phase proteins as markers for subclinical disease in young pigs

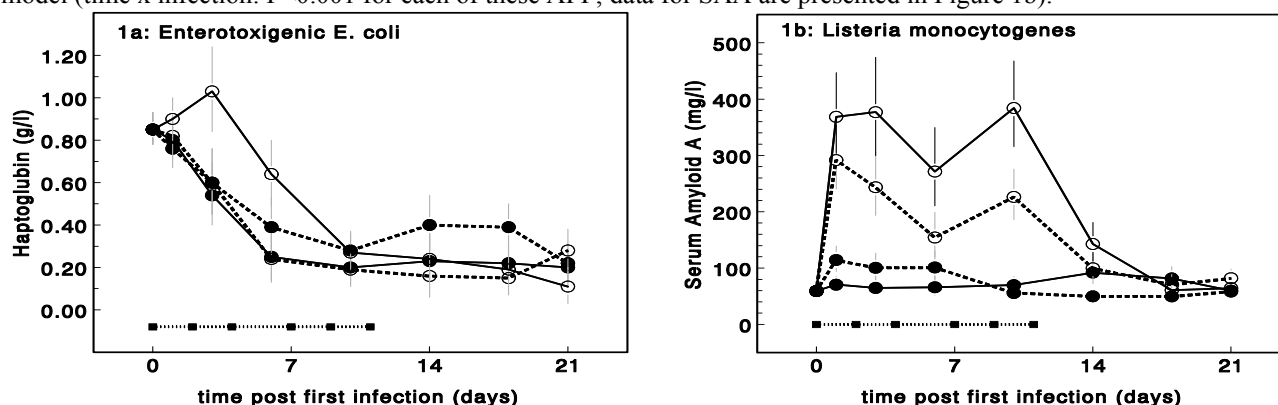
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**Introduction** Serum acute phase proteins (APP) are increasingly being recognised as useful markers for a range of clinical diseases in pigs, and are also sensitive to nutritional environment, husbandry and long-term stressors (Eckersall *et al*, 1996). However, it is less clear whether APP responses can be used as markers for sub-clinical disease, which often goes unrecognized due to absence of clinical signs and is a major contributor to a lower than expected pig performance, economic losses and reduction in pig welfare. Here, we assess APP responses and their potential as markers during experimental sub-clinical infections in young pigs.

**Materials and methods** A series of earlier studies (Athanasiadou *et al*, 2010) identified that enterotoxigenic *Escherichia coli* (ETEC) and *Listeria monocytogenes* (LM) may be appropriate pathogen models for inducing local and systemic subclinical disease in pigs, respectively. Five-wk old, weaned male pigs were trickle infected with Nal-resistant ETEC or LM, at one of four levels of infection, 0 (sham-infected controls),  $10^6$  (L),  $10^8$  (M) or  $10^{10}$  (H) cfu per day, on a Mon-Wed-Fri basis for two weeks (n=9 per treatment/pathogen). Feed intake was measured daily and pigs were weighed on regular intervals. Faeces consistency, cleanliness and health scores were taken daily, using an established, objective scoring system ranging from 1 (no symptoms) to 4 (pronounced symptoms) (Wellock *et al*, 2007). The ETEC and LM infections were monitored through faecal measurement of Nal-resistant ETEC and rectal temperatures around each infection, respectively. Blood samples were taken on d<sub>0</sub> (pre-infection measurement), d<sub>1</sub>, d<sub>3</sub>, d<sub>6</sub>, d<sub>10</sub>, d<sub>14</sub>, d<sub>18</sub> and d<sub>21</sub>. These were analyzed for six APP: haptoglobin (Hp), C-reactive protein (CRP), serum amyloid A (SAA), pig major acute phase protein (pig-MAP), apolipoprotein A1 (ApoA1), and albumin (Alb). Effects of infection type and level on daily weight gain and feed intake were assessed through ANOVA, using body weight taken three days before infection as a covariate. A repeated measures ANOVA, with pre-infection APP level as covariate, was used to assess effect of infection on APP concentrations.

**Results** A reduction in feed intake (~18%) and body weight gain (~30%) was observed during the first week of infection in ETEC-H pigs only (P<0.05), whilst LM infection did not affect performance. During the infection period, growth and intake of LM pigs were 10% lower than those of ETEC pigs (P<0.05). Nal-resistant ETEC excretion in the faeces during and after infection reflected closely the level of ETEC challenge in all pigs, and gradually reduced upon challenge cessation. LM-M and LM-H pigs had short-lived pyrexia; at 8 h after each challenge, rectal temperature was 39.2, 39.2, 39.4 and 40.2°C for LM-C, LM-L, LM-M and LM-H pigs, respectively (s.e.d. 0.08 °C; P<0.001). ETEC and LM challenge did not affect faeces, cleanliness and health scores (P>0.10). From all APP tested, only Hp responded in the ETEC model, which increased during the first week of pathogen challenge for ET-H pigs only (time x infection: P<0.05; Figure 1a). In contrast, pig-MAP and SAA, and to a lesser extent Hp and CRP, were increased during pathogen challenge in the LM model (time x infection: P<0.001 for each of these APP; data for SAA are presented in Figure 1b).



**Figure 1** Selected APP responses in pigs infected with ETEC (1a) and LM (1b) before, during and after trickle challenge (■) with at 0 (solid line ●),  $10^6$  cfu/day (broken line ●),  $10^8$  cfu/day (broken line ○) or  $10^{10}$  cfu/day (solid line ○).

**Conclusions** This experiment has assessed the feasibility of using APP as a marker to assess objectively the presence of sub-clinical disease in young pigs. It was observed that selected serum APPs increased in concentration at times of known sub-clinical exposure above a threshold level of infection. Furthermore, variation in Hp concentrations may be related to variation in feed intake in pig herds, at times of local infection. We also observed that measurement of Hp may be sufficient to assess objectively the presence of sub-clinical disease, as circulating Hp concentration increased in both enteric and systemic challenges.

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# Genetic associations of feed intake behaviour traits with piglet survival and litter size

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**Introduction** Piglet survival and litter size are reported to have low heritabilities and are associated with a long generation interval because these traits are recorded late in life (e.g. Kapell *et al.*, 2009). Therefore, traits recorded early in life, with higher heritabilities and genetically correlated to these reproduction traits, are of great interest for pig genetic improvement programmes. Feed intake behavioural traits, derived from electronic feeder information during performance tests, have been reported to be highly heritable (Schulze *et al.*, 2003). The genetic association of feed intake behavioural traits with piglet survival has not been investigated and will give further insight into the behavioural aspects associated with piglet survival. Therefore, the objective of this research was to estimate the genetic correlations of feed intake behaviour of boars tested in a central test station with reproduction traits (piglet survival and litter size) of sows closely related to those tested boars.

**Materials and methods** Feed intake behavioural traits were available from 2038 purebred boars of the dam line 03 of PIC Germany, tested at the central test station. Feed intake and its behavioural traits were recorded by using electronic feeders (ACEMA 48, ACEMO, Pontivy, France). The boars were penned in groups of twelve during the performance test from 100 to 170 days of age. Behavioural traits derived included: number of visits to the feeder per day ( $7.3 \pm 3.8$ ), time spent in the feeder per day ( $62 \pm 11$  min/d) and time per visit spent in the feeder ( $11 \pm 4$  min), feed intake rate as food consumed divided by time spent in the feeder ( $42 \pm 8$  g/min), and feed intake per visit ( $473 \pm 171$  g). Successive individual feed intake records differencing less than 40 s were condensed to one visit. Number of live born and stillborn piglets at birth was available for 5089 purebred primiparous sows of line 03. The years of recording were chosen in which numerous primiparous sows were highly related (full and half-sibs as well as progeny) to the boars recorded in the performance tests, in order to obtain high genetic connectedness between both datasets. A multiple trait analysis of all seven traits was carried out using VCE-6 (Version 6.0.2, Groeneveld *et al.*, 2008). The model for behavioural traits included; as fixed effects - birth farm (4), year-season of start of the performance test (16 quarterly year-season); as covariables - the weight and age at the start of test as well as the age at the end of test; and the animal and residual were included as random effects. Besides these random effects, the model for reproduction traits included the fixed effects of mating type (AI or natural service), boar line used for service (2) and herd-year-season (75 half year-seasons), with age at farrowing as a covariable.

**Results** The feed intake behavioural traits showed moderate to high heritabilities (Table 1) while litter size and piglet survival resulted in low heritabilities of 0.053 (s.e. = 0.017) and 0.036 (s.e. = 0.010), respectively. Significant moderate genetic correlations indicate that pigs spending a longer time per visit and a longer time per day in the feeder, and showing low feed intake rate had relatives with higher genetic potential in litter size. The difference in size of these correlations is consistent with negative genetic correlations of time per day or time per visit and feed intake rate of -0.892 (s.e. = 0.030) and -0.115 (s.e. = 0.048), respectively. Similarly to litter size, piglet survival was significantly positively associated with time per visit but non-significantly associated with time per day and feed intake rate. Piglet survival showed significant and higher correlations to feed intake per visit and visits per day than litter size did to these traits.

**Table 1** Heritabilities ( $h^2$ ) and genetic correlations ( $r_g$ ) between feed intake behavioural traits and litter size or piglet survival

Trait	Visits per day		Time per day		Time per visit		Feed intake rate		Feed intake per visit	
	$h^2$	s.e.	$h^2$	s.e.	$h^2$	s.e.	$h^2$	s.e.	$h^2$	s.e.
	0.316	0.023	0.396	0.043	0.330	0.044	0.365	0.031	0.362	0.041
	$r_g$	s.e.	$r_g$	s.e.	$r_g$	s.e.	$r_g$	s.e.	$r_g$	s.e.
Litter size	-0.119	0.165	0.472	0.099	0.413	0.172	-0.365	0.095	0.211	0.169
Piglet survival	-0.373	0.099	0.022	0.138	0.492	0.105	-0.046	0.137	0.401	0.102

**Conclusions** The moderate to high heritabilities of behavioural traits and their genetic correlations with litter size and piglet survival showed that these behavioural traits could be effectively utilised for early indirect improvement of litter size and piglet survival. In particular, pigs which spent a longer time in the feeder with higher average food consumption per visit as well as a low number of visits per day are expected to genetically improve both litter size and piglet survival. Based on the genetic associations of these behavioural traits with piglet survival, it may be hypothesised that boars, which showed less stress during feeding, may have female relatives that show less stress during farrowing, with the consequence of higher piglet survival, which warrants further research.

**Acknowledgements** The authors gratefully acknowledge the funding from the Scottish Government. We thank PIC for providing the performance data.

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## Is sheep BSE of greater risk to humans than cattle BSE?

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**Introduction** The Transmissible Spongiform Encephalopathies (TSE) are a group of fatal, infectious neurodegenerative diseases, which include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob disease (CJD) in humans. The nature of the infectious agent responsible for TSE disease is currently unknown, but the prion hypothesis predicts that the agent is a misfolded conformer of the host glycoprotein PrP. The misfolded isoform (PrP<sup>Sc</sup>) binds to and converts normal PrP<sup>C</sup> into PrP<sup>Sc</sup>, propagating the agent. Strain typing studies have shown that the same strain of TSE agent is responsible for BSE in cattle and variant CJD (vCJD) in humans, suggesting possible transmission of BSE from cattle to humans via contaminated food. However, studies which have attempted to model the transmission of cattle BSE to humans using transgenic mice expressing human PrP have shown limited transmissibility, suggesting the presence of a substantial transmission barrier. BSE has however been transmitted to a number of other species. These include naturally occurring transmissions to domestic and large cats and exotic ungulates, and experimental transmissions to small ruminants (sheep and goats). It is currently unknown whether these other sources of BSE infection pose a risk to human health. Here we aimed to further investigate the human transmission barrier following passage of BSE in sheep.

**Materials and methods** Groups of gene targeted transgenic mice (n=24) expressing human PrP with the codon 129 methionine/valine polymorphism (HuMM, HuMV and HuVV) were inoculated intracerebrally with 0.02ml of 10% brain homogenate from either cattle BSE (cBSE) or experimental Sheep BSE (Exp-ShBSE). Control 129/Ola wild type mice (n=24) and gene targeted transgenic mice expressing bovine PrP (n=24) were also inoculated as controls. Transmissions were performed from a single sample prepared from a BSE brainstem pool (supplied by VLA, Weybridge), and 2 separate samples from the same BSE infected sheep brain (produced at The Roslin Institute). All inoculated animals were monitored for lifespan (~700 days) for clinical signs of TSE disease, and were culled either at a pre-defined clinical endpoint, or due to welfare reasons. Average incubation times were calculated from mice in each group showing both clinical and pathological signs of TSE,  $\pm$  standard error of the mean (SEM). All mice were examined for TSE associated pathology by scoring the degree of vacuolation in 9 brain areas, and vacuolation profiles constructed from average scores  $\pm$  SEM. Mice were also examined by immunohistochemistry for the deposition of abnormal PrP (PrP<sup>d</sup>), and glial activation. Spleen tissues were analysed using the IDEXX Herdchek TSE diagnostic assay to detect peripheral accumulation of abnormal PrP. All mouse experiments were performed under licence from the UK Home Office and in accordance with the Animals (Scientific Procedures) Act 1986.

**Results** Inoculation of control 129/Ola wild type mice and transgenic mice expressing bovine PrP with cBSE and Exp-ShBSE did not reveal any significant differences in agent transmissibility or targeting of BSE neuropathological lesions from these two species. Hence BSE strain properties remained unchanged following transmission through sheep. However following inoculation with Exp-ShBSE, 16/23 HuMM transgenic mice showed positive TSE pathology in the form of vacuolation and/or PrP deposition, targeted mainly to the thalamic region (Plinston et. al., 2011). This deposition was evident in 50% of mice culled between 377-589 days post inoculation (n=14) and in all HuMM mice which survived >600 days post inoculation (n=9). Areas of the brain showing significant PrP deposition also showed glial activation, and abnormal PrP deposition was identified in the spleens of several, but not all HuMM mice with PrP<sup>d</sup> in the brain. In contrast, none of the 24 HuMM transgenic mice inoculated with cBSE showed any sign of vacuolar pathology or PrP<sup>d</sup> accumulation in brain despite surviving into and beyond times when Exp-ShBSE inoculated mice were shown to be affected. No PrP<sup>d</sup> was observed in HuVV or HuMV mice inoculated with either cBSE or Exp-ShBSE.

**Conclusions** Our data show that transgenic mice homozygous for human 129-M PrP are more susceptible to the BSE agent following transmission through sheep than to BSE from cattle. The reasons for this increased susceptibility are unclear. It is possible that sheep are able to replicate the infectious agent to a higher titre in the brain, or that transmission through sheep in some way alters the agent properties to allow a more efficient transmission to humans. These issues are currently subject to further investigation in our laboratory. Although sheep can be infected experimentally with cattle BSE, no cases of sheep BSE have ever been identified in the field. The possible increased risk of disease transmission to humans identified in these studies is thus not of major concern to the public at present. Natural BSE infection has however been identified in goats, indicating that small ruminants have been exposed to sources of contamination. While TSEs remain in the environment and continue to infect animals (even at low prevalence) there remains the potential for cross-species transmission and the emergence of TSE isolates with altered strain properties, or host ranges. Our data therefore emphasise the need for continued surveillance to identify, monitor and characterise any new emerging TSE agents that are identified in ruminants, and assess the potential risks posed to other species.

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## Developments in goat transmissible spongiform encephalopathy (TSE) research

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**Introduction** The BSE epidemic in cattle and subsequent emergence of vCJD in humans has been a warning that prion diseases have zoonotic potential. The fear for BSE being transmitted to small ruminant livestock was confirmed by the detection of (only) two cases of BSE in goats. Aware of such risks, the network “Neuropriion” (EU-project FOOD-CT-2004-506579) enabled a structured organisation of goat TSE research in Europe. A subsequent consortium “GoatBSE” (EU-project FOOD-CT-2006-36353) is underway to develop adequate tools for the diagnosis and prevention of TSEs in goats.

The main objective is to determine the tissue distribution of BSE, including muscle and milk after oral exposure of goats and to simultaneously generate data on genetic susceptibility in the most commonly used production breeds. These data will allow an improved risk assessment for human exposure and animal health.

**Materials and method** Goats for genetic frequency analyses were sourced in the participating countries from TSE affected and healthy herds according to disease diagnosis by Veterinary Services. Goats for BSE transmission studies were derived from French or UK herds of the following breeds: Saanen, Toggenburg and Boer.

**Results and Conclusions** Oral BSE transmissions to goats of various *PRNP* genotypes are progressing for both 1<sup>st</sup> and 2<sup>nd</sup> passage. A geographical study is underway to distinguish TSE isolates from the partner countries and to discriminate between scrapie strains and BSE involving bioassays in mice, bank voles, and *PRNP* transgenic mice. Studies of BSE infectivity in milk (‘cheese’) and muscle (‘meat’) following oral exposure are also in progress. Lastly the *PRNP* allele frequencies have been determined for each participating country.

All accumulated data on *PRNP* alleles and transmissibility of BSE indicate that susceptibility of goats to bovine BSE is similar to scrapie susceptibility established from field cases. A website has been created for public and expert information on all aspects of goat TSEs ([www.goattse.eu](http://www.goattse.eu)).

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## Early pre-clinical infection of peripheral tissues in sheep with experimental bovine spongiform encephalopathy

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**Introduction** In the UK cattle were infected by bovine spongiform encephalopathy (BSE) as a result of consumption of contaminated meat and bone meal feed supplements. Sheep were also fed the same supplements up to at least 1989. As sheep have been shown experimentally to be susceptible to BSE by oral exposure, there is therefore a theoretical possibility that some sheep in the UK became infected with BSE. The aim of this project was to describe the pathogenesis of BSE in sheep after oral exposure to identify how early the sheep tissues could be shown to be infected. The results of this study indicate which tissues might pose an infection risk to humans or animals, should BSE ever be identified within the sheep population, as it has in a small number of goats.

**Materials and methods** From the NPU Cheviot flock, 16 sheep (8 male, 8 female) of susceptible PrP genotypes (AXQ/AXQ) were challenged orally with BSE at 7-10 months of age. Two males and two females were culled at 3, 6, 9 and 12 months. The remaining 16 challenged sheep were left to develop clinical disease. Control animals were unchallenged and were matched for genotype and age. The inoculum ( $10^{4.2}$  infectious units/g) was a 10% homogenate of 6 pooled BSE cattle brain stems. A range of tissues was collected from each sheep and tested by immunostaining for the presence of abnormal accumulation of the prion protein (PrP<sup>Sc</sup>) which is regarded as a marker for infection. Tissue sections were labelled with the monoclonal anti-PrP antibody BG4. (TSE Resource Centre, The Roslin Institute). The secondary antibody, biotinylated rabbit anti-mouse IgG was linked to streptavidin-peroxidase with chromogen aminoethyl carbazole. In addition, 22 tissues from each of the four challenged sheep and the two control sheep in each serial kill group were bioassayed in mice, a more sensitive test for infectivity. A 10% homogenate of each tissue in physiological saline was injected into groups of 24 RIII mice by a combination of the intracerebral (0.02ml) and the intraperitoneal (0.1ml) routes. Mice were monitored for signs of neurological disease throughout their lifespan to determine incubation periods and brain tissues examined histopathologically to confirm BSE infection (Fraser and Dickinson, 1968).

**Results** In the end point group, 50% of challenged animals developed BSE clinical disease. This rate of transmission is standard when Cheviot sheep are challenged after the age of weaning, the reason is now thought to relate to PrP genetics but this was not known when the experiment was set up. Incubation periods in the affected sheep ranged from 19-25 months after infection although there was one additional outlier with incubation period 71 months. In clinically affected sheep, disease related PrP deposition (PrP<sup>Sc</sup>) was widespread in the brain and spinal cord and was also seen in the vagus in some of the sheep. Marginal staining was also seen in the dorsal root ganglia. Definite strong staining was observed in a number of lymph nodes, tonsil and Peyer's patches. Within these tissues the appearance and location of staining was consistent with follicular dendritic cells and/or tangible body macrophages. Marginal staining was also seen in some challenged sheep in the abomasum, duodenum, jejunum and distal ileum, associated with the enteric nervous system and in the celiac-mesenteric ganglia. The project aim however was to see if any of these tissues destined to become infected by clinical end point, would show up as positive at early preclinical stages. No PrP<sup>Sc</sup> or infectivity was found in tissues from the 3 month serial kill group. However in the 6 month cull group, one sheep showed definite accumulations of PrP<sup>Sc</sup> in retropharyngeal and submandibular lymph nodes and marginal levels in Peyer's patches. However the more sensitive method of mouse bioassay showed that two of the sheep in the 6 month cull group had a well established infection of peripheral tissues, with the highest levels of infectivity in spleen. Incubation period in RIII mice can give a rough indication of infectivity titre for comparison between tissues. For 6 month spleen the incubation period was 368 days post infection (dpi) with standard error (SE) = 5. At this time point brain was negative for infectivity. Low levels of infectivity were however detected in Peyer's patches (532 dpi, SE 11) and lymph nodes (484 dpi, SE 18), but infectivity was undetectable in the tonsils. Low infectivity levels were also detected in peripheral nerve ganglia (509 dpi, SE16) and livers (501 dpi, SE 19) and there was a trace of infectivity in the ileum and thoracic spinal cord (606dpi SE 48) of one of the sheep.

**Conclusions** This study confirms and extends the findings of our own previous work and that of other groups on the early distribution of BSE infectivity in sheep tissues following experimental infection. This study was designed to show how early infection could be found and is the only one which has tested sheep at 3 months after inoculation, at which stage infection was undetectable by Immunohistochemistry or by mouse bioassay. The results show that BSE in sheep, in contrast to cattle, involves a widespread infection outside the central nervous system. Should BSE ever be discovered in sheep, peripheral tissues, including liver (which might enter the human food chain) may contain quite high levels of infectivity as early as 6 months after exposure, at a time when the sheep appear clinically healthy. At this stage infectivity was, in our experiment, undetectable in tonsils, suggesting that tonsil biopsy may not always be appropriate for detecting early BSE infection in sheep.

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## Caprine prion gene polymorphism (I142M) associated with low scrapie susceptibility shows high allele frequency in British goat herds

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**Introduction** The goat population in the United Kingdom (UK) is small (~95,000 goats) compared to other European countries contributing only a minor fraction to the total UK livestock production. However, goat milk products are becoming increasingly popular and almost 20% of all goats end up as meat carcasses. In contrast to sheep, a natural case of bovine spongiform encephalopathy (BSE) has been detected in a UK goat and the number of scrapie-affected herds has significantly increased in the past 10 years. The combination of a zoonotic pathogen (BSE) and an animal health issue (scrapie) highlights the need for better understanding of the biology to transmissible spongiform encephalopathies (TSEs) also known as prion diseases in goats. A common feature of TSEs is the accumulation of the pathological prion protein (PrP<sup>Sc</sup>), an aberrant isoform of the normal, host-encoded prion protein (PrP<sup>C</sup>). PrP<sup>C</sup> is encoded by a single gene (*PRNP*), which shows considerable amino acid variability in domestic animal populations. *PRNP* genetic association with disease is well established for sheep and has led to large scale breeding programs in several EU countries. Similar scrapie association studies in goats are far more limited. Nonetheless, some caprine *PRNP* haplotypes have been implicated as providing increased resistance to disease relative to wild-type *PRNP*.

**Materials and methods** Blood samples were collected from a total of 22 UK holdings representing all commercially used breeds in the UK. DNA was extracted, the prion gene open reading frame was PCR-amplified and genotypes determined by direct sequencing.

**Results and conclusions** In this study about 1100 healthy animals have been *PRNP* genotyped for ten polymorphisms all leading to amino acid substitutions. The nine polymorphisms between codons 101 and 222 are all mutually exclusive, whereas proline or serine encoded in position 240 are found in various combinations with the other amino acids. They all have been described before to occur in goats (Vaccari *et al*, 2009) but Q101R, N146S and I218L were for the first time observed in British goats. Amino acid variants 222K, 146N, 211Q and 142M have previously been associated with protection from natural scrapie. The polymorphism I142M also lengthened the incubation periods of experimental scrapie and BSE in goats. We will present the allele and genotype frequencies in UK goats and discuss their potential for the establishment of a breeding programme for the eradication of scrapie in goats.

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## Listeriosis in farmed animals identified through Veterinary Laboratories Agency scanning surveillance over the last 9 years

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**Introduction** Listeriosis has long been recognised in farmed animals. The disease is chiefly caused by infection with *Listeria monocytogenes* which can be associated with several clinical and pathological manifestations, although it is uncommon to encounter more than one type in any outbreak. Less commonly *Listeria ivanovii* infection occurs, but only in sheep. Rare disease has also been reported due to *Listeria innocua*. A review was made of the incidence, pathology and epidemiological features of the various manifestations of disease in all farmed species submissions examined in the Veterinary Laboratories Agency (VLA) between 2002 and 2010.

**Materials and Methods** Submissions to the VLA, from which diagnoses of listeriosis were made, consisted of carcasses received dead or alive for pathological examination, the viscera or brains from animals examined *post mortem* by private practitioners, and ocular swabs. These samples were examined as appropriate using bacteriological and histopathological techniques within a quality framework accredited by UKAS to ISO 17025. The diagnoses are recorded in a database known as Veterinary Investigation Diagnosis Analysis (VIDA) according to standardised criteria.

**Results** Disease was predominantly encountered between December and April, usually peaking in March, and mostly in housed animals. Listeriosis was recorded in several species, most commonly in sheep, followed by cattle and goats with abortion and meningoencephalitis the most frequent manifestations.

**Table 1** VIDA recorded diagnoses of abortion and meningoencephalitis associated with listeriosis in cattle, sheep and goats in England and Wales between 2001 and 2010

Source	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Cattle	15	16	23	26	24	22	25	34	35	39
Sheep	53	67	85	113	75	97	55	73	75	84
Goats	3	3	2	9	4	7	8	8	8	8

In cattle abortion was the principal disease presentation, whereas in sheep and goats cases of meningoencephalitis predominated. There were 9 cases of listerial mastitis and 23 cases of eye infection in cattle. Since 2003, outbreaks of disease in sheep were identified where abomasitis and/or typhlocolitis was the main or sole presenting pathological manifestation. The most unusual form of infection in sheep was myelitis, occurring without brainstem lesions. *Listeria ivanovii* infection was only identified in sheep as a cause of abortion. Systemic infection resulting in hepatitis and/or septicaemia was diagnosed in several species, many of the cases occurring in neonatal animals. There were only 2 diagnoses of listeriosis made in pigs. Meningoencephalitis and systemic infections, but no listerial abortions, were diagnosed in camelids. Sporadic diagnoses were also made in chickens, pheasants, deer and bison.

**Conclusions** The true incidence of disease is difficult to ascertain as diagnostic specimens are not solicited, their numbers vary annually depending on farm profitability and other factors, and hence the submissions will inevitably be biased. Scanning surveillance samples are useful for monitoring the types of disease, patterns or trends of disease, and for identifying unusual or novel presentations. This review confirmed abortion and meningoencephalitis as the principal manifestations in farm animals. The higher prevalence of disease in the winter and early spring corresponds with the period of housing and probably mostly reflects the feeding of silage, a known risk factor. It is uncertain why abortion is the most common manifestation of listeriosis in cattle whereas in sheep and goats it is nervous disease. More unusual forms of disease were diagnosed in sheep, in the forms of alimentary tract, hepatic infection and myelitis. The rarity of disease in pigs, despite a large proportion of the national herd now being reared outdoors, is surprising and may suggest that the species is less susceptible to disease. Increasing numbers of diagnoses were made in the less commonly farmed animals such as alpacas, which are usually not fed silage and suggests that alternative risk factors are important for disease in these species.

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**Reservoir hosts of Leptospirosis Excrete Urinary IgG specific for Leptospira**

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Leptospirosis is a zoonotic disease of global significance. Pathogenic *Leptospira* colonize renal tubules of chronically infected reservoir hosts, in which leptospires proliferate and are shed via urine into the environment. Excreted leptospires can infect naïve reservoir hosts or incidental hosts which can develop acute leptospirosis. *Rattus norvegicus* is a reservoir host for pathogenic *Leptospira*, which due to their shared proximity to human habitats can have wide implications on human health. Experimentally infected rats shed high numbers of leptospires despite a specific host immune response. To further examine the reservoir host immune response to infection, urine was collected from experimentally infected rats for characterization of urinary IgG by gel electrophoresis and immunoblotting. Urinary IgG was detected in both experimentally infected and non-infected control rats. However, urinary IgG was not specific for *Leptospira* until 8-12 weeks post-infection, at which time urinary IgG, derived from experimentally infected rats but not from non-infected controls, reacted specifically with antigens of *Leptospira*. Urinary IgG from infected rats reacted with different antigens compared to serum IgG, suggesting a compartmentalized immune response. The identification of antigens reactive with urinary IgG compared to serum IgG can provide insights into differential antigen expression by leptospires during disease transmission.

## Pathogen-specific inhibition of the innate immune response during bovine mastitis

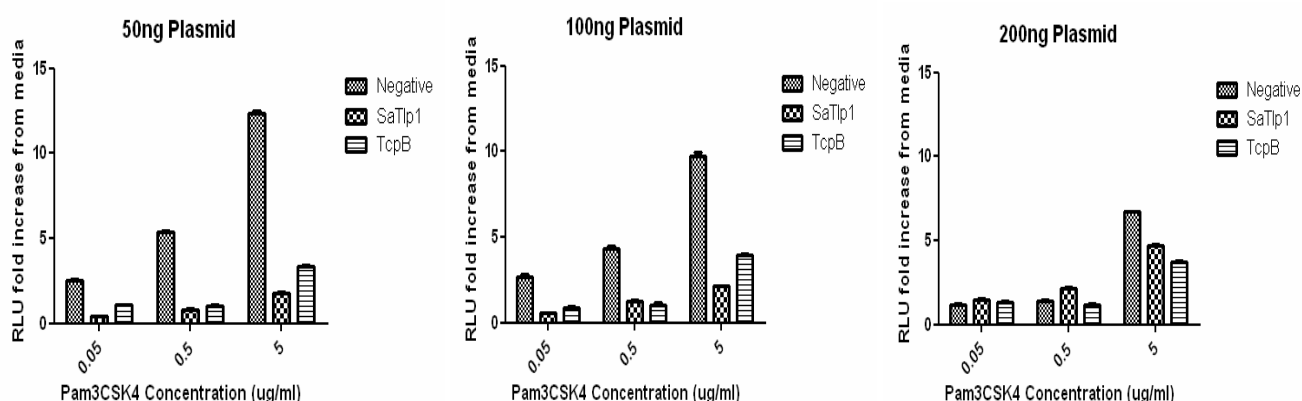
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**Introduction** A diverse range of bacteria express proteins containing Toll/Interleukin-1 Receptor (TIR) domains, the domain of Toll-like receptors (TLRs) responsible for signal transduction. Other members of the STIR (SEFIR, TIR) superfamily, which includes SEFIR (SEF/IL-17R) and DUF1863 (Domain of Unknown Function 1863) domains as well as TIR domains are also widely distributed in the bacterial domain. Some of these bacterial TIR domain containing proteins (Tcps) have been shown to have a subversive role, inhibiting TLR signalling and thereby dampening the innate immune response [Spear *et al*]. Some of these proteins, like the *Brucella melitensis* protein TcpB inhibit TLR signalling by interaction with MyD88 [Radhakrishnan *et al*]. This study aims to identify whether Tcps play a role in modulating the innate immune response during bovine mastitis.

**Materials and methods** Bovine and bacterial TIR domain sequences were used in protein PSI-BLAST searches of bovine mastitis relevant bacterial strains in order to identify any STIR superfamily containing proteins. Following the identification of a DUF1863 gene (SaTlp1) in *Staphylococcus aureus* a range of isolates were screened for this gene by PCR. The SaTlp1 gene as well as *tcpb* as a positive control were then cloned into the pcDNA3.3 TOPO (Invitrogen) expression vector. HEK293 cells stably transfected with boTLR2/YFP were transiently transfected with plasmids containing a firefly luciferase gene under the control of a NF- $\kappa$ B promoter and renilla luciferase under the control of a constitutive CMV promoter, as well as the SaTlp1, TcpB and negative control plasmids in the range of 50, 100 and 200ng. The cells were then stimulated with medium and 0.05, 0.5 and 5  $\mu$ g/ml of the TLR2 ligand Pam3CSK4. After 24 hours the luminescence was then measured using a Promega Dual-Luciferase kit to determine the level of NF- $\kappa$ B activity in each of these systems.

**Results** SaTlp1 has so far only been identified in ST398 isolates of *S. aureus* suggesting a recent acquisition. Preliminary results suggest that NF- $\kappa$ B activation was severely abrogated when the SaTlp1 plasmid was transfected into HEK-boTLR2/YFP cells, to a similar degree to TcpB.



**Figure 1** Graphs showing the RLU fold increase compared to media controls due to NF- $\kappa$ B activation in HEK-boTLR2/YFP cells transfected with 50, 100 and 200ng of negative control, TcpB positive control and SaTlp1 plasmids

**Conclusions** The results suggest clear down-regulation of NF- $\kappa$ B activity as a result of SaTlp1 being introduced into this experimental system, possibly due to the DUF1863 domain interacting with the TIR domain of MyD88 or one of its adapter molecules, thereby blocking signalling.

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## A probabilistic elicitation of expert veterinarians' beliefs regarding vaccination against bovine viral diarrhoea virus in dairy cattle and the effect on herd fertility performance

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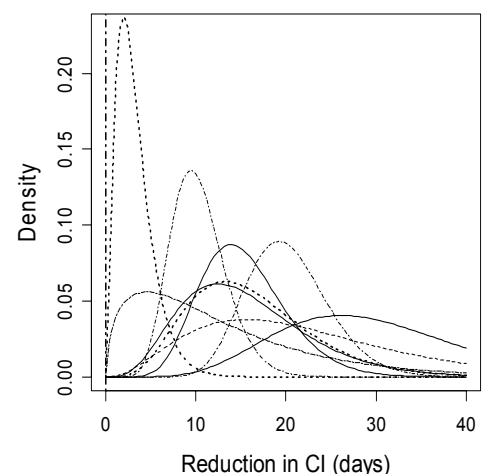
**Introduction** Scotland recently announced its compulsory bovine viral diarrhoea virus (BVD) eradication programme, bringing with it a multitude of questions regarding the formation of a national BVD strategy for England. To date, widespread vaccination alone has not improved the epidemiological situation in any area in which it has been applied and cannot supplant biosecurity measures. However, given large tracks of England have high cattle densities, vaccination (by affording fetal protection) would be a vital component of any future national control programme. Historically, common practice in England has been to BVD vaccinate dairy herds blindly (i.e. without any other BVD specific control measures). However since BVD infection is largely manifested sub-clinically and many other factors influence herd performance, in practice it is difficult to assess any improvement in herd health due to blind vaccination. Thus, in order to assess any observable effect on farm, the beliefs of practising “expert” veterinarians were investigated concerning the clinical impact on fertility performance attributable to blindly vaccinating dairy herds endemically infected with BVD ( $\geq 1$  persistently infected animals). This study aimed to quantify and assess their beliefs as probability density functions (pdfs) using a statistical technique called probabilistic elicitation.

**Material and methods** An “expert” was defined as holding  $\geq 1$  post-graduate qualification(s) from: RCVS Certificate in Cattle health and Production (CertCHP), RCVS Diploma in Cattle Health and Production (DCHP), Liverpool University Diploma in Bovine Reproduction (DBR). The RCVS database yielded 32 experts in the study area (100 mile radius, centred on Nottingham University). Of these, 3 had retired, thus 29 experts were assigned a random number, sorted in increasing order and voluntarily invited to participate in list order, until sample size (10) was achieved. The elicitation used specifically designed software, SHELF (O'Hagan and Oakley <http://www.tonyohagan.co.uk/shelf/>) and individual interviews ( $\leq 30$  minutes). The task considered three populations of dairy herds, starting at time  $>3$  years ago; (1) no BVD control measures and endemically infected, (2) blindly BVD vaccinating and endemically infected, (3) BVD free. “Overall fertility performance” was measured by:  $\theta^c$  = the current calving interval (in days) and  $\theta^p$  = the current maiden heifer pregnancy rate. Question 1 (Q1) concerned the reduction in  $\theta^c$  between populations (2) and (1), denoted  $\theta_2^c - \theta_1^c$ , and question 2 was  $\theta_2^p - \theta_1^p$ . Both questions were elicited by 5 probabilistic judgements (minimum, maximum, median, upper/lower quartiles) to which parametric gamma distributions were fitted. Similarly, questions 3 and 4 were the improvement in  $\theta^p$  between populations (i.e.  $\theta_2^p - \theta_1^p$  and  $\theta_3^p - \theta_1^p$ ) and beta distributions were fitted. Two pilot interviews tested the method. The pdfs were subsequently mathematically linearly pooled with each expert given equal weight.

**Results** 16 experts were contacted; non-participation was due to unavailability in the time frame. Of the 10 experts interviewed, four held the CertCHP only, four the DBR only, one held both CertCHP and DBR and one the DCHP. They worked in 8 veterinary practices located in 8 counties from Buckinghamshire to Yorkshire. Gender: two females and eight males. “Years qualified” varied from 7 to 46 (median 21.5); combined these experts had 220 years of clinical experience. Figure 1 shows the experts' beliefs as pdfs for Q1 and reveals *major* variations in their beliefs, both in centre of location (“best guess”) and variances (“how confident they are”). One expert was *certain* blind vaccination offered no reduction in  $\theta^c$  (single spike at zero, Fig.1). At the other extreme, one expert believed the median reduction was 30 days, but also showed *considerable* uncertainty (minimum to maximum: 15-45 days). Pooling the beliefs of the experts gave a 0.64 probability (P) to  $>10$  days reduction in  $\theta^c$ . For Q2-Q4 a similar diversity in beliefs was observed. For Q2 compared to Q1 (and Q3 compared to Q4) whilst all experts assigned more probability to larger reductions (improvements) they did so to very variable extents. The pooled distributions gave, Q2:  $P(\theta_2^p - \theta_1^p > 10 \text{ days}) = 0.73$ , Q3:  $P(\theta_3^p - \theta_1^p > 0.10) = 0.46$  and Q4:  $P(\theta_4^p - \theta_1^p > 0.10) = 0.65$ . Six of the 10 experts stated zero as their minimum elicited values for  $\theta_2^c - \theta_1^c$  and  $\theta_2^p - \theta_1^p$ ; the others stated positive numbers.

**Conclusions** Experts' beliefs can be usefully quantified by probabilistic elicitation. When combined mathematically, the experts' beliefs overall can be considered supportive of a discernable benefit to fertility performance from blind vaccination, at a herd level. However, *major* variations and *uncertainty* existed between experts. The practical importance of this is that considerably different advice is probably being given on-farm and the impact of blind vaccination with respect to observable improvements in herd fertility performance on farm is unclear. This has implications when considering the approach and delivery of an English national control policy. Investigating and understanding veterinary beliefs in the future will be essential.

**Acknowledgements** The authors would like to thank the 12 participating veterinarians. The Wellcome Trust and the University of Nottingham funded this research.



**Figure 1** Pdfs of the experts beliefs for  $\theta_2^c - \theta_1^c$

**An emerging haemorrhagic syndrome in young calves in Europe: pathology and pathogenesis**

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An unexplained haemorrhagic syndrome (bleeding calf syndrome, bovine neonatal pancytopenia) of calves less than 1 month old has been recorded in several European Countries since 2007/2008. The first cases were reported in Germany and cases have also occurred in other countries including the Netherlands, France, the UK, Ireland and Belgium. The presenting clinical signs are predominantly relate to external or internal haemorrhage.

Trilineage hypoplasia (TLH) involving extensive depletion of erythroid and myeloid precursors and megakaryocytes is the characteristic underlying pathology. The absence of megakaryocytes and resulting platelet deficiency provides an explanation for the haemorrhage observed.

TLH is the histological correlate of aplastic anaemia and indicates injury to pluripotential haemopoietic stem cells. In cattle and other species TLH / aplastic anaemia has been documented in association with genetic, viral (*eg* retroviruses, herpesviruses, parvoviruses, circoviruses), toxic (*eg* T2 toxin, chemotherapeutic agents) and immune-mediated (T cell) aetiologies. The latter includes many human cases previously categorised as idiopathic. To date, no evidence of toxic or infectious cause has been found. However, the pathology differs from that observed in T-cell associated aplastic anaemia. An alloimmune mechanism is proposed.

## Evaluation of the reliability, sensitivity and specificity of indicators of sheep welfare

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**Introduction** Sheep farmers and veterinary surgeons frequently judge the welfare of individual and groups of sheep by using animal-based measures, such as body condition and gait, to diagnose the presence or absence of a specific welfare condition. Principles used to evaluate the validity of diagnostic tests appear to be relevant to the validation of animal welfare indicators. Therefore, the aim of this study was to evaluate the diagnostic performance of animal-based indicators of sheep welfare.

**Materials and methods** Eight observers, with varying levels of experience and training, independently assessed indicators of demeanour, lameness, mastitis, body condition, wool loss, and pruritis on 1146 adult sheep (aged > 1 year) and growing lambs (aged > 6 weeks < 1 year). The level of inter-observer reliability was evaluated using Fleiss's kappa ( $\kappa$ ). Latent class analysis (LCA) determined the diagnostic sensitivity (Se) and specificity (Sp) of each observer and also predicted the test performance of randomly selected observers who may apply these indicators in the future.

**Results** Excellent levels of inter-observer reliability ( $\kappa > 0.75$ ) were identified for demeanour, mastitis, wool loss. Good levels of reliability ( $\kappa > 0.41 < 0.74$ ) were produced for tooth condition, pruritis and lameness and body condition assessment. LCA found that all indicators had high levels of diagnostic Sp ( $\geq 0.97$ ). Good levels of diagnostic Se ( $\geq 0.95$ ) were produced for the assessment of demeanour, wool loss, body condition ( $\geq 0.95$ ) and lameness (0.86 to 0.91). Lower levels of Se were identified for measures of mastitis (0.50 – 0.72) and pruritis (0.13 – 0.40). With the exception of pruritis, LCA predicted that future observers would have good levels of diagnostic Sp and Se.

**Conclusion** Overall, this study found that observers of varying experience and training had high levels of inter-observer reliability and diagnostic Sp. Results also suggested that future observers could also have high levels diagnostic ability when performing animal-based indicators of sheep welfare. The low prevalence of certain welfare conditions, such as pruritis, appeared to affect the level of diagnostic Se. Therefore, it may be useful to examine the test performance of these indicators on a population with a higher prevalence of sub-optimal welfare conditions.

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## Consequences of plant protein source on the periparturient resistance to parasites in ewes

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**Introduction** A large number of studies have suggested that an increased supply of metabolizable protein (MP) at times of MP scarcity reduces the degree of periparturient relaxation of immunity (PPRI) in sheep, as shown by reduced worm burdens and worm egg excretion (Kyriazakis and Houdijk, 2006). The magnitude of this effect varies between studies, which may be related to variation in amino acid (AA) supply of the provided MP (Sakkas *et al.*, 2010). Here we test the hypothesis that additional MP supply from xylose-treated soybean meal would be more effective than from field bean in reducing the degree of PPRI, as the latter contain less digestible undegradable protein that is also more deficient in methionine than the former.

**Materials and methods** Sixteen twin- and eight triple- bearing Mule ewes were trickle infected with *Teladorsagia circumcincta* every Mon-Wed-Fri from day<sub>-49</sub> until day<sub>31</sub> (day<sub>0</sub> is parturition). Infective larvae were superimposed on an existing natural infection and were given in increasing quantities to mimic the larval intake over time that would be associated with the periparturient increase in forage intake. On day<sub>-24</sub>, ewes were allocated to one of three feeding treatments (n=8), balanced for FEC, number of lambs carried and body weight (BW). Ewes were fed at 0.9 times their metabolizable energy requirement and at either 0.8 (LP) or at 1.2 times their respective MP requirements (AFRC, 1993) using either xylose-treated soybean meal (HPS) or field beans (HPB). Litter size was standardized to two at parturition. Ewe and litter BW and ewe condition score (CS) were measured weekly. Ewe faecal egg counts ((FEC, in eggs per gram (epg) fresh faeces)) were assessed twice weekly, and transformed via log (FEC+1) for statistical analysis. Regularly collected blood samples were analysed for plasma pepsinogen concentration. Data were analysed using repeated measures ANOVA for gestation and lactation separately, using diet as a factor and data collected before day<sub>-24</sub> as covariates.

**Results** Feeding treatment did not affect gestational ewe BW and CS, which changed from 75.8±1.29 kg and 2.3±0.05 before foods were offered to 77.4±1.39 kg and 2.1±0.04 just before parturition (P<0.001), and 63.4±1.13 and 2.0±0.05 at parturition. However, time interacted with feeding treatment on lactational ewe BW (P<0.001) and CS (P<0.05); by day<sub>31</sub>, HPS ewes were heavier than LP ewes, with HPB ewes being intermediate (63.1 vs 61.3 vs 58.8 kg; s.e.d. 1.50 kg; P<0.05), whilst HPS and HPB ewes had higher CS than LP ewes (2.1 vs 2.1 vs 1.8; s.e.d. 0.07; P<0.005). Time interacted with feeding treatment on litter body weight (P<0.001) reflecting a higher weight gain for HPS litters than for HPB and LP litters (Figure 1). Feeding treatments affected ewe FEC during lactation only (P<0.05); mean FEC of HPS ewes was lower than that of LP ewes (P<0.005) and tended to be lower than that of HPB ewes (P=0.08; Figure 2). Feeding treatment and time did not interact on plasma pepsinogen, which was similar for HPS and HPB ewes throughout and significantly elevated for LP ewes during both gestation (P<0.001) and lactation (p<0.005).

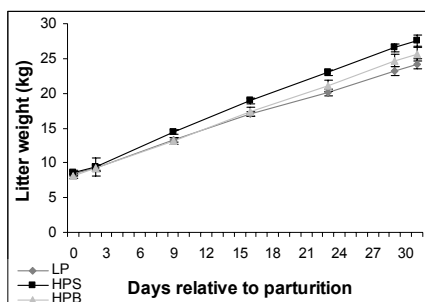


Figure 1 Litter growth

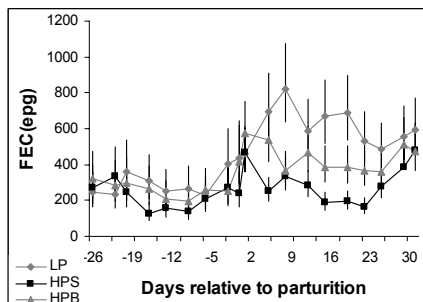


Figure 2 Faecal egg counts

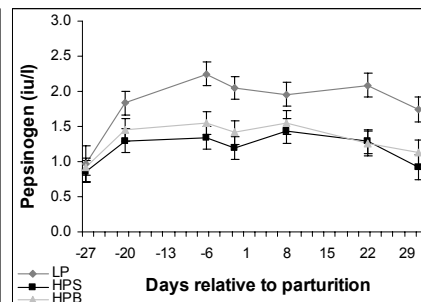


Figure 3 Plasma pepsinogen

**Conclusion** In agreement with our hypothesis, the results support the view that extra MP supply from field beans is less effective in reducing the degree of PPRI than MP supplementation from xylose-treated soybean meal. Whilst field beans did not significantly improve lactational performance, both protein sources resulted in reduced pepsinogen concentration which is indicative of reduced abomasal damage in response to abomasal parasitism. This suggests that additional MP from field beans was mainly directed to host maintenance functions rather than immunity to parasites. Thus, dietary protein quality needs to be considered when formulating high MP diets for worm control in sheep. Further research to assess variation in the ability of protein sources to improve host resistance to gastrointestinal nematodes is required, especially if reliance on imported soybean meal is to be reduced (Sakkas *et al.*, 2010).

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## An improved understanding of bacterial population dynamics in the development of ovine footrot

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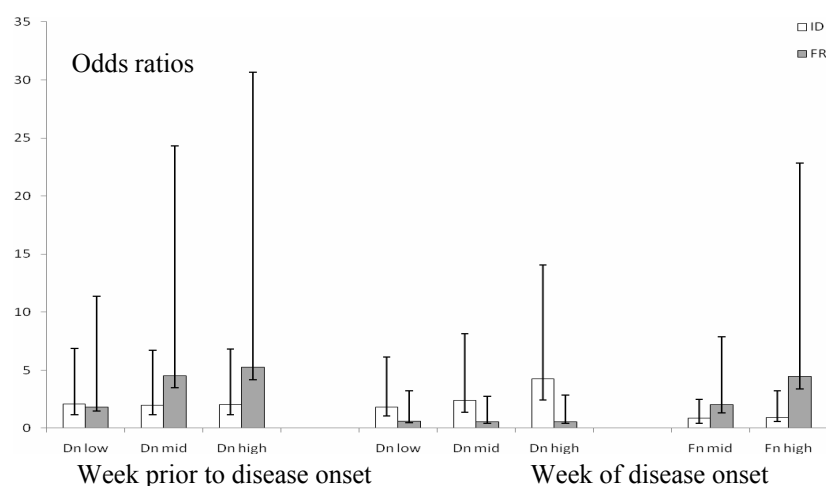
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**Introduction** Ovine footrot is caused by *Dichelobacter nodosus*, however there is still some debate regarding the role of *Fusobacterium necrophorum* in disease initiation. Disease begins as an interdigital dermatitis (ID) that may then develop to virulent footrot (VFR), a more severe presentation.

**Materials and methods** All 4 feet of 18 ewes, from a single flock were examined each week for 5 weeks in October 2006. The ewes were individually identified and their feet were coded as - healthy (H); no signs of clinical disease (sheep  $n = 3$ , foot observations  $n = 54$ ), ID; inflammation of the interdigital skin with no separation of hoof horn (sheep  $n = 7$ , foot observations  $n = 138$ ) and VFR; separation of the hoof horn from the sensitive underlying tissue (under-running) (sheep  $n = 8$ , foot observations  $n = 157$ ), by a trained researcher (9). On all but 11 occasions, a cotton swab sample was taken from the interdigital space in H and ID feet and from the site of hoof horn separation in sheep with VFR. The study was approved by the university local ethical committee. All swabs were stored in 0.5 ml sterile phosphate buffered saline (PBS) containing 20 mM Na2EDTA (pH 8.0). They were maintained at 4°C and then transferred to -80°C within 24 h and stored until required. Two real-time PCR (TaqMan) assays were developed to target *D. nodosus* (*rpoD*) and *F. necrophorum* (*rpoB*) DNA, in order to elucidate the temporal patterns between bacterial load and disease progression. An unordered multinomial mixed effects model accounting for repeated measures of feet over time clustered within ewes, was used to investigate the associations.

**Results** There was variation in load between sheep; however *D. nodosus* (*rpoD*) load was significantly higher in sheep with ID and VFR in comparison to healthy individuals. *F. necrophorum* (*rpoB*) load remained relatively stable irrespective of disease state, but was significantly higher in some cases of VFR. An increase in *D. nodosus* (*rpoD*) load was observed in feet with ID the week of disease, and a further increase in load correlated significantly with an increased risk of developing VFR one week later (Figure 1). An increase in *F. necrophorum* (*rpoB*) load was only observed after VFR developed. We hypothesise therefore that *F. necrophorum* is an opportunist and that *D. nodosus* burden is integral in the development of VFR, whilst *F. necrophorum* load is not.



**Figure 1** Output from the multinomial regression model of the abundance of *D. nodosus* and *F. necrophorum* the week before and the week of disease onset. Results displayed as odds ratios (ORs) with 95% confidence intervals. The explanatory variables were categorised under the following; undetectable (U), low ( $\leq 10^3$ ), mid ( $10^4$ ) and high ( $\geq 10^5$ ) abundance. Significance was set at  $P < 0.05$ .

**Conclusions** We conclude that change in *D. nodosus* load was associated with increasing severity of disease whilst there was no change in *F. necrophorum* load until disease had developed. This suggests that disease progression is driven by *D. nodosus* although this study does not rule out change in bacterial behaviour triggering disease progression.

**Practical implication** The results also indicated that sheep with ID harbour the greatest load of *D. nodosus*. Control and treatment strategies targeted at sheep with ID (isolation and treatment) might give the most efficient route to minimising disease severity and spread.

**Acknowledgments** Data for this work were collected during a Defra funded project AW0121 and analysed as part of BBSRC project BBE01870X1. Luci Witcomb is funded by a NERC CASE studentship with Pfizer.

## Management of over-weight and obese equids in a charitable trust setting: measures of success and failure

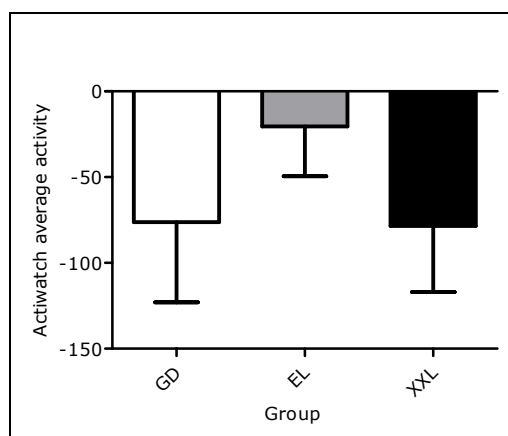
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**Introduction** Equine obesity is a growing welfare concern and charitable organisations are receiving an increasing number of obese animals. Concern about equine obesity is strongly supported by emerging studies, with prevalence parallels being drawn between the equid, smaller companion animals and humans, most notably in Western civilisation (Johnson and Wiedmeyer, 2009). As in other species, obesity is associated with significant metabolic disorders in the equid, including hyperlipaemia and laminitis. Overweight or obesity is assessed in equids using body fat scoring (BFS), this is a process of manually assessing subcutaneous fat and assigning a numerical value to particular anatomical points. When used correctly BFS is unaffected by the conformation or size of the horse and remains the single most accurate means of estimating body fat content. Weigh bridge and weigh tape measurements are also useful tools in assessing body weight change over time. Despite their best efforts, charitable organisations are currently struggling to manage overweight and obese equids. The aim of this project was to examine current management routines, effectively studying the success of management of the over-weight equine, in a charitable setting.

**Materials and methods** 15 ponies were selected from a group of 100 equids, established at a charitable organisation in the east of England. The ponies were selected and assigned to 3 groups by two members of senior staff: easy loser (EL), a group of ponies with no perceived concern regarding weight management; good doer (GD, a good doer typically describes a pony able to maintain bodyweight when calorific intake is limited), a group receiving some weight management and 'extra extra large' (XXL), a group being managed more rigorously than the GD ponies, in an attempt to promote significant weight loss. All animals were assessed over a 3 month period (September to November 2010), during which time individual body weight, BFS (1-5) and activity were assessed using weigh bridge and weigh tape, manual BFS and Actiwatch accelerometry respectively. Measurements were made by the same researcher on the first week of each month. Actiwatch accelerometers record movement levels in all directions (Cambridge Neurotechnology Ltd. Actiwatch User Manual: 4). Duration, intensity and amount of movement are recorded as a voltage, which is then converted to an activity count and recorded. Connecting the Actiwatch to appropriate software allows the total, peak and average Actiwatch to be visualised and the activity count can be expressed as an actogram. In this study, Actiwatches attached to the left forelimb, fixed on a strap fitting around Velcro brushing boots. Statistical analyses (repeated measures ANOVA and Spearman tests) were carried out using PASW Statistics (SPSS) (version 18.0).

**Results** There was no significant change in body weight assessed using weigh bridge or weigh tape during the study (body weight change by weigh bridge: GD,  $-7.6 \pm 5.9$ ; EL  $-11.2 \pm 15.7$ ; XXL  $5.5 \pm 12.5$  kg ( $P > 0.05$ )). Repeated measure ANOVA analysis (plus Bonferroni post hoc test) indicated significantly greater neck and shoulder BFS in the XXL compared to the EL ( $P < 0.05$ ). A similar increase was observed in the GD compared to the EL group in ribs and backbone BFS ( $P < 0.05$ ). There were no significant differences in average activity between groups during the study, although change in activity between September and November demonstrated a significant reduction in all groups ( $P < 0.05$ ) (Figure 1).



**Figure 1** Change in average Actiwatch activity.

**Conclusion** Despite careful management of equid obesity within this charitable setting, body weight and body fat score were maintained and activity levels reduced in all groups over the 3 month period. In accordance with other species, including humans, there appears to be a discrepancy between perceived exercise intensity and actual, meaning that there is huge scope for this charitable organisation to develop a regime that results in effective weight loss and is applicable for horse owners to use.

**Acknowledgements** The authors wish to thank the School of Veterinary Medicine and Science for funding and the charitable organisation (World Horse Welfare) for permitting the study.

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## Acute zinc intoxication in a dog – clinical and pathological findings

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**Introduction** Acute zinc toxicity has been reported in dogs in the USA on many occasions since 1982, predominantly associated with ingestion of zinc-rich coins (Gurnee and Drobatz, 2007). In Europe, fewer cases have been reported (Gandini *et al.*, 2002). Acute zinc toxicity in dogs usually results in haemolytic anaemia, gastrointestinal dysfunction, acute renal failure and, as reported in two cases, acute pancreatitis (Mikszewski *et al.* 2003; Weingert and Kohl, 2009). Zinc-associated pancreatitis has also been reported in humans, ferrets, sheep, cattle and rats. Presented here are the clinical and pathological features of the first case of canine zinc toxicity in the UK. The source of the intoxicant was an ingested metallic foreign body.

**Materials and methods** A 14 month old, female-neutered Cavalier King Charles Spaniel was admitted to the University of Liverpool Small Animal Teaching Hospital with a 48 hour history of haematochezia, icterus and collapse. Regenerative anaemia with a packed-cell volume of 7% was seen. Prior to referral, radiography had revealed a gastric, metallic foreign body which was removed at exploratory laparotomy. Clinical diagnostic investigations included serum biochemistry and haematology, a coagulation profile and abdominocentesis. The dog's condition deteriorated and she died several hours after presentation. Necropsy was performed followed by histopathological examination of all major organs. Liver and kidney underwent chemical analysis for zinc and copper.

### Results

**Clinical findings** On presentation the dog was comatose, hypothermic and bradycardic – resuscitation was performed successfully using Oxyglobin (OPK Biotech) and crystalloid. The dog displayed marked abdominal pain. Haematology showed a degenerative left shift and serum biochemistry revealed mild azotaemia, hyperbilirubinaemia and moderately elevated liver enzymes. Activated partial thromboplastin time was extended.

**Pathological findings** At post-mortem examination, the animal showed severe icterus. Both kidneys were diffusely dark red; the pancreas was diffusely pale and nodular. The bladder contained a small volume of red-tinged urine and the colon and rectum contained a moderate amount of soft, red faecal material, evidence of haematochezia. Histopathological examination of all major organs revealed evidence of intravascular haemolysis with blood vessel lumens containing faintly eosinophilic material (haemoglobin). The renal tubules also contained large amounts of intraluminal haemoglobin with multifocal haemoglobin crystals scattered throughout the renal cortex and medulla. Tubular epithelial cells showed evidence of haemoglobin uptake. The pancreas exhibited multifocal coagulative necrosis, surrounded by a neutrophil-dominated inflammatory infiltrate.

**Biochemical analysis** Fresh pieces of liver and kidney were analysed for zinc and copper levels, which revealed that zinc levels were markedly increased above the normal reference range in both organs (liver: 165 ppm; normal, 30-70 and kidney: 141 ppm; normal, 16-30) although below the reference range for toxic levels (204-436 ppm and 190-295 ppm for liver and kidney respectively). Copper was mildly elevated in the liver and normal in the kidney.

**Discussion** This report describes a case of acute zinc toxicity in a dog following ingestion of a metallic object which had been removed via a gastrotomy. The fact that the foreign body was present in the stomach is of importance in the development of zinc toxicity as an acidic pH facilitates absorption. The clinical signs of marked haemolytic anaemia are typical of previous cases of acute zinc toxicosis (Gurnee and Drobatz, 2007). Morphological changes of marked icterus, haematochezia and haemoglobinuria with histopathological evidence of intravascular and intratubular haemoglobin provide further evidence of intravascular haemolysis. The mechanism underlying zinc-induced haemolysis is not fully understood, but suggested mechanisms include a direct effect of the zinc on the erythrocyte membrane or inhibition of enzymes critical to their function. The animal had also developed an acute necrotising and purulent pancreatitis. The pathogenic processes leading to pancreatitis in association with zinc toxicity is not fully elucidated either, but may be related to the mechanism by which dietary zinc is excreted which includes excretion via the pancreatic duct after concentration in the pancreas. The levels of zinc determined in the liver and kidney in this case were raised above normal; but did not reach the toxic level given in the reference data. However, other cases of zinc toxicosis have demonstrated that serum zinc levels fall rapidly after removal of the source of the zinc (Weingert and Kohl, 2009). It is likely therefore that zinc levels in this animal were at a toxic level at the time the foreign body was present which lead to the observed pathological changes. The findings in this case indicate that zinc toxicosis must be considered as a differential diagnosis in cases of acute haemolytic anaemia in dogs, and that in such cases acute pancreatitis may also be present.

**Acknowledgements** The authors acknowledge the VLA Shrewsbury for biochemical analysis.

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## Paravertebral malignant schwannoma in a horse

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**Introduction** Peripheral nerve sheath tumours (PNSTs) are a heterogeneous group of neoplasms arising from cells surrounding the axons of neurons in peripheral nerves. They include schwannoma which originates from Schwann cells, and neurofibroma, which originates from both Schwann cells and fibroblast or perineurial cells (or a mixture of both). Schwannomas and neurofibromas are well characterized and, in human neuropathology, are well established as separate entities (Summers *et al.*, 1995; Weiss and Goldblum, 2001). In domestic animals, however, distinction between the two forms by light microscopy is less clear cut, and for this reason both are classified as PNSTs by the World Health Organization (WHO) (Koestner *et al.*, 1999). In the horse, schwannomas are rare (Cotchin E: 1977; Veazey *et al.*, 1993). This report describes the histological and immunohistochemical the first diagnosis of a malignant schwannoma in the epaxial musculature of a horse.

**Materials and methods** A 500 kg, 9 year-old Lipizzaner gelding with history of a chronic muscular weakness, dog sitting position and difficulties in urination was euthanased due to a radiographical soft tissue opacity with lysis of L5/6, and submitted for post-mortem examination. Tissue samples were fixed in 10% neutral buffered formalin and routinely processed in paraffin wax for light microscopy and immunohistochemical examination. Sections (4-5 µm) were stained with haematoxylin and eosin (HE), periodic acid-Schiff reaction (PAS), and PAS diastase reaction, Gomori trichrome stain, phosphotungstic acid-haematoxylin stain (PTAH) and Oil red – O stain. For immunohistochemistry (IHC) sections were labelled with commercially available monoclonal antibodies against Desmin, GFAP, Myoglobin, S100 protein and Vimentin. Visualization was achieved by 30 minutes incubation in Avitin Biotin Complex, nuclei were counterstained with Papanicolaou's haematoxylin.

**Results** Makroscopically a partly encapsulated, triangular shaped mass (~12x8 cm) on the left lumbosacral region, extending from L5 to S1, effacing the bulk of the deep axial musculature (multifidi muscles and superficial parts of longissimus dorsi muscles) and unilaterally diverting the processus spinosus of S1. On cut surface the mass was multilobulated, yellow-tan and of medium firm consistency, extending into the spinal canal of L6 through the ligamentum flavum.

Histological examination revealed a non-encapsulated poorly demarcated infiltrative mass of variable cellularity and pleomorphism, with 20-50 µm sized, spindeloid to stellate in cells arranged either in bundles or whorls, embedded in fine fibrovascular stroma and separated by collagenous tissue septae.

Immunohistochemically, neoplastic cells revealed strong positive reaction for vimentin, S100 protein, GFAP and myoglobin. Immunolabelling for pancytokeratin, Melan A, and desmin gave negative results. According to these findings the neoplasm was diagnosed as a malignant peripheral nerve sheath tumour (PNST).

**Discussion** In veterinary medicine no clear cut between the diagnosis of malignant schwannoma and neurofibrosarcoma is established and the classification of malignant PNST is proposed for malignant tumours assumed to be of peripheral nerve origin (Koestner *et al.*, 1999; 2002).

Malignant schwannomas are infiltrative tumours entirely composed of neoplastic Schwann cells with occasionally positive immunohistochemical reaction for myoglobin (Maxie *et al.*, 2007).

In the case presented, immunoreactivity of the majority of neoplastic cells to S100 protein in the absence of immunoreactivity to melan A suggests the diagnosis of a schwannoma. The distinction between benign and malignant schwannoma was based on morphological criteria (pleomorphism, anaplasia, epithelioid cells, multinucleated atypical giant cells), and biological behaviour (invasion of the surrounding soft tissues). In contrast to other studies however neoplastic cells were GFAP-positive, a feature more common in benign PNST's than in malignant PNST's (Chijiwa *et al.*, 2004).

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## Neonatal canine staphylococcal dermatitis

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**Introduction** The main species of *Staphylococcus* colonising the skin and hair of dogs are *Staphylococcus intermedius*, *S. pseudointermedius* and *S. delphini*. These species also colonise mucocutaneous sites, such as the upper respiratory tract, oral cavity and anus. *S. pseudointermedius* appears to be the main cause of superficial bacterial folliculitis (superficial pyoderma) in dogs, although *S. intermedius* may also cause superficial and deep pyoderma. Puppies are colonised by *Staphylococcus* spp. derived from the bitch immediately after birth. Neonatal mortality in puppies in the first two weeks following birth has been associated with umbilical infections, multifocal abscessation and septicæmia caused by *S. pseudointermedius*/*S. intermedius*. This report describes neonatal mortality in puppies associated with staphylococcal infections of the footpads and mentum (chin).

**Materials and methods** Postmortem examinations were performed on nine puppies that died within 14 days of birth during the period from 1986 to 2010. Affected breeds were Labradors (n = 6), a Gordon setter, a Beagle and a Foxhound. Two to seven puppies died in six litters and one puppy was submitted from each of three other litters. In two litters, one or more puppies were stillborn, followed by neonatal mortalities from 1-4 days of age. In one of these litters, the bitch had a history of vulvitis. Samples from the surface of the skin and from internal organs were inoculated onto sheep blood agar and cultured routinely. Isolates were identified as *Staphylococcus* spp. by colony morphology, Gram staining and biochemical tests. Selected isolates were identified using the bioMérieux ID 32 Staph kit and one isolate was submitted to the Scottish MRSA Reference Laboratory for typing. Samples of skin and internal organs were collected into neutral buffered formalin and processed routinely for histopathology. Histological sections were stained with haematoxylin and eosin and by the Gram method.

**Results** Nine puppies aged from 2-14 days (mean 6 days) from different litters had 2-4 mm diameter black spots or localised areas of pale yellow crusting with erythema on the foot pads (n = 6) or carpal pads (n = 4) of the forelimbs, along with 4-6 mm locally extensive pale yellow areas of crusting and erythema of the skin of the mentum (n = 6) and nares (n = 1). In addition to lesions on the skin, three of the nine puppies had pneumonia and one puppy had septicæmia. Affected regions of the foot pads, carpal pads and mentum exhibited segmental ulcerative dermatitis with necrosis of the dermis and adnexal elements. The surface of the skin and areas of dermal necrosis were colonised by large numbers of Gram positive cocci. Acute interstitial pneumonia was evident in the lungs of the three puppies with pneumonia. Leucocytosis and multifocal necrotising inflammatory lesions were evident in multiple tissues in the puppy with septicæmia. *Staphylococcus* spp. were recovered in profuse growth from lesions in the skin (n = 9) and from the lungs (n = 3) of puppies with dermatitis. Isolates were identified as *S. intermedius*/*S. pseudointermedius*.

**Conclusions** Ulcerative necrotising dermatitis and pododermatitis due to colonisation of the skin of the foot pads, carpal pads and chin by *Staphylococcus* spp. is a cause of neonatal mortality in puppies. In many cases, the bacteria remain localised to the skin and death appears to be due to toxæmia. Other cases develop pneumonia due to colonisation of the respiratory tract or septicæmia due to systemic spread of infection. Virulence factors produced by *S. pseudointermedius* include toxins such as haemolysins, exfoliative toxins and enterotoxins and enzymes such as coagulase, proteases and thermonuclease. Surface proteins expressed by *S. pseudointermedius* bind to fibrinogen and fibronectin and the bacterium also produces an immunoglobulin-binding protein similar to staphylococcal protein A. *S. intermedius* is able to bind to canine corneocytes. Puppies are colonised by *Staphylococcus* spp. from the bitch soon after whelping, but factors predisposing to disease are unknown. Staphylococcal dermatitis should be recognised as a cause of “fading puppy” syndrome.



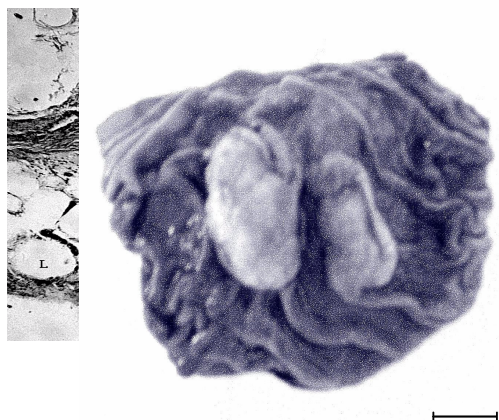
## Gastric lymphangioma in a dog

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**Introduction** Lymphangioma is a rare benign vascular swelling. It manifests clinically as an external disfigurement sometimes interfering with locomotion or associated with malfunction of an organ system. Gastric lymphangioma is very rare even in human medicine. This account is the first reported case of gastric lymphangioma in a domestic species.

**Clinical History and Gross Pathology** This greyhound was a three year old male in good athletic condition. The clinical history reported sporadic episodes of lameness associated with vigorous exercise but an absence of gastric or enteric disorders. Grossly there was a severe retroperitoneal haemorrhage which had caused immediate collapse and death after a race. The intestines, mesenteries and lymph nodes were unremarkable. The stomach lining was discoloured and between the gastric folds were two adjacent domed sessile white growths (Figure 1). The visceral peritoneal surface showed the emergence of vessels at this point continuous with the mesenteric vasculature.



**Figure 1** Dissected unfixed stomach wall showing a bilobed lymphangioma protruding into the fundic cavity and covered by a continuous mucosa. Bar = 10mm



**Figure 2** Mucosa from the centre of the tumour showing a denuded luminal surface covered with loose connective tissue of the lamina propria and a fragmented hypoplastic epithelium squeezed between dilated rounded fluid filled lymphatic capillaries. The muscularis mucosae is displaced by a dilated lymphatic vessel in the submucosa. 20µ section stained PAS and haematoxylin. Bar = 0.2mm.

**Histopathology** The growths were well delineated, unencapsulated and showed well defined tissue layers. The boundaries between normal and abnormal tissue were well defined. Each layer contained coiled irregularly arranged rounded dilated channels (Figure 2) which showed a graded increase in size from the mucosa to the serosa. The channels were lined sparsely by a single layer of uniform flattened endothelial cells enclosing empty lumina, completely devoid of blood and supported by a fine connective tissue stroma which under light microscopy showed an absence of muscle cells. There was a moderate lymphocytic infiltration and formation of lymphatic nodules. The dilated lymphatic vessels stained positive for pan-endothelial marker to Factor VIII related antigen but negative for CD31 antigen.

**Conclusion** A diagnosis of lymphangioma was made based on the gross appearance and the presence of a well delineated tumour showing a gradation of large coiled dilated vessels supported by fine loose connective tissue, devoid of blood, engorged with clear fluid and lined by a flattened uniform endothelium. There was a variable multifocal mucosal lymphocytic infiltration consistent with literature descriptions of human gastric lymphangioma.

**Acknowledgements** I wish to thank the National Greyhound Racing Club for inviting my participation in their survey into sudden death in racing greyhounds and the provision of this rare case.

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## Novel SNPs in sheep and cattle TLR5: potential biomarkers for disease resistance and susceptibility?

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**Introduction** Toll-like receptors (TLRs) are a family of proteins which are integral in the recognition of microbial pathogens and often referred to as ‘sentinels of the innate immune system’. TLRs are Pattern Recognition Receptors that detect microbial proteins; such as flagellin by TLR5. Following ligand binding, and dimerization, TLRs induce both, cell activation and the expression of pro-inflammatory cytokines. Their importance as strong Quantitative Trait Loci (QTL) candidates for disease resistance traits has also been reported (Jann *et al.*, 2009). Although these receptors are conserved across many species, it is clear that they vary in subtle ways both between species and also within species, indeed it has been reported that a single nucleotide polymorphism (SNP) can dramatically affect the function of a TLR (Brown *et al.*, 2010). Association studies indicate that a TLR5 SNP present in varying proportions between human populations, produces a premature stop codon that can for example increase susceptibility to legionnaire’s disease (Wlasiuk *et al.*, 2009; Hawn *et al.*, 2010). TLR5 is assumed to be under positive selection, this coupled with the potential impact that SNPs can have on the function of the protein, highlights the significance for investigating this gene further in other mammals. We identified polymorphisms in TLR5 cattle and sheep. We determined genetic variability and using a phylogenetic analysis we have compared the evolution of the TLR5 gene and identified regions of differential selection within mammals.

**Materials and methods** The entire coding sequence of TLR5 was PCR amplified in DNA samples from 110 individuals across 15 breeds of cattle and 87 individuals across 10 breeds of sheep. Sequence fragments were then assembled and SNPs identified using Staden Progam and MEGA4. The domain architecture was determined by LRR finder, SMART and TMHMM. This largely follows published predictions (Matsushima *et al.*, 2007). Tertiary structure predictions were generated using Swiss-Model.

**Results** A total of 65 SNPs were identified across cattle (40) and sheep (25) TLR5 in this study. In cattle, 11 SNPs have previously been reported, however, 29 are novel. In sheep, all 25 SNPs identified are novel. One sheep breed (Soay), shows no genetic variability. In cattle, there is a concentration of polymorphic variability within the flagellin binding region and a statistically significant enrichment of non-synonymous SNPs in this region (Fisher Exact P= 0.02). These include a selection of radical amino acid changes. Cattle breeds exhibit 4 polymorphisms producing a stop codon. As in human populations, cattle exhibit breed heterogeneity of these stop codons. In contrast, in the sheep breeds tested we did not detect mutations causing premature stop codons. Detected polymorphisms do not coincide in cattle and sheep homologous positions and distributions of sheep SNPs appears to be more stochastic.

**Conclusions** We have identified enrichment of SNPs within the flagellin binding region, and a number of stop codons, in cattle TLR5. These results indicate that a number of newly identified radical amino acid changes have the potential to affect the function of the protein. Functional studies are in progress to investigate their impact. The lack of polymorphisms seen in Soay breed supports the view that population crashes experienced by this breed have reduced genetic variability. Further comparison studies between cattle and sheep, along with other mammal datasets, will elucidate whether selective pressure is acting on TLR5 in general, whether it is specific to species or breeds as a consequence of geography. Determining genetic variability and the effects that polymorphisms may have on exposure to particular bacterial pathogens, within the livestock industry, could be of economic importance. The identified SNPs are good candidates for novel biomarkers allowing selection of more resistant livestock, and suggest that appropriate adjuvant ligands for vaccines need to be tailored to host species’ variation.

**Acknowledgements** This work was supported by a Genesis Faraday (now KTN Biosciences) facilitated project grant BB/D524040/1 jointly funded by Biotechnology and Biological Sciences Research Council (BBSRC), The Scottish Government Rural and Environment Research and Analysis Directorate (RERAD) and Pfizer Animal Health (PAH); BBSRC Institute Strategic Programme Grant funding, University of Nottingham School of Veterinary Medicine and Science.

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## Eliciting expert perceptions of the efficacy and practicality of pathogen control measures: *E. coli* O157 and human health

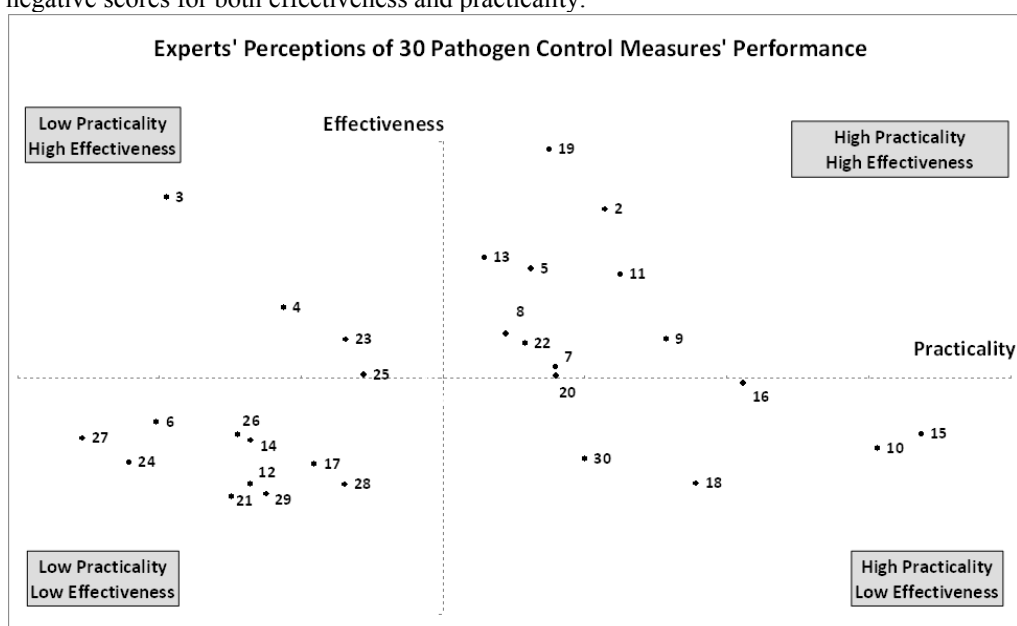
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**Introduction** In the absence of substantive empirical evidence policy makers require a future vision of how infectious zoonoses may develop and crucially, how science can effectively confront the new challenges that are contingent with novel pathogens (King *et al.*, 2006). Driven by the need to address new threats, scientists are often required to inform policy makers in the absence of such evidence. Uncertainty and partial evidence bases often prompt expert opinions to be elicited. In disease management settings there are many possible interventions, this makes it difficult to keep the elicitation process cognitively manageable. We address these issues using Best-Worst Scaling (BWS). BWS is designed to elicit scaled rankings over large sets via cognitively manageable tasks, avoiding the anomalies associated with conventional ranking methods. This paper reports the first use of BWS in veterinary and environmental epidemiology.

**Methods** Best-worst scaling is a multiple-choice based technique that requires respondents to make discriminating choices between a range of options (Auger *et al.*, 2007). In a typical study, respondents are presented with a set/subset of four or five items and asked to select their most preferred and least preferred choices. The views of 40 experts were elicited regarding the practicality and effectiveness of measures to reduce human exposure to *E. coli* O157. Bayesian analysis of the experts' ranking choices allows the measures to be located in practicality-effectiveness space.

**Results** Intervention 19 (*Vaccinate cattle to control pathogen colonisation and faecal excretion of E. coli* O157) was considered the most effective and intervention 21 the least (*Require In-house water troughs to be cleaned every day*). Intervention 1 (*Encourage Farmers and farm visitors to wash hands following contact with farm animals*) was considered the most practical and intervention 27 the least (*Reduce cattle stocking densities by 50%*). The most effective and practical interventions are those grouped in the upper right quadrant of Figure 1 whereas, those in the lower left quadrant had negative scores for both effectiveness and practicality.



**Figure 1** Bidirectional zero-centered scatter plot of expert opinion of the effectiveness and practicality of 30 interventions

**Conclusions** We find that the BWS technique aids in the management of uncertainty regarding pathogen control and identifies good/poor candidate measures for use and/or further research. We conclude that the technique is a potentially powerful tool in the communication and management of uncertainty between scientific and policy-based communities.

**Acknowledgements** The authors gratefully acknowledge funding from the Rural Economy and Land Use Programme (RELU)

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## Comparison of host pathogen interactions in two and three dimensional tissue culture systems: *Clostridium difficile* and porcine intestinal epithelial cells

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**Introduction** Bacterial infections remain major causes of human and animal disease and these may be exacerbated by interventions such as the use of antibiotic therapy. This is the case for many *Clostridium difficile* infections which affect aged hospitalized patients where reduction in the protective power of the gut flora by oral antibiotic administration may result in severe necrotic enteritis (Bartlett, 2002). In addition it is the cause of a severe form of neonatal porcine enteritis (Songer and Anderson, 2006). Three-dimensional cell culture systems are now available which facilitate considerable tissue differentiation (Choe *et al* (2006) and enable realistic interactions to be studied. Using the porcine intestinal epithelial cell line IPEC-J2 we compared host pathogen interaction with *Clostridium difficile* using cells cultured either in traditional flasks and 6-well plates (2D) or in gravity-free rotary culture (3D).

**Material and methods** IPEC-J2 cells were either cultured conventionally in tissue culture flasks or for 28-30 days in three-dimensional gravity-free rotary culture.  $10^6$  IPEC-J2 cells were infected with spores of *C. difficile* strains CD630 and R20291 for 24 h. Cell supernatants were collected for IL6 and IL-8 ELISA. To determine invasion levels of *C. difficile*, supernatants were removed and cells equilibrated in the anaerobic chamber for 1 h prior to lysis. Cells were fixed with 4% paraformaldehyde or 10% formalin prior to microscopy.

**Results** As expected there is clear difference in cell morphology depending on the culture method. IPEC-J2 cells cultured in 6-well plates have a 2D confluent monolayer consisting of undifferentiated cells. Cells grown on carrier beads in a micro gravity environment allow cells to form complex 3D structures promoting cellular differentiation. In response to infection with *C. difficile* spores, a proportion of cells started to detach from the carrier beads, while in control cultures no detachment was observed. Similar numbers of bacteria were isolated 24 h post infection from two-dimensionally and three-dimensionally cultured IPEC-J2 cells (Table 1). The release of the pro-inflammatory mediators IL6 and IL8 in both culture systems was compared and will be discussed.

**Table 1** Effect of culture conditions on *C. difficile* invasion

Strain	MOI	6-well plate (Log cfu/ $10^6$ cells) n=2	Rotary culture (Log cfu/ $10^6$ cells) n=3
CD630	0.2	4.85	5.03
	0.05	4.50	4.61
R20291	0.2	5.65	5.55
	0.05	4.27	4.76

**Conclusion** Rotary culture allows the development of multilayered organoids in contrast to a single layer in traditional culture vessels; however, these morphological differences did not affect the number of *C. difficile* invading the cells. Further experiments will show if there are differences in the host response to *C. difficile* infection (such as pro-inflammatory cytokine production) in the more differentiated 3D model compared to the traditional 2D model.

## Molecular insights into the innate immune response of bovine endometrial cells to the zoonotic abortifacient pathogen *Leptospira*

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**Introduction** *Leptospirosis*, caused by the abortifacient zoonotic pathogen *Leptospira*, is responsible for economic loss in the cattle industry and a serious public health issue, especially through urine-contaminated water. The role of the reproductive tract as a route of infection has been shown with experimental infection of cattle via the intrauterine routes but also in naturally infected cows. An important group of host receptors involved in bacterial recognition is the Toll-like receptor (TLR) family. Bacterial cell wall components such as lipopolysaccharide (LPS, endotoxin) or glycolipids are recognized by TLR receptors leading to innate immune and inflammatory responses. *Leptospira* signals through TLR2 (Werts *et al.*, 2001). The expression and function of TLR4 in *E. coli* infections has been characterized in bovine endometrial cells and hormones, such as oestradiol and progesterone, reduced the production of prostaglandins in response to LPS (Herath *et al.*, 2006). Here we report the host response of bovine caruncular epithelial cells to leptospiral infection with regards to TLR expression modulation and the impact on the production of pro-inflammatory mediator PGE<sub>2</sub>. The effect of pregnancy and stress on the cells was mimicked by pre-treatment with progesterone and cortisol.

**Material and methods** The bovine caruncular cell line BCECT1 was used to investigate the host response to *Leptospira interrogans* serotype Hardjo Bovis. Cells were pre-treated with progesterone (5ng/ml) and cortisol (5ng/ml) for 24 h prior to the addition of LPS (1ug/ml), *L. hardjo bovis* at MOI=1 or 10 and heat killed *L. hardjo bovis* at MOI=1 or 10 for 4h and 24 h. TLR2 and 4 mRNA expression was measured by quantitative PCR (qPCR) using dual-labelled probes,  $\beta$ -actin mRNA expression was used for normalisation. Prostaglandin E2 (PGE<sub>2</sub>) was quantified by competitive ELISA. Two-way ANOVA was used to investigate the effect of pre-treatment with progesterone and cortisol. Paired Student's T-test was used to compare responses of stimulation by LPS, live and heat-killed *L. hardjo bovis* with the PBS control. P-values of less than 0.05 were described as significant.

**Results** We demonstrated mRNA expression of TLR1-4, 6 and 9, oestrogen and progesterone receptor expression. While TLR2 mRNA expression was increased in response to lipopolysaccharide ( $p<0.001$ ), neither TLR2 nor TLR4 mRNA expression was modulated by *Leptospira* infection. Heat-killed and live *Leptospira* increased prostaglandin E2 release in a dose-dependent manner ( $p<0.05$ ). However, neither TLR expression nor prostaglandin production was affected by pre-stimulation with cortisol or progesterone, mimicking stress or pregnancy.

**Conclusion** The TLR repertoire expressed by BCEC cells, TLRs 1-4, 6 and 9, is similar to that of endometrial epithelial cells that were found to express TLRs 1-7 and 9 (Davies *et al.*, 2008) and, hence, BCEC cells are equipped to recognize bacteria. Infection with *Leptospira* or stimulation with heat killed *Leptospira* did not lead to a significant increase of TLR2 mRNA expression; TLR2 is the receptor recognizing leptospiral membrane proteins. Since *Leptospira* do not elicit an increased expression of its main innate immune receptor, it might be able to survive longer in the host.

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These results have been submitted to Veterinary Microbiology for publication.

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## Characterisation of a porcine small intestinal epithelial cell line (IPEC-J2), as a model to study host response to the probiotic *Lactobacillus rhamnosus*

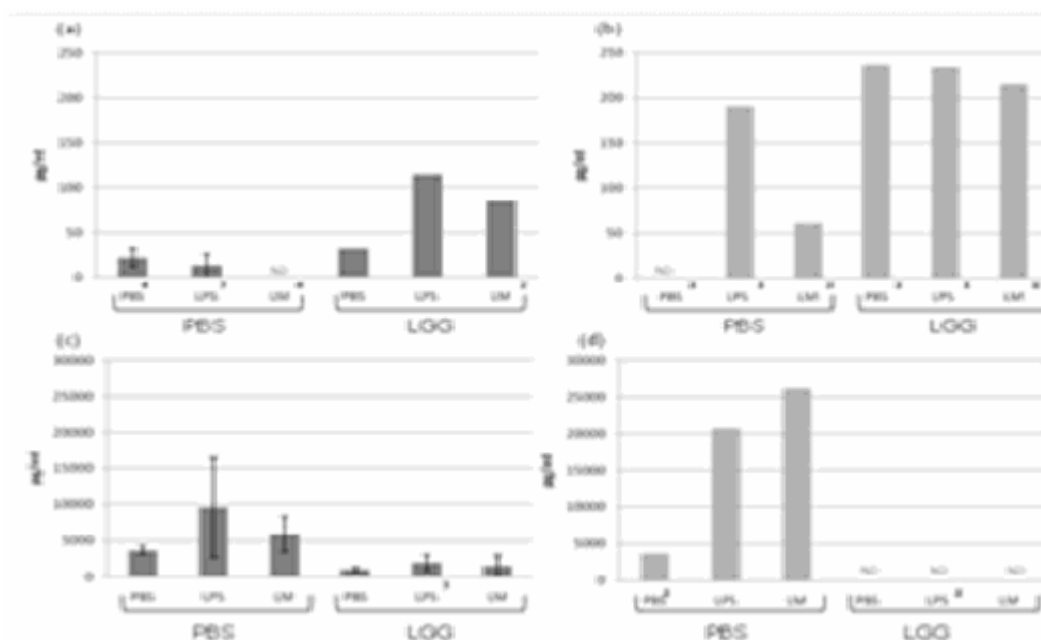
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**Introduction** The use of probiotics such as *Lactobacillus rhamnosus* (LGG), is of increasing interest with their potential benefit in the modulation of gut immune responses and the prevention of gastrointestinal infectious diseases. To reduce the number of animals used in research, good *in vitro* models are required to study host-probiotic-pathogen interactions. An important group of host receptors involved in bacterial recognition is the Toll-like receptor (TLR) family. Bacterial cell wall components such as lipopolysaccharide (LPS, endotoxin) or glycolipids are recognized by TLR receptors leading to innate immune and inflammatory responses. IPEC-J2 cells, a non-transformed intestinal porcine epithelial cell line that has been used to study host pathogen as well as host probiotics interactions. However, generally those studies are done under standard culture conditions with 5%CO<sub>2</sub> and atmospheric oxygen level of approximately 20%, although the oxygen level within the gut is much lower. In this study IPEC-J2 cells were cultured at both 20% and 5% O<sub>2</sub> levels. Toll-like receptor expression and the production of the proinflammatory cytokines IL-6 and IL-8 were investigated in response to culture conditions and the probiotics *L rhamnosus*.

**Methods** IPEC-J2 were cultured for 24h at 5% CO<sub>2</sub> prior to the addition of LGG; after an additional 24 h, cells were stimulated with Lipopolysaccharide (LPS, 1µg/ml) or heat killed *Listeria monocytogenes* (LM, MOI=10). Cell supernatants were collected and cells were lysed for RNA isolation 24h post stimulation. TLR2 and TLR4 mRNA expression was measured by quantitative PCR. IL-6 and IL-8 were quantified by ELISA. Cell morphology and confluency were assessed by microscopy.

**Results** Cell confluence decreased following culture at 5% oxygen and in response to subsequent LGG treatment. LGG pre-treatment increased IL-6 but decreased IL-8 production in response to LPS and LM. Initial results suggest that incubation at low oxygen, reduces the cell numbers but increases the release of IL-6 and IL-8 in response to LPS and LM, with LGG suppressing IL-8 release but increasing IL-6 release (Fig 1). TLR2 mRNA expression in IPEC-J2 is low with little variation due to LPS and LM stimulation and no effect by low oxygen levels.



**Figure 1** IL-6 Production by IPEC-J2 cells at (a) 20% O<sub>2</sub>, or (b) 5% O<sub>2</sub> for 72h and IL-8 Production at (c) 20% O<sub>2</sub> and (d) 5% O<sub>2</sub> for 72h, following pre-treatment for 24h with either Phosphate Buffer Solution (PBS) or *Lactobacillus rhamnosus* MOI 10 (LGG) and then stimulation with PBS, Lipopolysaccharide 1µg/ml (LPS) or host-killed *Listeria monocytogenes* (LM). Mean  $\pm$  Standard Error. <sup>1</sup>Number of replicates = 1. <sup>2</sup>n=2. <sup>3</sup>n=3. <sup>4</sup>n=4. ND = not detected, limit of detection 125pg/ml

**Conclusion** Presence of the probiotics LGG and reduced oxygen levels do not impact on TLR2 mRNA expression. Initial results suggest there is a marked difference in the effect of probiotics on the host response in the presence and absence of probiotics to bacterial ligands such as LPS and heat killed *L monocytogenes* if grown under reduced oxygen conditions. However the model needs to be optimized to prevent the large loss of viable cells due to the reduced oxygen environment.

## Apparent effects of post-natal infection with *Mycobacterium avium* on the susceptibility of sheep to copper toxicity one year later

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**Introduction** It is commonly assumed that lowered resistance to infection is an early consequence of trace element deprivation but evidence to support the assumption is scant (Suttle, 2010). Infections do affect trace element metabolism but there is again little evidence that this has clinical consequences in terms of the induction of trace element deficiencies or toxicities. During an experiment designed to develop specific diagnostic tests for Johne's disease, an uninfected lamb died from chronic (post-haemolytic) copper poisoning. A copper antidote, ammonium tetrathiomolybdate (ATTM) was given to all groups and the subsequent monitoring of treatment efficacy suggested that previous infection with *M. avium* lowered susceptibility to copper toxicity.

**Materials and methods** Of three groups of six cross-bred lambs, reared in a specific-pathogen-free (SPF) unit, one had been dosed orally and weekly for 10 weeks from birth with 5 ml saline (control group C) while the others were infected with saline containing  $5 \times 10^7$  colony-forming units  $l^{-1}$  of one of two subspecies of *M. avium*, *paratuberculosis* (Group *Map*) or *silvaticum* (Group *Mas*). Groups were reared separately and initially fed on condensed milk before moving to larger rooms after 27 weeks and weaning onto a complete, pelleted diet, containing 9.9 mg Cu  $kg^{-1}$  DM. In week 54, a Group C lamb died from chronic copper poisoning (plasma Cu 78.1  $\mu mol l^{-1}$ ; bilirubin 160  $\mu mol l^{-1}$ ). When blood tests on cohorts revealed 'raised' liver enzyme activities in all individuals, they were transferred to a hay diet, containing 3.7 mg Cu  $kg^{-1}$  DM, and treated conservatively with ATTM (1.7 mg  $kg^{-1}$  LW), given subcutaneously three times at three-day intervals. On the first day of injection (d 0), mean LW was  $49 \pm 7.3$  (s.d.) kg and did not differ significantly between groups. Sheep were blood sampled at irregular intervals (3-47 days) until d 111, frequency being highest during ATTM treatment. Plasma was conventionally retrieved, stored at  $4^{\circ}C$  and analysed within 1-2 days for a panel of five biochemical markers for liver disorder, including glutamate dehydrogenase (GDH) and bile acids (BA), using commercial assay kits C. Plasma Cu was determined by flame absorption spectrophotometry and was the only parameter that did not require  $\log_{10}$  transformation to give homogeneity to variances prior to statistical analysis by one-way ANOVA.

**Results** Initial copper status and 'liver health' of the sheep at 1 y.o. and the changes following intervention with the regimen of copper depletion are summarised at key stages in Table 1, different superscripts within rows indicating group differences ( $p < 0.05$ ).

**Table 1** Apparent effects of *M. avium* infections after birth on plasma copper (Cu) and two markers for liver disorder in sheep

Plasma marker	Day	Group			s.e. of means with n=6
		c(5)	Map (6)	Mas (6)	
Cu ( $\mu mol l^{-1}$ )	0	19.8a	15.2b	15.0b	0.86
	44	22.7a	15.9b	17.5b	1.67
	111	11.3	10.0	12.0	1.11
Log <sub>10</sub> BA ( $\mu mol l^{-1}$ )	0	2.037	1.579	1.841	0.121
	44	2.132a	1.440b	1.840ab	0.155
	111	1.699	1.743	1.652	0.102
GDH (IU $l^{-1}$ )	0	2.032	1.758	1.814	0.104
	44	2.237a	1.594b	1.649b	0.145
	111	1.510	1.386	1.418	0.49

On d 0 and d 44, Group C had higher mean plasma Cu than Groups *Map* and *Mas* and markers of liver disorder showed a similar ranking. On d 44, Group C had higher BA or GDH than infected groups and was showing no alleviation of liver injury. ATTM treatment was immediately repeated for all groups and by d 111, there were no differences between groups. However, 33% of infected lambs had become marginally hypocupraemic (plasma Cu  $< 9.4 \mu mol l^{-1}$ ) whereas uninfected lambs were all normocupraemic.

**Discussion** In SPF experiments, groups are housed in separate but identically serviced rooms to avoid cross-infection. In the absence of historical evidence of particular rooms causing copper poisoning, the higher severity of liver disorder and plasma Cu in uninfected sheep and their slower response to intervention suggests that chronic infection with *M. avium* had decreased liver copper burdens by either decreasing copper absorption and/or increasing endogenous copper loss. Increased requirements for copper after such infections are implied and natural infections may predispose to hypocupraemia.

**Conclusion** Post-natal infection with *M. avium* partially protected 1 y.o. sheep from copper toxicity.

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## Improving fatty acid profile in beef muscle

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**Introduction** Much emphasis has been placed on further improving the lipid profile of beef with much emphasis on n-3 PUFA. European Food Safety Authority (EFSA, 2009) have recently concluded on a level of long chain PUFA that a product must contain in order for it to be labelled as 'a source of' or 'high in' n-3 PUFA. Their conclusion was that it should be based around the requirement for 250mg per day of EPA plus DHA or 2g per day of  $\alpha$ -linolenic acid, and would require 40 or 80mg EPA plus DHA per 100g to be labelled as 'a source of' or 'high in' n-3 PUFA respectively. The objective of this paper is to summarise studies by our labs on manipulating fatty acid composition of beef, in relation to EFSA guidelines.

**Materials and methods** The fatty acid content and composition of retail beef was assessed in fifty beef sirloin steaks (Enser *et al.*, 1996). Subsequently, the effects of different sources of dietary lipids (i.e. megalac, linseed, fish oil and protected lipid; Scollan *et al.*, 2001; 2003), grass and white clover mixed swards (Scollan *et al.*, 2002), grass silage (Warren *et al.*, 2008) and lipid-rich plant extract (Kim *et al.*, 2010) were studied. Total saturated fatty acids (SFA) were calculated as sum of 14:0 + 16:0 + 18:0. Total ( $\Sigma$ ) PUFA = sum of all n-6 and n-3 PUFA. Total n-3 PUFA = 18:3n-3 + 20:4n-3 + 20:5n-3 + 22:5n-3 and 22:6n-3. P:S and n-6:n-3 were calculated according to Scollan *et al.* (2001).

**Results** All the beef analysed was lean with intramuscular fat ranging between 24 and 44 g/kg (see Table 1). Linseed relative to megalac not only doubled the levels of 18:3n-3 but also enhanced EPA. Fish oil doubled the proportion of EPA and DHA. The P:S was unchanged by feeding linseed or fish oil but n-6:n-3 ratio was markedly improved. Feeding ruminally protected lipid reduced total intramuscular fat and SFA and increased PUFA content. Grass silage and grass/white clover resulted in beef with high total PUFA, rich in n-3, contributing to a low n-6:n-3 ratio.

**Table 1** Effect of diet on the fatty acid composition of longissimus dorsi muscle (mg/100 g muscle)

	Total fat	18:2 n-6	18:3 n-3	EPA	DHA	$\Sigma$ SFA	$\Sigma$ PUFA	$\Sigma$ n-3 PUFA	P:S	n-6:n-3
Retail beef <sup>a</sup>	3835	89	26	10	1.6	1572	126	57	0.08	2.20
Megalac <sup>b</sup>	3359	78	20	10	2.5	1562	162	54	0.07	2.00
Linseed <sup>b</sup>	3618	69	38	15	2.7	1546	175	81	0.07	1.19
Fish oil <sup>b</sup>	4400	63	27	24	5.3	2089	187	80	0.05	0.91
Protected lipid <sup>c</sup>	2604	243	50	10	2.0	1064	361	77	0.27	3.59
Grass/white clover <sup>d</sup>	3411	94	66	27	3.6	1369	267	131	0.16	1.51
Grass silage <sup>e</sup>	3667	74	39	21	6.9	1501	210	97	0.08	1.20
Grass silage/plant extract <sup>f</sup>	2433	73	41	18	2.8	989	206	94	0.13	1.19

<sup>a</sup>Enser *et al.* (1996), <sup>b</sup>Scollan *et al.* (2001), <sup>c</sup>Scollan *et al.* (2003), <sup>d</sup>Scollan *et al.* (2002), <sup>e</sup>Warren *et al.* (2008) and <sup>f</sup>Kim *et al.* (2010)

**Conclusions** Retail beef contains significant quantities of n-3 PUFA and these may be further increased by feeding forage, concentrate-containing linseed or fish oil or lipid rich plant extracts. Diets containing only forage result in high PUFA meat. Ruminally protected lipids were very effective in increasing 18:2n-6 and 18:3n-3 but not long chain PUFA. It is evident that opportunities exist to deliver beef with higher P:S ratio and lower n-6:n-3 ratio in line with recommendations for the whole diet of humans described by World Health Organisation (2003). Based on the data and using 100 g/day as an appropriate figure for daily beef consumption then beef from the studies summarised may provide ~10-27 mg/d EPA and ~1.6-5.3 mg/d DHA. Hence the maximal levels of EPA + DHA delivered from beef from the studies reported would be ~31 mg/d. This is less than the ~15% of the daily recommended intake for LCPUFA (250 mg/d see above) for beef to be noted as a "source" of LCPUFA.

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## Carcasses of Belgian Blue culled cows and growing fattening bulls: 2 meat quality and fat and fatty acid composition in meat pieces

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**Introduction** Nowadays consumers attach great importance to tenderness and colour, two sensory traits of meat. In addition, attention is also paid to the dietetic aspects, particularly fat and fatty acids. Culled cows and young growing fattening bulls from the Belgian Blue breed represent a large proportion of beef meat in Belgium. The purpose of this study was to compare meat quality parameters in both types of carcasses. Furthermore, fat and fatty acid composition were also measured in beef meat cuts grouped according to culinary purposes.

**Materials and methods** A brief description of the management of the carcasses of the 32 culled cows and 20 growing fattening bulls is given in the companion paper. Meat quality was also measured on a sample of the Longissimus Thoracis on day 3 *post mortem*. For the 10 different meat classes, 3 major groups were constituted according to culinary purposes : fast cooking group with fillet, top loin and 1<sup>st</sup> class steaks, slow cooking group with dry heat roasts, moist heat roasts, 2<sup>nd</sup> class steaks while stew, boiled meat and minced meat were included in the third group. Fat and fatty acid contents were measured in 3 representative muscles of each group. The determination of the fatty acid profile in freeze-dried meat was performed using gas chromatography after extraction and trans-esterification to produce fatty acid methyl esters.

**Results** The meat of the cows was darker (lower L\*, P<0.001), redder (higher a\*, P<0.001) with a lower drip (3.6 vs 4.2%, P<0.01) but with higher cooking losses (32.5 vs 30.7%, P<0.05). There were no differences in tenderness (Table 1). The effects on the colour of the meat have to be related with the ages of the animals. On the whole, the fat content was low, the males being leaner than the females (Table 2). Further more, little differences were found between groups of cuts for the males while it was in the fast cooking pieces that the fat content was the lowest with the females (P<0.001). The differences observed for fat content between groups of cuts and sexes were similar to those found for the saturated fatty acids (SFA). The total n-6 fatty acid content ( $\Sigma$ n-6) was not influenced by the groups of cuts but was significantly higher with the males than with the females (P<0.001). By contrast, the cows were characterized by higher total n-3 fatty acid contents ( $\Sigma$ n-3) than the males (P<0.001) and it was with the fast cooking pieces that the  $\Sigma$ n-3 content was the highest. Finally, the C18:3 n-3 content was not affected either by the sex or by the group of cuts.

**Conclusion** The fat and fatty acid contents of meat cuts grouped according to culinary purposes did not vary in the male carcasses. With cows, the group of fast cooking cuts appeared to provide healthier meat owing to lower contents in fat and SFA and a higher n-3 fatty acid content than the 2 other meat groups. The meat of the cows was darker but not different in tenderness as compared with males. Regardless of these differences, carcasses of both sexes are of great value.

**Table 1** Meat quality characteristics measured at day 3 post mortem in Belgian Blue culled cows and fattening bulls offered a fattening diet based on sugar beet pulp

	Cows	Bulls	SEM	P>F
Meat colour L* (%)	38.4	43.9	0.27	***
Meat colour a* (%)	24.4	19.5	0.18	***
Drip (%)	3.6	4.2	0.07	**
Cooking losses (%)	32.5	30.7	0.27	*
Tenderness (N)	43.6	45.9	0.89	NS

NS: non significant; \* P<0,05; \*\* P<0,01; \*\*\* P<0,001

**Table 2** Weight, fat and fatty acid contents in meat pieces of culled cows and of growing fattening bulls grouped according to culinary purposes

	Fast cooking		Slow cooking		Minced		P>F			
	cows	bulls	cows	bulls	cows	bulls	SEM	Cuts	Sex	Inter
Weight (kg)	50.5	40.2	48.7	45.1	45.2	30.4	0.93	***	***	***
Fat (g/kg DM)	27.8	13.4	39.1	14.3	42.5	17.3	1.68	***	***	***
Fatty acids (mg/100g fresh meat)										
SFA	289.6	135.2	377.9	131.7	385.9	142.9	15.81	***	***	**
□n-6	125.0	156.6	129.2	155.7	126.0	151.4	2.35	NS	***	NS
□n-3	51.1	37.3	47.9	35.4	45.8	35.9	1.35	*	***	NS
C18:3 n-3	18.6	17.7	19.3	17.0	17.8	17.7	0.86	NS	NS	NS

NS: non significant; \* P<0,05; \*\* P<0,01; \*\*\* P<0,001

## Application of near infrared hyperspectral imaging to the prediction of meat quality

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**Introduction** The application of near infrared spectroscopy (NIR) to predict meat tenderness has been recently reviewed by Prieto *et al.* (2009). Hyperspectral imaging in the NIR region provides the potential to identify and measure spectra for specific regions of interest. and application to several food quality areas have been reviewed by Gowen *et al.* (2007). For beef, NIR hyperspectral imaging has the potential to identify independent spectra relating to subcutaneous fat, marbling fat and lean (Whitworth *et al.*, 2010). The aim of this study was to predict meat quality from the spectra obtained from the whole longissimus dorsi (LD) surface and also from the LD surface from which the contribution of marbling had been removed.

**Materials and methods** Foreribs and corresponding sirloins were obtained from a random selection of 50 bulls, 50 steers and 50 heifers on six separate slaughter days from a commercial abattoir at 2 days post slaughter.. A slice 25 mm thick was removed from the caudal surface of the forerib and an image of the exposed surface of the forerib taken within 2 minutes of cutting and again after allowing the meat surface to bloom for 1 hour at 4°C. Images were taken using an instrument described by Millar *et al.* (2008), as described by Whitworth *et al.* (2010). After NIR scanning, the LD muscle was removed from the forerib and two 25 mm thick slices were removed, vacuum packed and stored for 14 and 21 days at 4°C. After this time the Warner-Bratzler shear force (WBSF) values were measured (Keady *et al.* 2008). After aging for 14 days the sirloins were prepared for consumer evaluation as described by Farmer *et al.* (2009). The LD was identified in the NIR image and an average spectrum (total LD) which included contributions from marbling fat was calculated for each sample. The marbling fat was identified from the spectral features at 1674 to 1743 nm (Whitworth *et al.* 2010) and a new average for the lean portion of the LD which did not include pixels identified as fat was calculated (lean) for each sample. Models were developed to predict WBSF and eating quality of the meat using both the total LD and lean spectra using partial least squares regression after appropriate mathematical treatment of spectra (WINISI software, Foss NIR systems, Infrasoft International, LLC).

**Results** A number of bull carcasses with high ultimate pH values (pHu) were identified in the random sampling. Given the possible curvilinear relationship (Peachey, 2002), only carcasses which had pHu values of ≤5.8 were used for prediction purposes. The spectra obtained from the LD of a freshly cut surface and 1 hour after being allowed to bloom were not markedly different. Prediction models were developed for unbloomed, bloomed and combined spectra (bloomed and unbloomed). Table 1 shows that the regression coefficient ( $R^2$ ) for the prediction of shear force at 14 and 21 days ranged from 0.41 to 0.56 when the combined spectral data (unbloomed and bloomed) were used. The prediction for WBSF at 14 days was improved when the lean spectra (i.e. based on exclusion of marbling fat) were used ( $R^2=0.56$ ); however using the lean spectra made no difference to the prediction of WBSF at 21 days. The error in prediction represented around 15% of the mean WBSF value (5.1 kg force).

Prediction of eating quality at 14 days resulted in  $R^2$  values ranging from 0.44 to 0.51 (Table 1). There was little difference between the prediction of eating quality assessed by consumers obtained when the total LD spectra or lean only spectra were used for calibration. The error of prediction for consumer assessment of eating quality was around 15 to 18 % of the mean values (tenderness 50.5, juiciness 48.9, flavour 52.3 and overall liking 50.8).

**Conclusion** The results show that hyperspectral NIR imaging (900 to 2300 nm) has the potential to predict meat quality for carcasses with pH range up to 5.8. The effect of measurement time post-cutting had little effect on the ability to predict meat quality. Exclusion of the marbling fat from the spectra had little effect on prediction ability for WBSF and eating quality.

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## Application of near infrared hyperspectral imaging to the prediction of intramuscular fat and fatty acids in beef longissimus dorsi

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**Introduction** In a recent review Prieto *et al.*, (2009) concluded that near infrared reflectance (NIR) was much better at predicting compositional aspects of meat than quality aspects. Several workers have developed prediction models for fatty acids (FA) from the NIR spectra of meat (Sierra *et al.*, 2008). Monounsaturated FA (MUFA) increase with intramuscular fat (IMF) content (De Smet *et al.*, 2004) thus the ability to predict FA composition from NIR of lean meat may be a reflection of the relationship between FA composition and IMF content. Hyperspectral imaging in the NIR provides the potential to identify and measure spectra for specific regions of interest. For beef, NIR hyperspectral imaging has the potential to identify spectra relating to subcutaneous fat, marbling fat and lean (Whitworth *et al.*, 2010). The aim of this study was to predict major FA groups in the muscle from spectra obtained from the whole longissimus dorsi (LD) surface and also from spectra identified as marbling fat within the LD.

**Materials and methods** Foreribs were obtained from a random selection of 50 bulls, 50 steers and 50 heifers on six separate slaughter days from a commercial abattoir at 2 days post slaughter. A slice 25 mm thick was removed from the caudal surface of the forerib and an image of the exposed surface of the forerib taken within 2 minutes of cutting and again after allowing the meat surface to bloom for 1 hour at 4°C (Whitworth *et al.*, 2010). The LD was identified in the NIR image and an average spectrum (total LD), which included contributions from marbling fat, was calculated for each sample. The marbling fat was identified from spectral features at 1674 to 1743 nm (Whitworth *et al.* 2010) and average spectra for marbling fat calculated for each sample. The 25 mm slice removed from the caudal surface of the forerib was trimmed free of fat and the IMF content (g/100 g fat) and FA concentrations (mg/g muscle; g/100g fat) were measured as described by Dawson *et al.* (2010). Models were developed to predict the major FA groups (saturated FA (SFA); MUFA; polyunsaturated FA (PUFA); conjugated linoleic acid (CLA)) from the total LD and marbling spectra using multivariate statistical methods (Foss NIR, Infrasoft International, LLC).

**Results** Using the spectra from the total LD (includes lean and marbling fat) high  $R^2$  values were obtained for the prediction of IMF and MUFA (Table 1). Lower  $R^2$  values were obtained for the prediction of PUFA and CLA from the total LD spectra. Using the marbling fat spectra, the  $R^2$  values (Table 1) for the prediction of PUFA and CLA increased; however the prediction of SFA was very low ( $R^2=0.1$ ) compared to its prediction from the total LD spectra ( $R^2=0.68$ ). In samples of low marbling fat relatively few pixels were identified as marbling fat, leading to greater sensitivity to noise and misclassified pixels. To reduce these possible problems the prediction models were re-run using only those samples in which > 50 pixels were classified as marbling fat. This resulted in an increase in the  $R^2$  value for the prediction of SFA from the marbling fat ( $R^2=0.66$ ), with smaller increases in  $R^2$  values for the prediction of MUFA ( $R^2=0.44$ ) and CLA ( $R^2=0.62$ ) and a decrease in the  $R^2$  value for prediction of PUFA. The higher  $R^2$  obtained for the prediction of MUFA when the spectra of the total LD is used may reflect the ability to predict IMF and the relationship between IMF and MUFA. Using the marbling fat spectra permitted major FA groups to be predicted independently from IMF content.

**Table 1** Prediction of major FA groups from the total spectra of the LD and the spectra of marbling fat

Variable predicted	mean	Total LD		Variable predicted	$R^2$	SECV
		$R^2$	SECV			
% IMF (g/100 g fat)	2.98	0.73	0.71	NA		
SFA (mg/g muscle)	13.01	0.68	3.27	SFA (g/100 g fat)	0.1	1.92
MUFA (mg/g muscle)	11.46	0.75	2.86	MUFA (g/100 g fat)	0.39	2.12
PUFA (mg/g muscle)	1.49	0.38	0.34	PUFA (g/100 g fat)	0.63	1.39
CLA (mg/g muscle)	0.34	0.49	0.09	CLA (g/100 g fat)	0.54	0.33

SECV: standard error of cross validation. NA: not applicable.

**Conclusion** Hyperspectral NIR imaging (900 to 2300 nm) has the potential to predict IMF and the major FA groups from total LD spectra. Use of the marbling fat spectra improved the prediction of PUFA and CLA. Prediction models were improved when samples with very low marbling fat were excluded. Further work is required to determine the ability of hyperspectral imaging to predict individual FAs.

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## Contribution of ruminal protozoa to the duodenal flow of polyunsaturated fatty acids following feeding on low and high chloroplast-containing diets

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**Introduction** Rumen protozoa contain 75% of the total microbial lipids; 90% of which are unsaturated fatty acids (Devillard *et al.*, 2006), maybe due to engulfment of chloroplasts, which are rich in polyunsaturated fatty acids (PUFA) and in particular 18:3 n-3 (Huws *et al.*, 2008). We evaluated protozoal contribution to duodenal n-3 PUFA flow as a result of their intracellular chloroplast content.

**Materials and methods** The experiment consisted of 8 Holstein x Friesian steers following a two-period change-over design with two diets; straw : concentrate (low chloroplast, hence low 18:3 n-3) and fresh grass (high chloroplast, hence high 18:3 n-3). Following 14d diet adaptations duodenal fatty acid flow were monitored using chromium and ytterbium as markers. On the last day of diet adaptations protozoal samples were taken and purified by centrifugation ( $5 \times$  in Coleman's buffer). Duodenal samples were also taken at the same time. Fatty acid content of rumen protozoal samples were assessed (Huws *et al.*, 2008) and protozoal qPCR was performed (Huws *et al.*, 2008) on rumen protozoal and duodenal samples, enabling calculation of the ratio of protozoal DNA : individual fatty acid. These values could be used to calculate duodenal protozoal DNA : individual fatty acids because Denaturing Gradient Gel Electrophoresis based-dendograms revealed that ruminal and duodenal protozoal diversity were similar on each diet. Based on calculated total fatty acid flow data, contribution (%) of protozoa to individual fatty acid flows were calculated. Fatty acid and protozoal flow to the duodenum as well as fatty acid content of the protozoa on both diets were subjected to Analysis of Variance (ANOVA)

**Results** Protozoal fatty acid data coupled with microscopic observations revealed that protozoa were enriched with 18:3 n-3 due to an abundance of intracellular chloroplasts on the grass diet compared with the straw : concentrate diet. However, protozoal 18S rDNA based qPCR data for duodenal samples obtained post-grass feeding of steers were low indicating retention of the protozoa within the rumen on the grass diet. The relatively low abundance of protozoal 18S rDNA at the duodenum following perennial ryegrass feeding represented protozoal contribution to the flow of all fatty acids being exceptionally low on this diet (Table 1).

**Table 1** Duodenal and of protozoal flow of, long-chain fatty acids, biohydrogenation intermediates and protozoal fatty acid in steers offered straw/concentrate (S/C) or fresh Perennial Ryegrass (PRG)

	Duodenal flow (g/d)				Protozoal flow (g/d)				Contribution*	
	S/C	PRG	SED	P	S/C	PRG	SED	P	S/C	PRG
Fatty acid										
14:0	2.29	1.72	0.39	<0.01	0.02	0.00	0.00	<0.01	1.00	0.01
15:0	1.44	1.27	0.28	<0.01	0.03	0.00	0.00	<0.01	1.90	0.01
16:0	24.9	25.5	4.31	<0.01	0.55	0.00	0.04	<0.01	2.19	0.00
17:0	1.32	1.56	0.30	<0.01	0.01	0.00	0.00	<0.01	0.89	0.00
18:0	88.6	102	18.3	<0.01	0.73	0.00	0.00	<0.01	0.85	0.00
trans-11 18:1	5.20	24.0	4.37	<0.01	0.12	0.00	0.00	<0.01	2.33	0.00
18:2 n-6	10.2	2.21	0.62	<0.01	0.12	0.00	0.01	<0.01	1.14	0.00
18:3 n-3	1.66	3.06	0.44	<0.01	0.01	0.00	0.00	<0.01	0.69	0.01
cis-9, trans-11 CLA	0.10	0.08	0.04	<0.01	0.12	0.00	0.00	<0.01	19.6	0.00
trans-10, cis-12 CLA	0.00	0.06	0.02	<0.01	0.00	0.00	0.00	<0.01	0.00	0.00
Total fatty acids	173	196	29.4	<0.01	2.11	0.01	0.31	<0.01	1.23	0.00

\*% Protozoal contribution to individual fatty acids flowing to the duodenum comparative to total fatty acid flows. S/C – straw: concentrate diet, PRG – perennial ryegrass.

**Conclusions** Feeding fresh grass compared with straw : concentrate increased the 18:3 n-3 concentration of rumen protozoa as a consequence of chloroplast engulfment. Protozoal flow to the duodenum was minimal on the fresh grass diet resulting in little contribution of protozoa to duodenal PUFA flow. Conclusively we have the ability to increase the chloroplast content of protozoa, thus increasing 18:3 n-3 content. The challenge is to develop strategies to increase protozoal flow to the small intestine, whilst maintaining sustainable rumen densities, in order to increase the n-3 content PUFA availability to tissues..

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## Finishing lambs on chicory increases killing out percentage and carcass conformation score without detrimental effects on sensory meat eating quality

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**Introduction** Finishing lambs on chicory is increasingly popular as it may support higher growth rates than grass/clover, especially in the face of gastrointestinal nematode parasitism (Kidane *et al.*, 2010). However, impact on meat eating quality is largely unknown. We have reported that chicory grazing increased killing out (KO) percentage, without detrimental effects on meat eating quality but in fact with increased loin juiciness although in female lambs only (Houdijk *et al.*, 2010). However, castrated and female lambs were allocated to different taste panels, and lambs were not finished to commercial standards. Here, we have assessed the effect of sex and chicory grazing on KO percentage, carcass conformation score and sensory meat eating quality in commercially finished lambs.

**Materials and methods** Lambs grazed pure stands of chicory (CH, 18 castrates and 12 females) or grass/clover (GC, 16 castrates and 14 females) from birth to slaughter at 193 days of age. Body weight (BW) taken just before slaughter and carcass weights (CW) defined KO percentage as (CW/BW)\*100%. EUROP carcass grades taken were transformed into scores for analysis (E=5, U=4, R=3, O=2 and P=1 for conformation grades; 1=4, 2=8, 3L=11 and 3H=13 for fat grades). Carcasses were hip-suspended at 2° C for 24 h before posterior 20 cm of left loins were retrieved, matured at 2° C for another 9 days in vacuum bags and frozen pending sensory quality assessment at University of Bristol. Loins were thawed overnight, de-boned on the assessment day, cut in 8-10 2-cm thick samples and cooked until internal temperature reached 75° C. Samples were placed in an incubator (60 °C) prior to sampling by 10 qualified assessors, who were asked to rate 8 point category scales for tenderness, juiciness, lamb flavour intensity, abnormal lamb flavour intensity and two hedonic scales for flavour liking and overall liking. A 13 descriptor flavour profile, using unstructured 100 mm intensity scales, was also used (0: nil intensity; 100: extreme intensity). Lambs were allocated to different sub-panels, balanced for sex and forage type as much as possible. Reported results were derived from a 2 x 2 factorial ANOVA using REML.

**Results** CH and GC lambs weighed 46.9 and 45.1 kg, respectively (S.E.D. 0.53 kg; P<0.001) at slaughter, and their KO were 46.8 and 44.6%, respectively (S.E.D. 0.55%; P<0.001). Carcass fat scores were similar, averaging at 10.3±0.18, but CH had higher conformation scores than GC (3.8 vs 3.4; S.E.D. 0.12; P=0.003). Thus, CH and GC lambs had an average carcass grading of U3L and R3L, respectively. Sex did not affect BW, KO and carcass grades. Table 1 shows the sensory assessment results. CH loins had higher lamb flavour than GC loins. Forage and sex tended to interact for some traits; for female lambs only, CH loins had reduced grassy and fatty/greasy flavour (Table 1) but increased acidic flavour (data not shown). Castrated lamb loins scored lower for texture, abnormal flavour and kidney flavour but higher for sweet and soapy flavour than female lamb loins. Forage and sex did not affect loin hedonic flavour, overall liking and other flavour descriptors (livery, bitter, metallic, rancid, ammonia, fishy and dairy).

**Table 1** Effect of forage type and sex on sensory quality of loins from commercially finished lambs.

	Chicory		Grass/clover		S.E.D.	P-values		
	Castrates	Females	Castrates	Females		Forage	Sex	Forage x Sex
<b>8 point scale used</b>								
Texture	5.7	6.1	5.8	6.1	0.20	0.728	0.016	0.523
Juiciness	5.1	5.1	5.0	5.1	0.14	0.618	0.436	0.529
Lamb flavour	4.7	4.7	4.5	4.4	0.11	0.002	0.395	0.876
Abnormal flavour	2.3	2.4	2.2	2.5	0.11	0.487	0.019	0.287
<b>Hedonic</b>								
Flavour liking	5.3	5.2	5.2	5.1	0.12	0.434	0.112	0.692
Overall liking	5.2	5.1	5.2	5.1	0.12	0.959	0.217	0.663
<b>100 mm line scale used</b>								
Fatty/Greasy	12.2	9.7	11.1	11.1	1.12	0.899	0.112	0.095
Kidney	7.3	9.6	6.1	8.4	1.12	0.143	0.004	0.979
Sweet	14.0	12.6	13.6	12.3	1.14	0.545	0.099	0.545
Grassy	5.9	5.6	5.8	6.6	0.42	0.186	0.381	0.067
Soapy	4.0	3.4	3.9	3.8	0.33	0.706	0.080	0.311

**Conclusion** Finishing lambs on chicory resulted in heavier carcasses with better KO percentages and conformation scores, without detrimental effects on overall sensory meat eating quality. As such, they are in agreement with those reported earlier (Houdijk *et al.*, 2010) and contribute to a growing body of evidence of chicory as an alternative crop for finishing lambs.

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# Stearidonic acid biohydrogenation by the mixed rumen microbial population *in vitro*

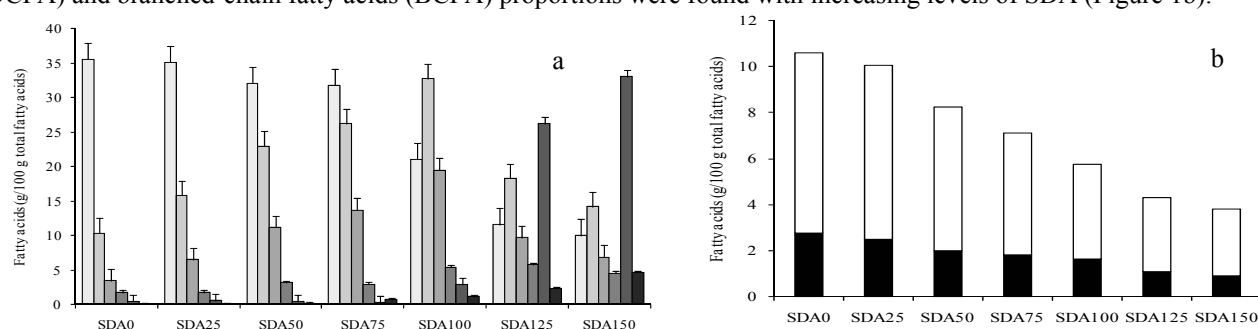
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**Introduction** Rumen hydrogenation of dietary unsaturated fatty acids is one of the key reasons why ruminant fats tend to be highly saturated and complex in nature. During this microbial process, unsaturated fatty acids become more saturated by the addition of hydrogen which also results in the formation of various intermediates. Most biohydrogenation studies focused on the main polyunsaturated fatty acids present in plants, linoleic (C18:2 *n*-6) and linolenic acid (C18:3 *n*-3) but information is lacking on the fate of the *n*-3 fatty acid with more unsaturations naturally occurring in plant, stearidonic acid (SDA; C18:4 *n*-3). The aim of the present study was to evaluate SDA biohydrogenation by the rumen microbial population *in vitro*.

**Material and methods** Stearidonic acid biohydrogenation was evaluated in *in vitro* batch incubations. A commercial total mixed ration (TMR) for dairy cows was used as substrate and increasing concentrations of SDA were added (0, 25, 50, 75, 100, 125 and 150 mg/ g dry matter TMR). Strained ruminal fluid obtained from three adult dairy cows at the slaughterhouse was diluted anaerobically in Goering and Van Soest (1970) medium, and the buffered ruminal fluid dispensed anaerobically into Hungate tubes containing the dietary treatments. Tubes were incubated in triplicate at 39°C for 72 h. Lipids were extracted from whole freeze-dried fermentation media (Folch *et al.*, 1957) and fatty acid methyl esters prepared by the combined transesterification method (Raes *et al.*, 2001) to prevent isomerisation of conjugated fatty acids. Fatty acid methyl esters were analyzed using an HP6890A gas chromatograph equipped with a flame-ionization detector and a fused silica capillary column (CP-Sil 88, 100 m × 0.25 mm × 0.20 µm), and helium as the carrier gas. Peak identification was based on chromatography with known standards. Fatty acids results were analysed using the GLM procedure (SAS Institute, Inc., Cary, NC, USA.). The model included the fixed effect of treatment and residual error.

**Results** After 72 h incubations, SDA was only detected at SDA50 level or greater (data not shown). Additionally, a complex mixture of C18 fatty acids, including several C18:4, C18:3, C18:2 and C18:1 isomers, were found (Figure 1a) particularly at higher addition levels, clearly suggesting extensive biohydrogenation of SDA *in vitro*. The decrease of the end-product (C18:0) proportion and the increase of the putative intermediates with increasing SDA levels, suggests inhibition of the biohydrogenation activity, being more marked at SDA100 level and greater. Lower odd-chain fatty acids (OCFA) and branched-chain fatty acids (BCFA) proportions were found with increasing levels of SDA (Figure 1b).



**Figure 1** Fatty acids profile of whole fermentation media following increasing supplementation levels of stearidonic acid to a basal TMR-diet. **a)** Major C18 fatty acid profiles. In order of increasing shading density: C18:0, total C18:1 fatty acids, C18:1 *trans*-11, total C18:2 fatty acids, total C18:3 fatty acids, total C18:4 fatty acids. **b)** Total BCFA (□) and OCFA (■) profile.

**Conclusions** Rumen microorganisms have the ability to biohydrogenate SDA. *In vitro*, SDA hydrogenation promoted an increase in fatty acid intermediates without increasing the final end-product. Inhibition of the biohydrogenation might be explained by a possible toxic effect on the rumen microbial population at higher SDA addition levels. Indeed, changes in OCFA and BCFA, mainly of bacterial origin, may reflect modifications in the relative concentration or metabolic activity of a group of bacteria. Overall results suggest a possible toxic effect of SDA on the rumen microbial population at higher addition levels, which may explain the biohydrogenation inhibition.

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## Comparison of wheat- versus corn-based dried distillers' grains with solubles on muscle fatty acid composition of feedlot cattle

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**Introduction** Production of biofuels in Canada and USA as a renewable energy source has increased creating opportunities for substituting barley with lower cost dried distillers' grains plus solubles (DDGS) in cattle finishing diets. Ethanol production removes starch from grain and increases crude protein, fat and fibre levels. Fatty acid (FA) composition of beef is heavily influenced by rumen microflora. Bacteria isomerize and hydrogenate dietary polyunsaturated FAs (PUFA) and metabolites can accumulate. The profile of *trans*-18:1 depends mainly on diet, and high levels of rapidly fermentable carbohydrates produce increased 10*t*-18:1 and lower rumen pH, while forage has been shown to increase 11*t*-18:1 under neutral pH. DDGS has increased PUFA available for biohydrogenation and increased level of crude fibre that could possibly foster a more neutral rumen pH favouring 11*t*-18:1 production. The objective of this study was to determine the effect of replacing a barley-based control diet with corn (C) or wheat (W) DDGS at 20% and 40% of DM, on the muscle FA composition with special emphasis on the *trans*-18:1 isomeric profile.

**Material and methods** This trial involved 100 crossbred steers and 5 feed treatments (20 steers/treatment). Diets included: control (86.6% rolled barley, 7.7% barley silage in DM), 20 and 40% W DDGS and 20 and 40% C DDGS substituted for barley in DM. Animals were transported (~6h) and held in lairage overnight with free access to water and slaughtered the next day at a target end-point of 645kg live weight. After 24h, the left *longissimus thoracis* was removed and the first steak from the posterior end was used for FA analysis. Lipids were extracted using a mixture of chloroform-methanol (1:1, v/v) and methylated separately using acidic (methanolic HCl) and basic (sodium methoxide) reactions. FAMES were analyzed using GC and Ag<sup>+</sup>-HPLC (Kramer *et al.*, 2008). Data were analyzed as a one-way ANOVA including diet as the main effect and kill day and pen as random variables using PROC MIXED of SAS (2001). A W vs C pre-planned comparison was conducted.

**Results** Replacing barley with DDGS had no effect on total saturated (SFA), branched-chain (BCFA), *c/t*-diene and conjugated linoleic acid (CLA) contents of muscle (Table 1). *Cis*-monounsaturates (MUFA) were highest in control animals ( $P<0.01$ ) while *trans*-MUFA were highest in C DDGS fed animals compared to animals fed the other diets ( $P<0.001$ ). Within *trans*-MUFA, beef from C DDGS fed animals showed higher total and individual *trans*-18:1 contents, and highest levels were found in 40% C DDGS fed animals ( $P<0.001$ ). Even though there were not significant differences in the 11*t*-/10*t*-18:1 ratio between treatments, the ratio was higher in beef from steers fed W DDGS due to the lower levels of 10*t*-18:1 compared to C fed steers. Levels of total PUFA were in agreement with levels of DDGS inclusion in the diet. Animals fed DDGS diets had significantly higher PUFA (10.2%;  $P<0.001$ ) than control animals (6.9%). Similar differences were also observed for n-6 content, but not for n-3 PUFA. The n-6/n-3 and P/S ratios reflected the differences reported in the PUFA content.

**Table 1** Fatty acid composition (groups and ratios) of muscle from steers fed different diets

Fatty acids	Control	20% W	40% W	20% C	40% C	s.e.m.	P value	W vs C
SFA	41.5	41.6	41.3	42.0	40.7	0.704	0.629	0.933
<i>cis</i> -MUFA	44.8 <sup>a</sup>	42.9 <sup>ab</sup>	41.4 <sup>b</sup>	41.3 <sup>b</sup>	38.8 <sup>c</sup>	0.916	0.001	0.021
<i>trans</i> -MUFA	3.53 <sup>bc</sup>	3.44 <sup>c</sup>	3.37 <sup>c</sup>	4.23 <sup>b</sup>	5.30 <sup>a</sup>	0.253	<0.001	<0.001
10 <i>t</i> -18:1	1.45 <sup>bc</sup>	1.21 <sup>c</sup>	1.08 <sup>c</sup>	1.77 <sup>b</sup>	2.37 <sup>a</sup>	0.165	<0.001	<0.001
11 <i>t</i> -18:1	0.58 <sup>b</sup>	0.63 <sup>b</sup>	0.66 <sup>b</sup>	0.68 <sup>b</sup>	0.83 <sup>a</sup>	0.034	<0.001	0.004
11 <i>t</i> -/10 <i>t</i> -18:1	0.63	0.59	0.70	0.43	0.39	0.092	0.070	0.010
PUFA	6.87 <sup>d</sup>	9.04 <sup>c</sup>	10.8 <sup>ab</sup>	9.22 <sup>bc</sup>	11.6 <sup>a</sup>	0.538	<0.001	0.353
n-6	5.73 <sup>d</sup>	7.95 <sup>c</sup>	9.61 <sup>ab</sup>	8.23 <sup>bc</sup>	10.6 <sup>a</sup>	0.492	<0.001	0.210
n-3	1.15	1.09	1.17	1.00	1.02	0.064	0.278	0.082
CLA	0.56	0.57	0.57	0.64	0.66	0.041	0.220	0.041
<i>c/t</i> -dienes	0.83	0.79	0.84	0.86	0.85	0.030	0.461	0.170
n-6/n-3	4.92 <sup>c</sup>	7.30 <sup>b</sup>	8.30 <sup>b</sup>	8.34 <sup>b</sup>	10.4 <sup>a</sup>	0.389	<0.001	<0.001
P/S	0.17 <sup>c</sup>	0.22 <sup>b</sup>	0.26 <sup>a</sup>	0.22 <sup>b</sup>	0.29 <sup>a</sup>	0.015	<0.001	0.368

**Conclusions** Muscle levels of total SFA, BCFA, CLA and dienes were unaffected by substituting barley with C or W DDGS. *Cis*-MUFA were higher in control samples while PUFA were higher in DDGS samples. Overall, beef from control and W DDGS fed animals had lower *trans*-18:1 content and consequently lower levels of individual *trans*-18:1 isomers and an improved (higher) 11*t*-/10*t*-ratio compared to beef from C DDGS fed animals.

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## Physical qualities, proximate chemical composition and fatty acid profiles of three freshwater fish species harvested from upstream and downstream locations

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**Introduction** Fish can supply high quality protein and fatty acids into animal and human diets. Fish may bridge the nutrient deficit for the growing human populations which cannot meet their nutrient needs from livestock derived foods alone. It may help if fish are farmed on the basis of their growth and nutritional potentials. This study compared some selected profiles of three freshwater fish (*Cyprinus* or *C. carpio*, *Labeo* or *L. rohita* and *Oreochromis* or *O. mossambicus*) which are frequently harvested from the Indus River in Mianwali district of Pakistan. These fish were selected for their productivity, resistance to harsh environments, taste and economic importance for this area.

**Materials and Methods** A 3 x 2 factorial design was used to compare 3 fish species (*C. carpio*, *L. rohita* and *O. mossambicus*) from 2 locations (SK=upstream and CH=downstream) of Indus River in Mianwali for their physical, chemical and fatty acid profiles. The fish were collected in cooperation with local fishermen by involving twenty seven fish of each species of similar sizes comprising nine fish per net as replicates per location on ice before recording their wet weight (WW), length, and width. Each fish was dissected to collect muscles and fat which were freeze dried to determine their moisture, crude protein (CP) and fatty acid (Sukhija and Palmquist, 1988) profiles. Individual fatty acids were quantified by comparing their peaks with the peak areas of the corresponding fatty acid standards and expressed as % of total fatty acids (TFA). The fatty acids were then grouped as saturated (SFA), mono-unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. The data were statistically analysed by Minitab to compare the effects of fish species (S), location (L) and S x L interaction on different physical, chemical and fatty acid profiles at P<0.05 (\*), P<0.01 (\*\*) and P<0.001 (\*\*\*). Tukey's test was used to compare means by using relevant standard errors of means (SEM) at P<0.05.

**Results** Table 1 shows significant differences between fish species for their length, width and weight (P<0.01) but not for location or L x S interaction (P>0.05). Fish muscles did not differ for location, species, and the L x S interaction (P>0.05). While *C. carpio* and

**Table 1** Physical, chemical and fatty acid parameters of three fish species from two locations (CH and SK) of Indus River

Parameters	C. carpio		L. rohita		O. mossambicus		SEM and Significance		
	CH	SK	CH	SK	CH	SK	Location	Species	LxS
Length, cm	34.9	33.4	41.5	35.6	19.8	20.2	0.74	0.91**	1.28
Width, cm	11.1	11.4	11.9	10.3	7.9	7.6	0.20	0.25**	0.35
Wet Weight (WW), g	633	568	900	600	207	143	103	150**	212.7
Muscles, % WW	65	63	65	61	60	61	1.13	1.4	1.95
Moisture, % WW	78	79	80	78	75	76	0.33	0.4**	0.6*
Crude Protein, % DM	46	54	50	57	50	40	1.2	1.21**	2.1**
Fat, % DM	10	14	9.9	12.6	14.1	18.8	0.64***	0.8***	1.1
SFA, % TFA	59.1	52.3	48.6	52.5	65.7	60.3	3.9	3.5*	4.9
MUFA, % TFA	31.3	34.5	28.3	26.2	22.0	27.7	2.6	2.8	3.7
PUFA, % TFA	9.6	13.2	23.1	21.3	12.3	12.0	1.7	2.2*	2.8

*L. rohita* had comparable muscle moisture content, they differed significantly from *O. mossambicus* (P<0.001). The fat contents differed significantly between locations and species (P<0.01). Here *L. rohita* was highest in crude protein but lowest in fat whereas, *O. mossambicus* was lowest in crude protein but highest in fat (P<0.05). *O. mossambicus*, *C. carpio* and *L. rohita* were high in saturated, mono-unsaturated and poly unsaturated fatty acids, respectively. In addition, Palmitic acid (not in Table 1) was the major fatty acid in fish species ranging from 32 to 46% of TFA. While these fish had variable amounts of PUFA such as docosahexaenoic, eicosapentaenoic and arachidonic acids, *L. rohita* had more PUFA and protein and thus may be more attractive to the fish producers and consumers.

**Conclusion** The high, though variable, levels of fatty acids such as PUFA suggested that these fish especially *L. rohita* could be used as part of a healthy diet in addition to livestock derived foods for humans. These findings may benefit the fishing industry, nutritionists and researchers who are striving to improve the nutritive value, processing and marketing of selected fish species for their optimum utilization in different regions of global importance. The data of this study may help fish producers to promote farming on the basis of more desirable traits of these fish.

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## Meat quality of bulls, heifers and steers sampled at a commercial abattoir

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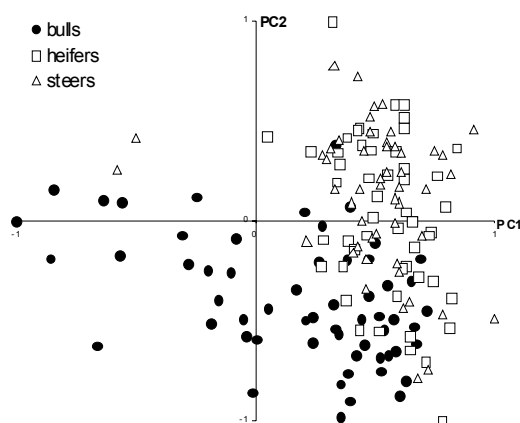
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**Introduction** Kirkland *et al.* (2005) found no significant differences in Warner Bratzer shear force (WBSF) of longissimus dorsi (LD) from bulls compared to steers at 450 kg slaughter weight, however the acceptability of tenderness of LD of bulls was lower than steers at this weight (Moss *et al.*, 2005). In a commercial sample, Moss *et al.* (2009) found significantly lower slice shear force at 2 days post slaughter for LD of steers compared to bulls. Fatty acid (FA) profiles are related to a number of factors including intramuscular fat (IMF) content and diet (Wood *et al.*, 2008). The aim of the current study was to assess the meat quality of bulls, heifers and steers selected at random at a commercial abattoir.

**Materials and methods** Foreribs and corresponding sirloins were obtained from a random selection of 50 bulls, 50 steers and 50 heifers on six separate slaughter days from a commercial abattoir. The joints were obtained at 2 days post slaughter and a slice 25 mm thick was removed from the caudal surface of the forerib for IMF and FA analysis (Dawson *et al.*, 2010). Two more 25 mm thick slices were removed, vacuum packed and stored for either 14 or 21 days at 2°C prior to Warner-Bratzler shear force (WBSF) measurements being made. After aging for 14 days the sirloins were prepared for consumer evaluation as described by Farmer *et al.* (2009). Analysis of variance was used to determine the effects of sex type on meat quality and principal component analysis (PCA) used to study the effect of sex type on FA profiles.

**Results** The incidence of carcasses with pHu > 5.8 was higher in bulls (18%) than steers (4%) or heifers (2%). Given the unsuitability of high pHu carcasses for vacuum packing and exclusion of high pHu from a number of meat quality systems (e.g. Meat and Livestock Australia), statistical analysis was undertaken only on those carcasses with pHu < 5.8. Bulls sampled were younger with significantly ( $P < 0.05$ ) greater hot standard carcass weight (HSCW) than heifers or steers (Table 1), with HSCW of steers significantly ( $P < 0.05$ ) higher than heifers. Even when carcasses with pHu > 5.8 were excluded, the pHu of bulls was significantly ( $P < 0.05$ ) higher than steers or heifers. WBSF values of bulls at day 14 were significantly ( $P < 0.05$ ) higher than steers or heifers, however there was no significant effect of sex type on cooking loss at day 14. Bulls had significantly lower ( $P < 0.05$ ) tenderness, juiciness, flavour and overall liking than steers and heifers.



PCA scores of FA profiles for bulls (fig 1) were located mainly in the lower half (PC2 scores < 0) of the plot, with steers and heifers mainly in the right hand side (PC1 > 0). Those carcasses positioned in the lower left quadrant were associated with a number of FAs (C18:1n-7; C20:4n-6; C18:1n-9) which would be expected to be higher in cattle finished on concentrates rather than grass.

ns  $P > 0.05$ ; \*  $P < 0.05$ ; \*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

**Figure 1** PCA scores plot for fatty acid profile (mg/g muscle)

**Conclusion** In the weight ranges and age represented in this commercial study, the meat quality of bulls was poorer than that of steers and heifers. The FA profile of bulls was different to that of steers and heifers, and may reflect different dietary conditions. Further work is required to elucidate the reasons for these sex type differences in FA profile and meat quality.

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## Meat quality characteristics from lambs and sheep produced in the mountainous and the semi-mountainous area in North Greece

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**Introduction** Sustainable animal production in otherwise marginal areas is promoted by the European Union (Cifuni *et al.*, 2000). Meat from small ruminants produced in such systems is an important economic activity in the rural areas of North Greece. Animal production is based on natural rangelands. The animals belong to local breeds and are either moved short distances in the mountainous and/or the semi-mountainous areas for grazing or they stay in the local villages and they graze in the nearby areas (Zervas, 1998). When grazing is not continuous animals receive a concentrate supplement. Changes in the soil properties and the climatic conditions that lead to diverse vegetation and to subsequent differences in the chemical composition and the nutritional value of the ingested pasture affect both animal performance and product quality. This work aimed to define quality characteristics of lamb and sheep meat from animals produced in the mountain and the semi mountainous area of the region of West Macedonia in Greece.

**Materials and methods** *M. longissimus lumborum* samples from 12 lambs and 12 sheep were taken from a local abattoir in the period from the beginning of May to the beginning of July. The samples came from animals reared in the mountainous and semi-mountainous area of West Macedonia (region of Grevena; latitude 40.08°, longitude 21.25°, average altitude 700 m) according to their slaughter certificates. Samples were air packed and stored at 4°C for 4 days to simulate retail conditions in traditional butcher shops in Greece. Lean meat colour was measured using CIELAB L\*a\*b\* colour space. The oxidative stability was determined as thiobarbituric acid reacting substances (TBA value). The fatty acid composition of the same muscle was also determined. Analysis of variance (SPSS version 13.0, 2004) was used to analyse differences between treatments and within the same treatment.

**Results** Colour reported as redness did not change during storage in lamb meat (NS, s.e.d. 1.082) whereas there was a significant change in sheep meat ( $P < 0.01$ , s.e.d. 0.927). Lipid oxidation was greater in lambs but there were no changes in the TBA value within the same animal type (NS, s.e.d. 0.291 for lambs and NS, s.e.d. 0.044 for sheep) during storage. The nutritional quality of the meat from both types of animals was good and in general agreement to the guidelines of the Department of Health (1994) regarding fat consumption. The atherogenicity and thrombogenicity indices of both types of meat can be considered as low and in compliance with the recommendations of Ulbricht and Southgate (1991).

**Table 1** Shelf life parameters and nutritional indices of *m. longissimus lumborum*

Shelf life parameters	Lamb	Sheep	Significance	s.e.d.
Redness (a*) Storage Day 2	18.15	23.39	***	1.070
Redness (a*) Storage Day 4	18.70	20.67	NS	1.022
TBA value Storage Day 2 (mg/kg muscle)	0.692	0.391	*	0.117
TBA value Storage Day 4 (mg/kg muscle)	1.139	0.475	**	0.221
Nutritional indices				
Total fatty acids (mg/100 g fatty acids)	1133	2049	NS	530.95
CLA (cis-9, trans-11) <sup>1</sup> (mg/100 g fatty acids)	1.65	1.32	NS	0.946
PUFA:SFA	0.15	0.14	NS	0.02
$\sum n-6 : \sum n-3$	4.01	5.73	NS	1.39
Atherogenic Index (AI) <sup>2</sup>	1.2	0.8	*	0.14
Thrombogenic Index (TI) <sup>3</sup>	2.4	2.1	NS	0.39

<sup>1</sup> Conjugated linoleic acid; <sup>2</sup> AI = (C12:0 + 4×C14:0 + C16:0)/[( $\sum$  MUFA +  $\sum$  PUFA (n-6) and (n-3)];

<sup>3</sup> TI = (C14:0 + C16:0 + C18:0)/[(0.5× $\sum$  MUFA + 0.5× $\sum$  PUFA (n-6) + 3× $\sum$  PUFA (n-3) + (n-3)/(n-6)]

NS not significant; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

**Conclusions** The results suggest that the lamb and the sheep meat produced in the mountainous and the semi-mountainous area of North Greece has good quality characteristics. Samples are quite variable and therefore a study with a greater number of animals has been planned. Currently work is carried out to study the effect of season (winter) on meat quality from animals produced in the same region.

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