

The effects of dietary nitrate addition and increased lipid concentration on sensory attributes of loin steaks from beef cattle

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Application Inclusion of nitrate or increasing the dietary lipid content in the diet of finishing beef cattle, with the primary aim to mitigate methane emissions, does not adversely affect sensory quality of meat. Thus, these strategies can be recommended for methane mitigation provided its use is economically competitive.

Introduction Incorporating nitrate into the diet and increasing the level of dietary lipid have been shown to be effective at reducing methane from beef cattle without adversely impacting on performance (Troy *et al.*, 2015; Duthie *et al.*, 2016). It is also important that any recommended strategy does not negatively affect product quality. The objective of this study was to investigate the effect of these key nutritional strategies on the sensory quality of meat from finishing beef steers.

Material and methods The experiment was a 2x2x3 factorial design comprising 2 breed types (CHx, Charolais cross; and LUI, Luining); 2 basal diets consisting of (g/kg DM, forage:concentrate) 520:480 (Mixed) or 84:916 (Concentrate); and 3 treatments: (i) control with rapeseed meal as the protein source, (ii) rapeseed meal replaced with nitrate (18 g nitrate/kg DM) or (iii) rapeseed cake (increasing the oil from 25 to 48 g/kg DM). Steers (n=84) were group-housed in even numbers of each breed across 6 pens; each diet x treatment was allocated to 1 pen. Steers were slaughtered in a commercial abattoir in 4 batches on trial days 99, 120, 141 and 162, respectively. At 48 hours *post-mortem*, samples from the loin eye muscle were obtained, chilled and aged at 0 to 2°C to 10 days post slaughter. Sensory analysis was carried out by a 10-person trained taste panel. Steaks were grilled to an internal temperature of 74 °C, after which, all fat and connective tissue were trimmed and the muscle was cut into blocks of 2 cm³. The blocks were wrapped in pre-labelled foil, placed in a heated incubator and then given to the assessors in random order chosen by a random number generator. Assessors are asked to rate the samples on eight point category scales for texture, juiciness, flavour intensity, and abnormal flavour intensity. Two additional hedonic questions relating to flavour liking and overall liking were also used. Data was analysed using ANOVA including diet, treatment and breed as factors (IBM SPSS v21).

Results There was no effect of treatment on sensory attributes of loin muscle. The LUI breed produced meat which was more tender, juicy and of higher flavour than meat from the CHx steers. Consequently the flavour of the LUI meat was preferred and they were more liked overall. The Mixed-fed animals were less tender than the Concentrate-fed animals and although the flavour was not significantly different, the panel preferred the flavour of the Mixed-fed animals and liked them best overall. Therefore, flavour out-weighed tenderness, or they were both sufficiently tender that flavour predominated.

Table 1 Effect of basal diet, treatment and breed on sensory attributes of steaks from loin eye muscle

	Basal Diet		Treatment			Breed		Significance		
	Mixed	Conc	Control	Nitrate	Lipid	CHx	LUI	Diet	Treatment	Breed
Tenderness	5.36	5.66	5.52	5.41	5.60	5.10	5.92	***	NS	***
Juiciness	5.35	5.29	5.31	5.27	5.38	5.19	5.45	NS	NS	***
Beef Flavour	4.50	4.42	4.44	4.44	4.49	4.36	4.56	NS	NS	***
Abnormal flavour	2.14	2.28	2.17	2.20	2.26	2.34	2.08	*	NS	***
Hedonic										
Flavour liking	5.57	5.38	5.48	5.49	5.46	5.22	5.73	**	NS	***
Overall liking	5.40	5.26	5.36	5.31	5.32	5.00	5.65	*	NS	**

* <0.05 , ** <0.01 , *** <0.001 , NS = not significant ($P>0.05$). No significant interaction effects ($P>0.05$).

Conclusion This experiment is part of a larger study in which supplementing nitrate or increasing the level of dietary lipid have been shown to be effective at reducing methane emissions from beef cattle without negatively affecting performance. This experiment demonstrates that these strategies also have no adverse effects on sensory attributes of meat.

Acknowledgements The authors gratefully acknowledge funding from AHDB Beef & Lamb, the Scottish Government and by DEFRA and the devolved administrations through the UK Agricultural Greenhouse Gas Inventory Research Platform.

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Alternative growth path finishing systems and their effects on animal performance in Limousin cross (LIMx) steers and heifers

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Application Beef producers should be encouraged to adopt efficient, short duration finishing systems that deliver high growth rates and quality carcasses with greater profit potential compared to longer duration finishing systems of production

Introduction A wide variety of finishing systems and growth paths (GP) are employed in UK beef production. Objectives of this study were to compare short, medium and long duration GPs with regard to animal performance and potential profitability when comparable animals from common sires were managed to slaughter at a wide range of ages.

Material and methods A 3 x 2 factorial continuous design experiment examining three alternate GP and using 36 LIMx steers (S) and 36 LIMx heifers (H) was undertaken to compare short, medium and long duration GPs. The short GP animals were finished indoors on an intensive concentrate based system and slaughtered at 12-16 months of age. The medium GP animals were turned out to graze a high quality reseed from 12-17 months of age and then finished indoors during the subsequent winter period when offered a mixed forage:concentrate (F:C) finishing diet and slaughtered at 18-24 months of age. Long GP animals were grazed for two summers on poor quality grassland, stored on a forage based diet for the intervening winter and finished during their final winter, again on a mixed F:C diet to slaughter at 28-36 months of age.

Results Days on trial (DOT), average daily liveweight gain (DLWG), age at slaughter (AGE), slaughter LW (SLW), cold carcass weight (CCW), killing out proportion (KO), fat score (FAT), and conformation score (CONF) are given in Table 1 whilst the economic parameter "Feeders Margin" is shown graphically (Figure 1) in relation to average GP system daily costs. GP system significantly increased ($P < 0.001$) DOT and AGE but decreased ($P < 0.001$) DLWG as finishing duration increased from an average of 86 to 286 to 622 days across the short, medium and long duration systems respectively. Both SLW and CCW increased generally as GP duration increased whilst short duration heifers had lower FAT scores ($P < 0.05$) compared to all other treatment groups. Steers generally grew faster and had significantly higher ($P < 0.001$) SLW and CCW compared to heifers. Feeders Margin (£/head/day) exceeded either actual SRUC costs during the trial or the average of industry costs (above the line = profit: below the line = loss) in only some individual animals finished on the short and medium, but not the long duration GP systems.

Table 1 Animal and slaughter performance in LIMx steers (S) and heifers (H) finished using alternative growth paths

	Short		Medium		Long		s.e.d.	Sig. of effects		
	S	H	S	H	S	H		GP	Sex	GPxSex
DOT	81 ^a	90 ^a	271 ^b	300 ^b	610 ^c	635 ^c	20.1	***		***
DLWG (kg/d)	1.72 ^a	1.43 ^b	1.05 ^c	0.88 ^d	0.58 ^e	0.50 ^e	0.059	***	***	**
AGE (months)	15.4 ^a	14.8 ^a	21.5 ^b	22.0 ^b	32.8 ^c	33.0 ^c	0.70	***		***
SLW (kg)	592 ^a	465 ^d	670 ^b	579 ^a	712 ^c	631 ^e	15.9	***	***	*
CCW (kg)	337 ^{bc}	258 ^a	384 ^d	328 ^b	398 ^d	359 ^c	11.6	***	***	*
KO (g/kg)	570	554	572	566	558	570	12.9			
FAT (1-15)	7.62 ^a	5.88 ^b	7.50 ^a	8.12 ^a	7.38 ^a	7.75 ^a	0.714	*		
CONF (1-15)	9.92	8.92	10.25	9.50	9.50	9.75	0.652			

Values within rows not sharing common superscripts differ significantly

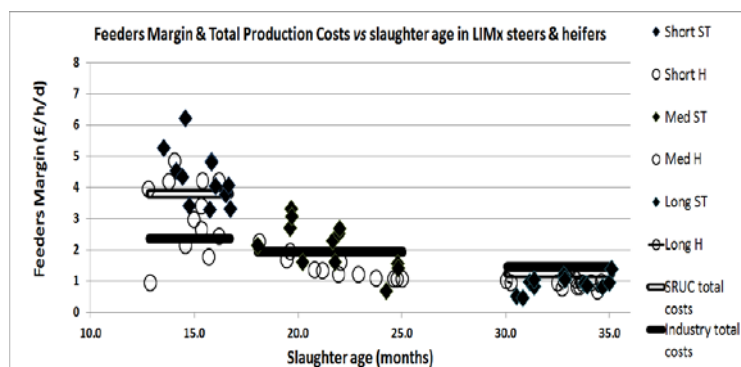


Figure 1 Daily feeders margin & total production costs in LIMx finishing cattle (£/head/day)

Conclusion Short duration finishing systems result in fast, consistent growth paths to slaughter whereas longer duration growth paths will result in much slower, more intermittent growth paths to slaughter. Acceptable carcasses can be produced with all alternate growth path finishing systems but longer duration systems usually increase carcass weights. However, profit potential (feeders margin > total costs) only exists where shorter duration (<24 months AGE) growth paths are used.

Acknowledgements This work was funded by AHDB Beef & Lamb.

Quantifying the impact of Environmental Stewardship on pasture-based cattle production

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Application The results can be utilised to evaluate the cost effectiveness of UK public expenditures on agri-environmental schemes, understanding of which is essential for the design of policy to successfully induce sustainable intensification.

Introduction With the annual throughput of 2.94 million head, the UK beef industry and its associated sectors produce meat products worth £7.4 billion per year, and its existence is widely considered to be indispensable for provision of high-quality protein as well as various ecosystem services. At the same time, beef production is responsible for 41% of global emissions of greenhouse gases from livestock and also known to be a leading polluter of water systems. In order to maintain food security without compromising environmental integrity, the concept known as sustainable intensification needs to be adopted on the national scale. The Environmental Stewardship (ES) initiative was set up to induce this change, with the stated objective to ‘conserve wildlife and biodiversity; maintain and enhance landscape quality and character, and protect the historic environment and natural resources’. Despite being in existence since 1991, however, there has been little work examining the quantitative impact of this scheme upon the structural change in the British farming industry (Quillérou *et al.*, 2011; Courtney *et al.*, 2013).

Material and methods The effect of participation in the Higher Level Scheme (HLS) subprogramme (Natural England, 2013) on successful reduction in livestock number per area, one of the most important target variables related to the degree of sustainable intensification, was quantified using the framework of propensity score matching (PSM: Rosenbaum and Rubin, 1983; Austin, 2011). An anonymised dataset for the 2013 edition of the June Agricultural Survey was combined with a Natural England register for the HLS membership to create a sample of 29,160 agricultural producers randomly selected across England. From this sample, 14,025 producers who (1) own or manage cattle; and (2) own or rent in grassland (including rough grazing land) were screened to provide a subsample relevant to the analysis of pasture-based cattle production systems. Furthermore, producers below the 10th centile and above the 90th centile in terms of their cattle holdings per hectare were removed so as not to include operations too small (‘hobby farms’) or too large (feedlots) to be considered as extensive production. The final dataset encompassed 437 HLS participants and 10,784 non-participants, whose stocking rates ranged from 0.39–4.07 head per hectare of grassland. Each participant in this dataset was then matched against a non-participant through the nearest-neighbour algorithm, whereby the propensity score was derived by a probit estimation using biophysical, geographical and socioeconomic characteristics of farms. The balancing property was satisfied between the participants and the counterfactuals.

Results The pre-matching difference in stocking rate between the participants (mean = 1.38) and non-participants (mean = 1.80) was –0.42 head per hectare ($p < 0.001$). The mean stocking rate for the matched counterfactuals (1.58) was considerably lower than the full set of non-participants, resulting in a lower programme impact of –0.20 head per hectare ($p = 0.029$). Participation in HLS was thus shown to contribute, on average, to a 13% reduction in grazing intensity. Neither the cattle number ($p = 0.726$) nor the area of grassland ($p = 0.242$) was significantly different between the participants and the counterfactuals, although the participants showed a tendency to have slightly larger cattle herds (mean difference = 4.61 head) on slightly larger grasslands (mean difference = 12.8 ha).

Conclusion While the pre-matching and post-matching results would lead to the identical conclusion that the ES initiative has had a statistically significant effect to reduce the level of production intensity, the quantitative impact shown post-matching was 48% smaller than the value derived from unprocessed data. The suggested overestimation by nominal calculation is likely attributable to a greater selection, which stems from the fact that the likelihood of the HLS enrolment is higher amongst producers with large landholdings. The future research will link the stocking rate reduction brought about by HLS to the social benefit of the corresponding reduction in pollution, with the view to investigate the optimal rate of government payment for each specified ‘environmental stewarding’ activity.

Acknowledgements The authors gratefully acknowledge Defra and Natural England for the provision of data.

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Effect of encapsulated nitrate (EN) and elemental sulphur (S) on methane (CH₄) production and microbial protein synthesis in beef cattle

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Application Encapsulated nitrate is a potential additive to reduce enteric CH₄ emissions from beef cattle. Furthermore, EN may serve as an alternative non-protein nitrogen (NPN) source to urea, for cattle diets, and decrease the amount of true protein needed for animal production.

Introduction Methane produced from domestic ruminant enteric fermentation is the largest anthropogenic source of CH₄. Alternative sinks need to be investigated to redirect metabolic rumen hydrogen (H₂) away from methanogenesis and provide an energetic gain for the host animal (Malik *et al.*, 2015) This study investigated the effect of using EN as a replacement for urea (U) or dietary protein, plus the addition of inorganic S, on enteric CH₄ emission, nutrient digestibility, N utilization and microbial protein synthesis from crossbred beef steers. In addition, nitrate toxicity and eating behaviour were investigated.

Material and methods This experiment was conducted at UNESP Brazil in 2015. Five crossbred Angus x Nelore steers fitted with permanent rumen cannulae were used in a 5x5 Latin square design with 21 day periods (14 days adaptation and 7 days measurement) Steers had average initial and final body weights (kg) of 327 ± 20.1 and 423 ± 41.3, respectively (mean ± SD). Animals were fed *ad libitum* (1.10 times average daily intake) during adaptation and restricted to 90% of voluntary feed intake during sampling. The five iso-nitrogenous treatments were: control (C) which consisted (g/kg DM) of Tifton 85 hay (500), maize (570) and soya-bean meal (SBM, 100); U, SBM partially replaced by 8 g U/ kg DM; US, as U plus 2.4 g S /kg DM; EN, SBM partially replaced by 20 g EN (equivalent to 14.3 g nitrate) /kg DM; ENS, as EN plus 2.4 S g/kg DM. Stepwise adaptation to nitrate was done by increasing the level of EN by 25 % of final dose every 4 days. Feed offered and refused was recorded daily and intake was measured 3 h after daily feeding on days 3, 6, 9, and 12. Blood samples were taken 3 hours after feeding on days 1, 4, 7, 10 and 14. Enteric CH₄ production was measured from day 15 to 20 using the sulphur hexafluoride technique (Johnson *et al.*, 2007). Total daily faecal and urine collections were done from days 16 to 21. On day 20, rumen samples were collected at 0, 2, 4, 6, 12, and 24 h after feeding. Rumen fluid volatile fatty acids (VFA) and ammonia (NH₃N), urine purine derivatives and blood methaemoglobin (metHb) concentrations were determined. Data were analysed as a 5*5 Latin Square and orthogonal contrasts calculated. Rumen fermentation data were analysed by repeated measures Anova with time point 0 included as a covariate.

Results DM intakes (DMI) and feed intake patterns during adaptation to EN were not affected by treatments (P>0.05). Steers receiving EN had MetHb levels below the threshold for toxicity and did not show any symptoms of toxicity. (Maximum, 13.7% total Hb). Apparent digestibilities were not different between treatments (P> 0.05). Although not significant, daily CH₄ production (Table 1) was lower for EN treatments. Rumen NH₃N concentrations were lower with NPN treatments than with C (16.5 vs 21.5 mg/dl; P=0.03) and were lower in the urea-containing diets than EN treatments (P=0.02). However, total VFA concentrations were not affected by treatments (P>0.05). Inorganic S showed no effect on any parameter studied.

Table 1 Methane production by steers fed different sources of N

Methane	Treatments					SEM	P-value for contrasts			
	Control	Urea	Urea+S	Nitrate	Nitrate+S		C v NPN	EN v U	Added S	NPN * S Interaction
g/day	121	139	85	73	71	30.1	0.40	0.21	0.36	0.41
g/kg DMI	13.6	15.9	10.4	9.6	9.4	2.72	0.47	0.21	0.32	0.35

Conclusion The inclusion of EN in this experiment showed a potential reduction in CH₄ production, providing an alternative source of N for protein metabolism for beef cattle. EN had no adverse effects on rumen fermentation or N metabolism. The level of addition of EN (14.3 g nitrate /kg DM) and the time of adaptation chosen for this study were adequate to avoid nitrate toxicity. Inorganic S did not show any effects.

Acknowledgements The author acknowledges funding by SRUC International Engagement Strategy Fund.

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Prediction of methane (CH₄) emissions by individual beef cattle from ruminal volatile fatty acids (VFA) using compositional linear mixed models

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Application VFA compositional data have the potential to be used as a predictor for CH₄ emissions in individual steers.

Introduction Enteric CH₄ is a major contributor to greenhouse gas emissions from the livestock sector. While modifying the diet of cattle is an effective short-term method for mitigating CH₄ emissions, longer-term the wide variation in CH₄ yield (g / kg dry matter intake, DMI) between individual animals should be exploited. A limitation to exploitation is the slow output and cost of measurements of CH₄ made using indirect respiration chambers. Therefore rapid and low cost proxy measures are required. Here we investigate the relationship between VFA molar proportions and CH₄ yield with the aim of assessing the potential of VFA as a proxy for methane emissions.

Material and methods The dataset available consisted of 284 measurements (from 6 experiments) for beef cattle of CH₄ yield measured in indirect respiration chambers at SRUC between 2011 and 2014. Data available were: for individual animals; live-weight, DMI, CH₄ yield and VFA molar proportions (single sample taken by stomach tube on exit from chamber) and for diets; forage proportion, starch, neutral detergent fibre and metabolisable energy (ME) concentration. Preliminary analysis revealed that forage proportion was a satisfactory descriptor of diet composition and for further analysis, diet-class was used as an explanatory variable where diet-class Concentrate included diets with <150 g forage /kg DM; Mixed, 400-600 g forage /kg DM and Forage >700 g forage/kg DM. A novel compositional modelling approach was employed where VFA were considered as a whole, instead of in isolation, emphasizing their multivariate relative scale. Data analysis was based on “balances” representing log-contrasts between subsets of associated VFA components obtained by a sequential binary partition (Fig 1). Linear mixed models (LMMs) were then used to explore the contribution of VFA balances to CH₄ yield while accounting for the influence of covariates such as steer live-weight, DMI and diet ME (CH₄ yield and covariates were log-transformed prior to analysis).

Results

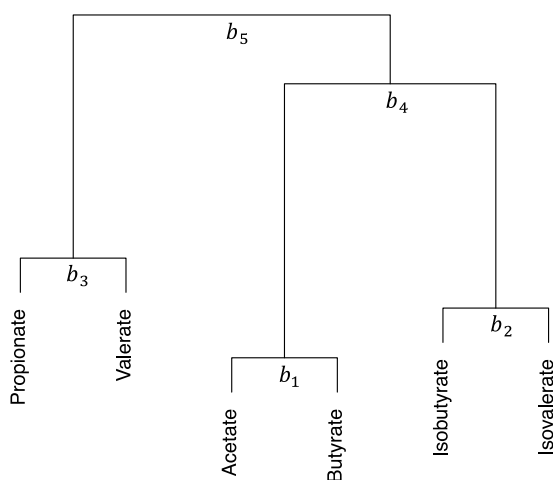


Figure 1 Balances describing log-contrasts between VFA from compositional analysis

The balances that best described the variability of the VFA compositional data are shown in Fig. 1 and clearly segregated the VFA into pairs associated with hydrogen consumption (b_3), hydrogen production (b_1) and protein catabolism (b_2). When these balances were incorporated into LMMs, balances b_3 ($P=0.005$), b_4 and b_5 (both, $P<0.001$) contributed significantly to the model together with diet-class, steer DMI and diet ME and explained 69% of the variation in CH₄ yield. Further evaluation of possible binary partitions revealed that when a balance (b_6) representing (acetate + butyrate / propionate) was specified it became the only balance to contribute significantly to the model. The LMM was:

$\text{Log CH}_4 \text{ (g/kg DMI)} = -0.84 + 0.24 b_6 \text{ (} P<0.001 \text{)} + 0.37 \text{ Mixed (} P<0.001 \text{)} + 0.73 \text{ Forage (} P=0.002 \text{)} + 1.7 \text{ log ME (} P<0.001 \text{)} + \text{log DMI (} P<0.001 \text{)}$ where Mixed and Forage are dummy variables accounting for the effects of moving from diet-type Concentrate. To check whether the relationship between the VFA balance and CH₄ yield of an individual animal was consistent within diet-type, the analysis was repeated for Concentrate ($n=88$) and Mixed ($n=151$) diet-types separately and balance (b_6) was significant ($P<0.001$) within both diet-types.

Conclusion Compositional analyses accounted for the relative nature of VFA and generated balances which described the underlying biology well. When incorporated into LMMs, a single balance (acetate + butyrate / propionate) was a significant predictor for CH₄ yield from individual steers and therefore shows potential as a proxy in identifying animals which emit less CH₄.

Acknowledgements The authors gratefully acknowledge funding from AHDB Beef and Lamb, the Scottish Government and by DEFRA and the devolved administrations through the UK Agricultural Greenhouse Gas Inventory Research Platform) and the Spanish Ministry of Economy and Competitiveness.

Alternative growth path finishing systems and their effects on beef eating quality in Limousin cross (LIMx) steers and heifers

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Application Beef producers should be encouraged to adopt efficient, short duration finishing systems that deliver higher eating quality beef compared to longer duration finishing systems of production.

Introduction A wide variety of finishing systems and growth paths (GP) are employed in UK beef production but relatively few studies have detailed effects of alternative GPs on beef eating quality parameters with comparable animals of common genotype blocked across GP treatments in a comprehensively balanced way. Objectives of this study were to compare short, medium and long duration GP finishing systems with regard to the eating quality of the beef produced.

Material and methods A 3 x 2 factorial continuous design experiment examining three alternate GP and using 36 LIMx steers (S) and 36 LIMx heifers (H) with known sires was undertaken to compare short, medium and long duration GPs. The short GP animals were finished indoors on an intensive concentrate based system and slaughtered at 12-16 months of age. The medium GP animals were turned out to graze a high quality reseed from 12-17 months of age and then finished indoors during the subsequent winter period when offered a mixed forage:concentrate (F:C) finishing diet and slaughtered at 18-24 months of age. Long GP animals were grazed for two summers on poor quality grassland, stored on a forage based diet for the intervening winter and finished during their final winter, again on a mixed F:C diet to slaughter at 28-36 months of age. Beef eating quality was assessed by human taste panel and by the mechanical slice shear force (SSF) measure of tenderness.

Results Sensory taste panel measures of eating quality and gristle contents (% of L.dorsi weight) are given in Table 1 whilst the SSF measure of tenderness is shown graphically in Figure 1. Steers generally produced tougher beef with lower overall liking and higher gristle contents compared to heifers, although this was primarily associated with animals from the long duration GP. Long duration GP steers had higher scores from both the taste panel and SSF mechanical measures of toughness and gristle content was also higher for the long duration GP system. Age at slaughter *per se* explained < 20% of the variation in SSF measures across the dataset as a whole, confirming that many factors other than age at slaughter are likely to influence the toughness aspects of beef eating quality.

Table 1 Taste panel measures of beef eating quality in LIMx steers and heifers finished using alternative growth paths

	Short		Medium		Long		GP	s.e.d.	Sig. of effects	
	S	H	S	H	S	H			Sex	GPxSex
Toughness (0-80)	39.0 ^a	38.5 ^a	42.7 ^a	42.4 ^a	51.7 ^b	42.1 ^a	2.24	*	*	*
Juiciness (0-80)	50.2	52.7	54.6	52.1	52.9	55.3	1.93			
Beef Flavour (0-80)	43.9 ^a	44.9 ^a	45.2 ^{ab}	49.2 ^b	46.8 ^{ab}	46.4 ^{ab}	1.45	*		*
Abnormal “ (0-80)	23.2 ^a	24.2 ^a	20.8 ^{ab}	20.3 ^b	19.8 ^b	19.6 ^b	1.75	*		*
Flavour liking (0-80)	43.1 ^a	44.4 ^{ab}	46.0 ^{ab}	48.9 ^b	47.7 ^{ab}	47.9 ^{ab}	1.87	*		*
Overall liking (0-80)	40.9 ^a	44.1 ^{ab}	43.8 ^{ab}	47.1 ^b	42.3 ^{ab}	46.1 ^b	1.79	*	**	*
SSF (kg force)	10.6 ^a	10.9 ^{ab}	10.2 ^a	10.7 ^{ab}	12.2 ^b	11.8 ^{ab}	0.67	*		*
Gristle:L.dorsi (%)	1.65 ^{ab}	1.61 ^{ab}	1.75 ^b	1.49 ^a	2.10 ^c	1.82 ^b	0.113	***	**	*

Values within rows not sharing common superscripts differ significantly

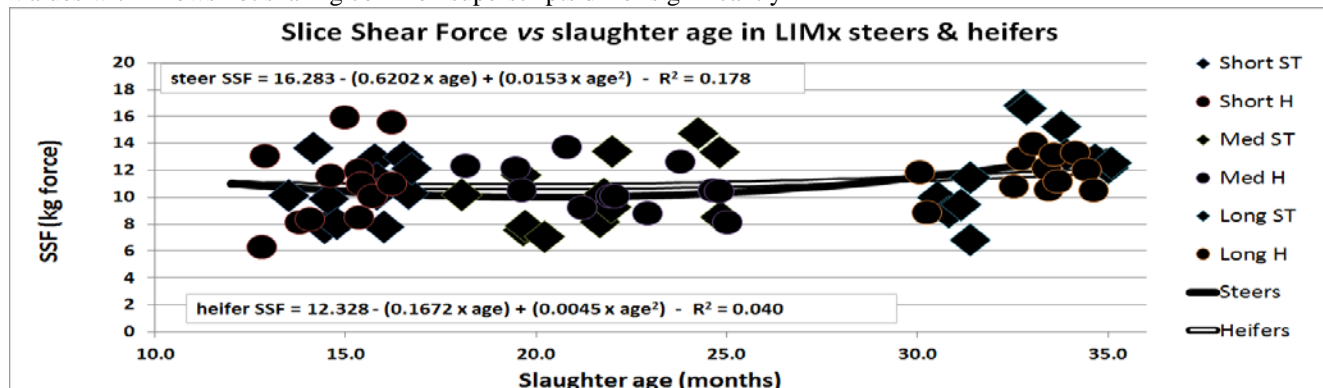


Figure 1 Slice shear force vs slaughter age in LIMx finishing cattle (kg force)

Conclusion Beef eating quality that is acceptable to the consumer (< 18 kg SSF) can be produced from GPs of all durations. However, long duration GPs may result in beef being tougher with higher gristle content than shorter GPs.

Acknowledgements This work was funded by AHDB Beef & Lamb.

Effect of beef fat class on *post-mortem* tissue glutathione peroxidase activity

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Application *Post-mortem* meat quality and oxidative stability may be adversely affected by sub-optimal *post-mortem* glutathione peroxidase activity

Introduction: Glutathione peroxidases (GSH-Px) are a group of selenium dependent enzymes that protect against oxidative damage. It is fairly well established that tissue GSH-Px (GSH-Px4) activity persists *post-mortem* and reduces the production of thiobarbituric acid reactive substances (TBARS) (DeVore *et al.*, 1983; Juniper *et al.*, 2011) which are associated with the development of off-flavours in retail cuts of meat (Morrissey *et al.*, 1998). Studies in human subjects have shown that GSH-Px4 activity is modulated by alterations to lipid profiles (Sneddon *et al.*, 2003). This would suggest that tissue GSH-Px activity might be influenced in animals that have greater levels of adiposity. The aim of this study was to determine the relationship between tissue lipid content and *post-mortem* tissue GSH-Px activity in the loin muscle of finished beef cattle of contrasting fat classes.

Material and methods Loin samples were taken *post-mortem* from 16 beef cattle of mixed breed, eight from fat class 2 and eight from class 4H. Cattle were from different farms but were all transported to and slaughtered at the same abattoir on the same day; carcasses were selected based on *post-mortem* EC fat class classification. All carcasses were chilled and deboned according to industry standards at two days *post-mortem*, at which time loin samples were taken from the left side of each carcass, vacuum packaged and chilled on ice. Samples were transported to the University on ice where on arrival samples were removed from packaging and all visible traces of fat were removed from each loin sample prior to homogenisation. Samples were homogenised using a domestic blender which was cleaned thoroughly between samples. Following homogenisation, samples were freeze dried, ground and analysed for lipid content (using the Soxhlet method, Brown and Mueller-Harvey, 1999) and tissue GSH-Px activity (by ELISA, AB102530 kit, Abcam, Cambridge, UK). Samples were analysed in quadruplicate. The effect of fat class on the lipid content and GSH-Px activity of the tissue was determined by analysis of variance (Minitab 17, Minitab Inc, PA, USA).

Results The results of this study are summarised in Table 1. Although subcutaneous fat was removed from all samples, the lipid content of 4H samples was 2.5 times higher than that of fat class 2 samples. This was associated with nearly a two fold increase in GSH-Px activity in the fatter samples.

Table 1 Effect of fat class on tissue dry matter, lipid content and tissue GSH-Px activity

	Fat class		SEM	P-Value
	2	4H		
Dry matter content (g/kg FW)	261.2	296.9	7.51	0.007
Lipid content (g/kg FW)	31.36	80.40	7.72	0.001
GSH-Px activity (nmol NADPH/mg tissue)	363.4	684.1	67.8	0.007

Conclusion The findings of this study indicate that tissue GSH-Px activity appears to be associated with the lipid content of the tissue. This would suggest that the relationship between tissue selenium and tissue GSH-Px activity is more complex than simple tissue selenium concentration alone. Furthermore, the findings would also suggest that fatter animals could be more likely to be prone to issues of selenium deficiency, and this may then result in reduced shelf-life of the meat produced.

Acknowledgements The help and provision of tissue samples from Dawn Meats is gratefully acknowledged.

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Effect of dietary crude protein level on the performance of cereal fed Continental cross Holstein bulls

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Application According to AFRC 1993 young cereal fed bulls from 7 months old to slaughter should be fed a diet containing 120g/kg crude protein (CP). Contrary to this recommendation the results from this study shows that 300kg continental dairy-bred beef bulls should be fed a diet containing 140g/kg CP.

Introduction There is increasing concern that the stated energy and protein requirements for beef cattle (AFRC, 1993) are now underestimated for today's modern high genetic merit beef cattle, especially for young fast growing intensively fed bulls. The current recommendation for the crude protein (CP) content of cereal based rations for young bulls from 3 to 7 months old is 140g CP/kg which can then be reduced to 120g through to slaughter. In a study by Marsh *et al.*, (2009) 285kg Holstein bulls were intensively finished on cereal based rations containing 120, 140 or 160g CP. There was no response to feeding elevated levels of CP. However genetic improvement and sire selection for higher productivity and lean tissue deposition may have substantially increased the protein requirement with Continental bred cattle. With the increased costs of protein feedstuffs relative to cereals and the requirement to determine the optimum protein content of finishing rations for continental bred beef cattle the objective of this experiment was to evaluate different protein levels for intensively finished Continental cross Holstein bulls from 7 months old to slaughter.

Material and methods Thirty six British Blue x Holstein bulls weighing 320kg were reared through to slaughter on a cereal beef system and fed *ad libitum* diets containing either 120 or 140g/kg dietary CP. The 120g/kg CP ration contained the following ingredients (g/kg): rolled barley 750, molassed sugar beet pulp 100, soyabean meal 75, molasses 50, and mineral 25. The 140g/kg CP diet was formulated with 125g/kg soyabean meal replacing an equal quantity of barley. The cattle were housed in straw-bedded pens and were selected for slaughter at EUROP fat class 3. The data were analysed using ANOVA.

Results The bulls fed the 140g/kg CP ration recorded performance that exceeded recognised targets for cereal beef production. The bulls fed the 140g/kg CP ration recorded significantly higher ($P < 0.05$) daily carcass (DCG) and liveweight gains (DLWG).

Table 1 Animal performance

Crude protein (g/kg)	120	140	s.e.d	Sig
Slaughter weight (kg)	587	596	8.1	NS
Days to slaughter	202	196	5.4	NS
DLWG (kg)	1.33	1.42	0.042	*
Carcass weight (kg)	320.7	328.1	4.75	NS
Kill out (g/kg)	547	551	3.4	NS
Carcass daily gain (kg)	0.85	0.92	0.034	*
Conformation class ¹	4.2	4.3	0.17	NS
Fat class ¹	3.1	3.2	0.19	NS

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

Table 2 Feed use and feed conversion ratio (FCR)

Crude protein (g/kg)	120	140
Daily feed intake (kg)	8.24	8.18
Total feed intake (kg)	1,666	1,605
FCR (kg feed: kg LWG)	6.24	5.82
FCR (kg feed: kg carcass gain)	9.69	8.90

Based on the costs prevailing at the time of the experiment the bulls fed the 140g CP ration recorded an increase in carcass value worth £43. However increased feed costs of £11 per bull reduced the margin over feed to an extra £32 per bull.

Conclusions It is recommended that 7 month old intensive cereal finished dairy-bred Continental bulls are fed diets containing 140g/kg CP rations compared to the AFRC 1993 recommendation of 120g/kg.

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Relationship between net feed efficiency (NFE) and scrotal circumference in young Stabiliser breeding bulls

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Application The results suggest that selecting for lower NFE and therefore more feed efficient Stabiliser cattle in future generations can be applied without adverse changes in scrotal circumference (and by inference fertility) in young bulls.

Introduction Earlier work has shown that substantial variation in NFE exists within the Stabiliser cattle population in the UK (Hyslop *et al*, 2013) and that NFE is a moderately heritable trait suggesting that genetic selection based on traditional quantitative genetic approaches will lead to future generations of Stabiliser cattle with improved feed efficiency. However, it is important to assess the possibility of inadvertently selecting for any associations in any additional traits such as scrotal circumference that may be associated with low NFE animals. Consequently the objective of the current study was to examine the potential relationship between NFE measured in young Stabiliser breeding bulls and scrotal circumference (SC) as an indicator trait of fertility when SC was measured in the same individual bulls at NFE determination.

Materials and method NFE was determined in 388 young Stabiliser breeding bulls (9-12 months old) as described previously (Hyslop *et al*, 2013) during an 8-week measurement period. At the end of the NFE measurement period SC was determined on each individual bull using a simple measurement tape. All data was grouped into three groups where low NFE = < -0.5 sd of mean NFE, mid NFE = > -0.5 but < +0.5 sd of mean NFE and high NFE = > +0.5 sd of the mean NFE value respectively. Differences and relationships between and amongst these three groups for NFE & SC were examined using the residual maximum likelihood (REML) and linear regression facilities in Genstat 16.

Results Despite highly significant differences ($P < 0.001$) in actual NFE values across the low, mid and high NFE groups (Table 1) there were no significant differences in SC between the three NFE groups. The simple linear relationship between NFE and SC also showed no significant relationships between NFE and SC (Figure 1).

Table 1 NFE and SC in young Stabiliser breeding bulls

	low NFE	mid NFE	high NFE	sed	Sig		low NFE	mid NFE	high NFE	sed	Sig
NFE(kg/DMI/d)	-0.82 ^a	-0.02 ^b	+0.84 ^c	0.046	***	SC (cm)	36.4	36.2	36.9	0.44	

Values not sharing common superscripts differ significantly.

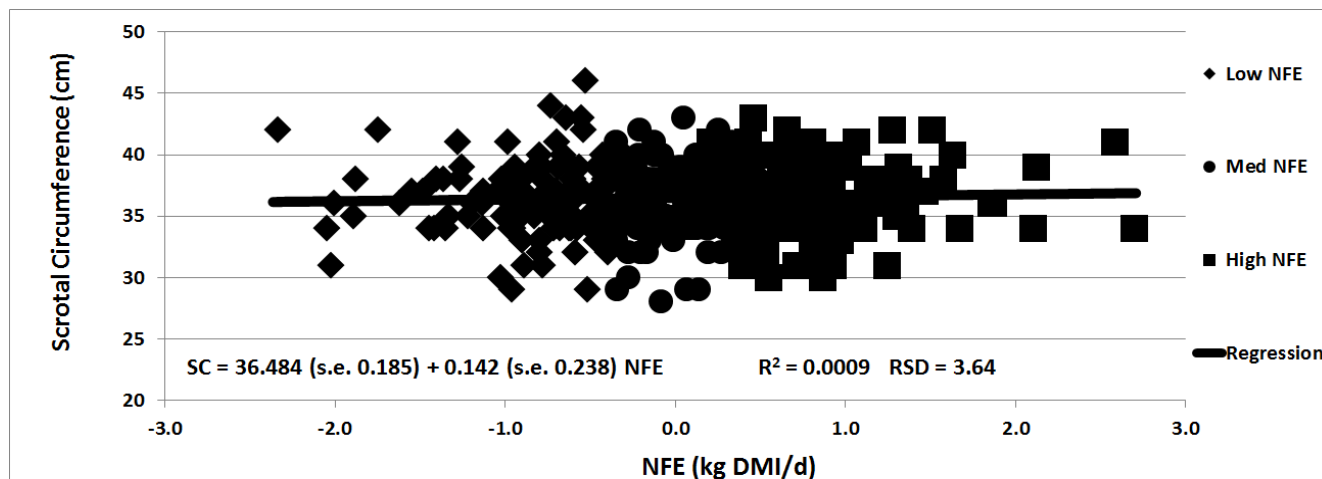


Figure 1 Linear regression relationship between NFE & scrotal circumference in young Stabiliser breeding bulls.

Conclusion No significant relationship was seen between NFE and SC in young Stabiliser breeding bulls regardless of the fact that there were significant differences ($P < 0.001$) in their underlying net feed efficiency values.

Acknowledgements This work was funded by an Innovate UK project grant under the SAF-IP programme

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Growth response to creep feeds containing different protein levels in autumn born suckled calves

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Application Autumn born continental cross native breed suckled bull calves should be fed creep diets containing at least 160g/kg crude protein (CP) 'air dry basis'.

Introduction The benefits of feeding creep to suckled beef calves are well recognised with improved growth rates, efficient feed conversion and minimising growth checks at weaning. However there is a paucity of information on the effect of different formulations of creep feed for suckled calves. The objective of this experiment was to evaluate 130 versus 160g/kg CP 'air dry basis' creep feeds with autumn born suckled calves.

Material and methods Forty autumn calved pure bred Stabiliser cows (the Stabiliser is a four breed composite based on continental and native breed genetics including Red Angus, Hereford, Simmental and Gelbvieh) with Stabiliser calves (bulls = 20, heifers = 20 with a mean age of 58 days) were randomised according to cow live weight, condition score, calf liveweight and sex into two balanced groups with calves offered either 130g or 160g/kg CP (149 and 184g CP/kg DM respectively) creep offered *ad libitum*. The 130g/kg CP creep ration contained the following ingredients (g/kg): rolled barley 575, molassed sugar beet pulp 250, soyabean meal 50, rapeseed meal 50, molasses 50, and mineral 25. The 160g/kg CP diet was formulated with 100g/kg soyabean meal and 100g rapeseed meal replacing an equal quantity of barley. The cattle were housed in straw-bedded pens and the cows were fed *ad lib* grass silage (30.8% DM, 10.6 ME MJ/kg DM, 127g CP/kg DM) and 0.6kg of mineralised barley. The calves had access to the silage. The trial commenced on the 22nd of November and ended on the 26th of March 2014 prior to turn-out to grass. The data were analysed using ANOVA.

Results The calves fed the 160g/kg CP ration recorded higher ($P < 0.05$) weaning and 200 day weights and significantly higher ($P < 0.01$) daily liveweight gains (DLWG). Analysis of the performance of the bull and heifer calf performance showed a significant response to feeding an elevated CP creep to bull calves with no response recorded by the heifers.

Table 1 Growth performance of suckled calves offered creep feeds at two CP levels

Creep feed CP level (g/kg)	130	160	s.e.d	Sig
Start weight (kg)	126	126	8.0	NS
Weaning weight (kg)	305	324	8.7	*
DLWG (kg)	1.45	1.60	0.076	**
Calf weaning age (days)	184	185	4.9	NS
Adjusted 200 day wt (kg)	328	348	9.7	*
Bull calf DLWG (kg)	1.45	1.72	0.122	*
Heifer calf DLWG (kg)	1.44	1.44	0.131	NS

Significance levels - NS: >0.05 ; *: <0.05 ; **: <0.01 .

There were no significant differences in cow liveweight or condition scores between the treatments. The mean cow liveweight and condition score at the start of the experiment were 660kg and 2.73 respectively.

Table 2 Feed intakes (kg/calf)

Creep feed CP (g/kg)	130	160
Daily feed intake	3.15	3.54
Total feed intake	390	439

Overall performance of the cows and calves was excellent with calf DLWGs exceeding commercial lowland suckled calf producers recorded by AHDB Beef & Lamb of 1.11kg. The 200 day weights for the calves fed the 130 and 160g/kg CP creep were calculated to be 51.6% and 57.5% of the cow weight at weaning. The recognised industry target is 50%. Based on the costs prevailing at the time of the experiment the calves fed the 160g/kg CP ration recorded an increase in gross margin worth £25 per calf.

Conclusions Autumn born continental cross native breed suckled calves should be fed creep diets containing at least 160g/kg CP. If commercial suckler producers can house cows with bull calves separate from heifers the latter can be fed 130g/kg CP creep rations. Further work is required to evaluate alternative protein sources and levels for creep fed suckled calves with continental and native breed types.

Acknowledgements The authors gratefully acknowledge the financial support from Rumenco Ltd.

Differences between fresh ryegrass and hay in the dynamics of feed colonization and utilization by rumen microbes using a rumen simulation technique

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Application Fresh grass promotes a more rapid feed colonization and utilization by rumen microbes than grass hay.

Introduction The decision of feeding ruminants with fresh pasture vs hay is often arbitrary based on the farm management without taking consideration of its impact on rumen microbiota. Similarly, supra-nutritional levels of vitamin E are often used to improve product quality regardless of its potential impact on the rumen function. This study investigates the differences between fresh ryegrass and hay in the dynamics of plant colonization and feed utilization by rumen microbes.

Material and methods Fresh ryegrass (GRA) and ryegrass hay (HAY) from the same pasture (AberMagic) and harvest were used. Fresh ryegrass was immediately frozen in liquid N while hay was dried in the field for 2 days and then oven dried at 25°C. Experimental diets consisted of two forage types supplemented with concentrate (20% in DM basis) containing zero (-) or 50 IU/d (+) of added vitamin E as dl- α -tocopheryl acetate. The experiment was carried out using a rumen simulation technique with 16 vessels inoculated with rumen fluid from four rumen-cannulated cows fed with the same forage/concentrate ratio. Rumen fluid was filtered through muslin, diluted with anaerobic artificial saliva (McDougal) and vessels were kept at 39°C under constant vertical agitation. A nylon bag (100 μ m² pore size) containing feed (11g DM) was supplied daily into each vessel and incubated for 48h. The experiment lasted 13 days: 9 for the microbial adaptation to the *in vitro* system and Experiment consisted in 13 days: 9 for the microbial adaptation to the *in vitro* system and 4 for measurements. Plant residues were sampled at 2, 4, 8 and 48h after incubation to determine microbial colonization and feed degradability. Plant residue was frozen in liquid N and RNA was extracted, reverse transcribed and used for microbial characterization. Bacterial diversity was studied by 16S amplicon sequencing of RNA samples using Ion Torrent Next Generation Sequencing. Forage colonization by the main microbial groups (i.e. bacteria, anaerobic fungi, methanogens and protozoa) was measured by qPCR and using ¹⁵N as microbial marker.

Results Small differences were observed in the chemical composition between GRA and HAY which could explain part of the differences observed in the plant colonization pattern by rumen microbes. This pattern was divided in 3 colonization phases: primary colonization (up to 2h after feeding) led to the greatest bacterial diversity across diets, with *Lactobacillus* and *Streptococcus* being the most abundant primary colonizers in GRA and HAY diets, respectively. Secondary colonization occurred between 4 and 8h after feeding and was characterized by a progressive increase of fibrolytic bacteria (*Ruminococcus* and *Butyrivibrio*) in GRA diets and *Lachnospiraceae* in HAY diets. Tertiary colonization (>8h after feeding) was dominated by Firmicutes across treatments. GRA promoted a more rapid feed colonization and digestion during the primary and secondary colonization. On the contrary, HAY required a formation of a complex bacterial network during the primary and secondary phase which could delay its utilization. However once this bacterial consortium was stabilized the HAY colonization and utilization was accelerated leading to a similar bacterial structure and feed digestibility at 48h after feeding. Vitamin E had minor effects and only seemed to slightly accelerate the plant colonization process for HAY diets.

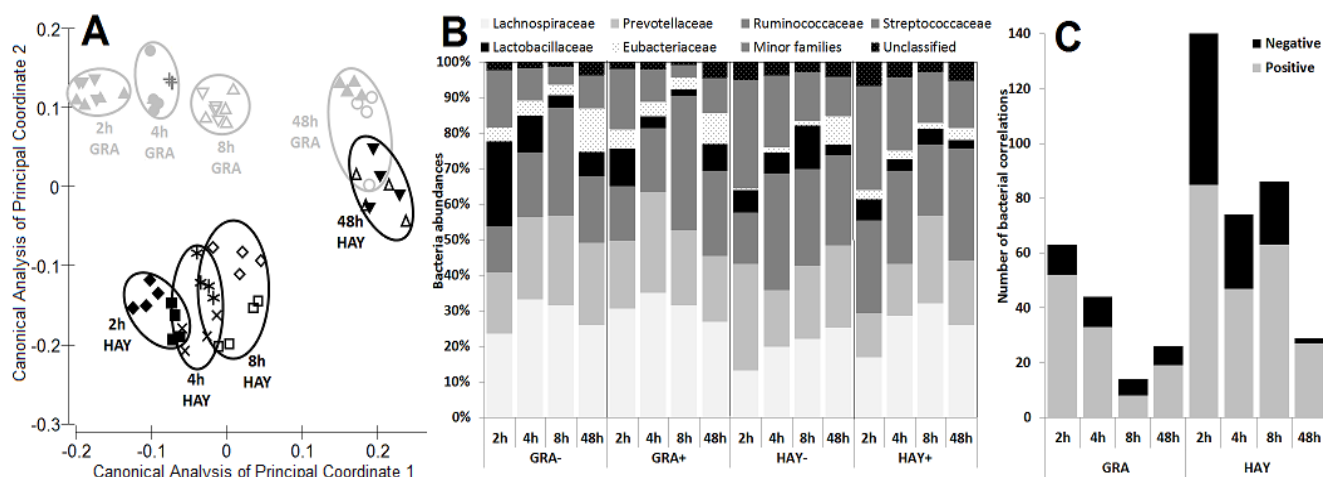


Figure 1 Effect of experimental diets and incubation time on the structure of the bacterial community (A), abundance of the main bacterial families (B) and number of correlations in the bacterial network (C).

Conclusions The study of the feed colonisation pattern by rumen microbes can help to understand the mode of action of nutritional interventions such forage preservation method and feed additive utilization on feed utilization by ruminants.

Acknowledgements This work was supported by the WISE Network (ERDF) and by the BBSRC (BBS/E/W/10964A01).

Prediction of methane emissions and feed conversion efficiency of beef cattle based on the rumen microbial community at phylum or genus level

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Application Methane emissions and feed conversion ratio (FCR) of beef cattle can be predicted using the rumen microbial community from rumen samples taken in the abattoir. The information can be implemented into genetic improvement programmes or nutritional interventions as a highly cost-effective prediction strategy for these traits.

Introduction In previous studies, it has been found that the relative abundance of rumen microbial genes can be used to predict methane emissions and FCR (Roehé *et al.*, 2016). It was not analysed whether the relative abundance of the rumen microbial community can also be used to predict these traits. Therefore, the object of this study was to predict these traits using the relative abundance of rumen community at the phylum and genus taxonomic levels.

Material and methods Data are from an experiment ($n = 72$) of 2x2 factorial design comprising of 2 breed types (Aberdeen Angus and Limousin rotational crosses); 2 basal diets consisting of (g/kg DM, forage:concentrate) 520:480 (Mixed) or 84:916 (Concentrate). FCR was calculated based on average DM intake per day (individually recorded by electronic feeders and analysis of the DM content twice weekly) and average daily gain (linear regression of weekly body weight measures on test date) during the performance test period of 56 days. Methane emissions were measured for 48 hours within open-circuit respiration chambers. Eight extreme animals (4 high and 4 low) for methane emissions, balanced for breed type and diet, were used in a metagenomic study of rumen samples taken at slaughter, in which deep sequencing was applied (43.4 to 72.7 million paired reads per sample). Taxa of the rumen microbial community were determined using 16S rRNA gene analysis, by aligning the genomic reads to the GREENGENES database (Roehé *et al.*, 2016). Partial least squares (PLS) analysis in SAS was used to identify the phyla and genera significantly associated with methane emissions and FCR. The PLS estimates were based on predictors and responses centred and scaled to have a mean of 0 and standard deviation of 1. Variable Importance for Projection (VIP) statistic of Wold, which summarizes the contribution of a variable maker to the model were used to identify the significant microbial genera or phyla associated with methane emissions. The R^2 values were obtained by fitting a general linear model (GLM, SAS) including a genus or phylum as well as breed and diet effects.

Results There were 6 genera significantly associated with methane emissions, with *Methanospaera*, *VadinCA11* and *Methanobrevibacter* the most important microbes identified in the PLS analysis (Table 1). The most important genera associated with FCR were *Sphaerochaeta*, *Ruminobacter*, *Succiniclasticum*, and *Dialister*. The PLS models explained 89.7% and 86.9% of the variation in model effects as well as 84.5% and 73.6% of the variation in methane emissions and FCR, respectively. The Phyla significantly associated with methane emissions were Verrucomicrobia, Proteobacteria and Euryarchaeota and with FCR were Lentisphaerae and Proteobacteria.

Table 1 Microbes classified at genus level associated with methane emissions (g/kg dry matter intake) and feed conversion ratio (kg DMI intake /kg growth rate)

Methane Genus	Methane			Feed conversion ratio			
	Estimate	VIP	R^2	Genus	Estimate	VIP	R^2
<i>Methanospaera</i>	0.360	1.15	0.84	<i>Sphaerochaeta</i>	0.224	1.09	0.82
<i>VadinCA11</i>	0.279	1.07	0.77	<i>Ruminobacter</i>	0.206	1.06	0.84
<i>Methanobrevibacter</i>	0.190	1.05	0.92	<i>Succiniclasticum</i>	0.360	1.04	0.80
<i>Moryella</i>	0.098	0.98	0.77	<i>Dialister</i>	0.277	1.01	0.73
<i>Megasphaera</i>	-0.092	0.90	0.83	<i>Clostridium</i>	0.156	0.95	0.83
<i>Desulfovibrio</i>	-0.027	0.81	0.98	<i>Bifidobacterium</i>	0.074	0.83	0.66

Conclusion The PLS analyses indicate that the microbial community classified at the genus level has the potential to predict accurately methane emissions and FCR. Additionally, the results give insight into which genera variation are closely associated with those traits.

Acknowledgements This project was funded by the Scottish Government, BBSRC, Scottish Funding Council, Defra and the UK Agricultural Greenhouse Gas Inventory Research Platform.

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The effect of essential oils on rumen bacteria and protozoa in dairy cattle

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Application Essential oils can change the rumen microbial population; however more trials including the possibility that longer periods are needed, are required to determine situations under which this may lead to production benefits.

Introduction A wide range of plant extracts including essential oils have been used to modify rumen fermentation. Here we have investigated the effect of a commercial essential oil product on milk yield and the microbial population in the rumen.

Material and methods Eight, high yielding Holstein/Friesian cows fed a TMR consisting of grass and maize silage, were used in a duplicated 4 by 3 Youden square with 35 day periods to investigate the effects of 0, 0.5, 1 or 2.5g/ d of a commercial essential oil (Agolin Ruminants, AGOLIN S.A, Switzerland, EO) on milk yield and rumen microbial populations. Protozoal numbers were counted microscopically and bacterial diversity estimated from next generation sequencing (PGM Ion-Torrent) of the 16S rDNA gene as previously described (Veneman *et al.*, 2015).

Results EO had no effect on milk yield, but at 1 g/d did increase both milk fat and total solids (Table 1, $P < 0.05$). The number of holotrich protozoa in rumen increased in animals fed 1 g/d EO (8.5, 17.3, 27.8, 15.3 SED 5.86×10^3 /ml for the control, 0.5, 1 and 2.5 g/d treatment respectively), but had no effect on the numbers of entodimorphid protozoa ($9.0, 7.9, 11.4, 8.7$, SED 1.91×10^4 /ml). There was also a trend towards a slightly lower abundance of Bacteroidetes and Proteobacteria in cattle receiving 1 g/d EO (Figure 1, $P < 0.1$)

Table 1 The effect of essential oils on milk yield and milk composition in Holstein/ Friesian cattle fed a grass and maize silage based TMR

	0	0.5	1	2.5	SED
	Additive (g/d)				
Milk yield (kg/d)	26.5	26.9	26.2	27.0	0.63
Lactose (g/kg)	46.8	46.8	46.2	47.2	0.41
Protein (g/kg)	33.6	33.6	34.5	33.8	0.46
Fat (g/kg)	45.2	44.5	46.5	44.0	0.67*
Total solids (g/kg)	132.9	132.2	134.9	132.3	0.73*

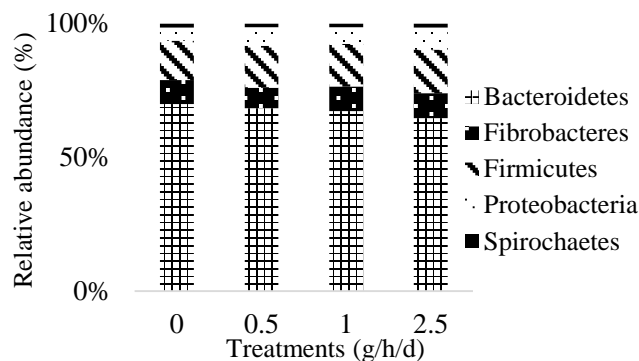


Figure 1 The effect of essential oils on the abundance of the main bacterial phyla in the rumen

Conclusion The essential oils product used had a major effect on the rumen protozoal population and more limited effect on the structure of the rumen bacterial population. However, this resulted in no change in milk yield although milk composition was altered.

Acknowledgements This study was funded by Climate KIC as part of the project Reduced Methane Emissions from Ruminants (RuMeClean).

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Antiprotozoal effect of saponins in the rumen is enhanced by chemical modifications to their structure

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Application Hederagenin bis-esters could potentially be used in ruminant diets as effective defaunation agents to, ultimately, increase nitrogen utilization, decrease methane emissions, and enhance animal production.

Introduction The antiprotozoal effect of saponins is transitory, as when saponins are deglycosylated to the sapogenin by rumen microorganisms they become inactive (Newbold *et al.*, 1997). We postulated that the substitution of the sugar moiety of the saponin with small polar residues would produce sapogen-like analogues which might be resistant to degradation in the rumen as they would not be enzymatically cleaved. The aim of this study was to test the antiprotozoal effect and the stability of this effect with fifteen hederagenin bis-esters (TSB24, TSB33, TSB34, TSB35, TSB36, TSB37, TSB38, TSB44, TSB45, TSB46, TSB47, TSB50, TSB51, TSB52 and TSB58) and five cholesterol and cholic acid derivatives (TSB39, TSB40, TSB41, TSB42 and TSB43).

Material and methods Protozoal activity was measured from the breakdown of [¹⁴C] labelled bacteria by rumen protozoa as described by Wallace and McPherson (1987). Strained rumen fluid (SRF) from four cows (n=4) was diluted in Simplex-type Salt Solution and incubated at 39°C with [¹⁴C]leucine-labelled *Streptococcus bovis* in the presence or absence of hederagenin, cholesterol and cholic acid derivatives at 0.5 or 1 g/L. Samples were taken at 0, 1, 2, 3 and 4 h of incubation, acidified and centrifuged. Radioactivity released in the supernatant was measured by liquid-scintillation spectrometry and transformed into bacterial breakdown rate (% of the initial radioactivity released per hour). To estimate the stability of the antiprotozoal effect, SRF was diluted 1:2 in artificial saliva solution and 30 mL aliquots were incubated with 0.3 g of diet under CO₂ and at 39°C for 24 h. Samples at different time points (0, 4, 8 and 24 h) were collected for visual assessment of protozoa motility (Newbold, 2010). A stability index, to estimate the persistence of the saponin effect over time, was calculated as the percentage of the motility at 8 h that remained at 24 h. Data were analysed statistically by ANOVA with treatments as main factor and cow as the block term. LSD test was used to establish comparisons between treatments.

Results The acute antiprotozoal effect of hederagenin derivatives was more pronounced than that of cholesterol and cholic acid derivatives (P<0.001). Modifications in the structure of hederagenin, cholesterol, and cholic acid derivatives resulted in compounds with different biological activities in terms of acute effect and stability, although those which were highly toxic to protozoa were not always the most stable over time.

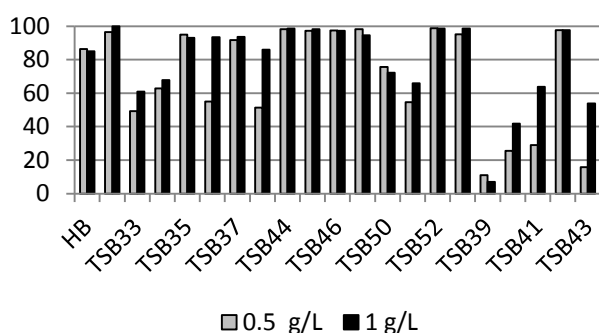


Figure 1 Percentage of decrease in protozoa activity relative to the control in the presence of the derivatives and hederoside B (HB), a natural saponin, at 0.5 and 1 g/L.

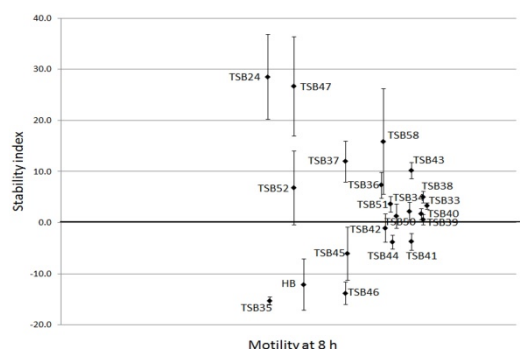


Figure 2 Stability index against motility scores at 8 h in the presence of the derivatives and hederoside B (HB), a natural saponin, at 1 g/L.

Conclusion Most of the hederagenin bis-esters, and in particular TSB24, TSB37 and TSB47, had a persistent effect against rumen protozoa *in vitro*.

Acknowledgements This work has been funded by Innovate UK, Project “Ivy for ruminants”, Ref: 101091.

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Antiprotozoal effect of saponins improves when combined with iminosugars from Stevia

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Application Saponins combined with iminosugars could potentially be used in ruminant diets as effective defaunation agents to, ultimately, increase nitrogen utilization, decrease methane emissions, and enhance animal production.

Introduction The elimination of the ciliate protozoa has been shown to increase microbial protein supply to the host by up to 30% and reduce methane production by up to 11% (Newbold *et al.*, 2015). The antiprotozoal effect of saponins is transitory, as when saponins are deglycosylated to sapogenins by rumen microorganisms they become inactive (Newbold *et al.*, 1997). We hypothesised that the combination of saponins with glycosidase-inhibiting iminosugars might potentially increase the effectiveness of saponins over time by avoiding their deglycosylation in the rumen. The aim of this *in vitro* study was to evaluate the effect of ivy saponins, either alone or combined with a stevia extract rich in iminosugars, on rumen protozoa and fermentation parameters.

Material and methods Ivy fruit extract, rich in saponins (15% DM), obtained after three consecutive extractions with ethanol, petrol ether and n-butanol, was provided by Bangor University. Stevia extract, obtained by ethanol extraction and particularly rich in DMDP (0.2%) and other iminosugars, was provided by PhytoQuest Ltd. Strained rumen fluid from four cows (n=4) was diluted 1:2 in artificial saliva solution. Aliquots of 30 mL were incubated with 0.3 g of diet (barley:alfalfa hay, 60:40) under CO₂ and at 39°C for 24 h. Treatments consisted of control incubations (diet only) and incubations with ivy extract (1 g/L) and stevia extract (2 g/L), either alone or combined. Protozoa numbers, volatile fatty acids and ammonia concentrations were determined and pH measured after 24 h. Data were analysed by ANOVA with treatments as main factor and cow as the block term. Fisher's unprotected LSD test was used to determine comparisons between treatments.

Results Unexpectedly, stevia extract alone had an antiprotozoal effect decreasing pH values and ammonia (-44%) and butyric acid (-17%) concentrations and increasing propionic acid concentration (+35%). The combination of ivy and stevia was more effective in shifting the fermentation pattern towards higher propionate (+38.6%) and lower butyrate (-32.5%) and ammonia (-64.5%) production than ivy and stevia incubated separately.

Table 1 Effect of Ivy (1 g/L) and Stevia (2 g/L) extracts, either alone or combined, on protozoa and rumen fermentation parameters after 24 h of incubation.

	Treatment				SED	P
	Control	Ivy	Stevia	Ivy+Stevia		
pH	6.33 ^d	6.27 ^c	6.17 ^b	6.11 ^a	0.016	<0.001
NH ₃ -N (mmol/L)	9.56 ^d	4.16 ^b	5.34 ^c	3.39 ^a	0.263	<0.001
Total VFA (mmol/L)	98 ^a	99.2 ^a	105 ^{ab}	110.8 ^b	3.56	0.02
VFA (mmol/mol)						
Acetate	654 ^{bc}	627 ^a	656.8 ^c	642.8 ^b	5.61	0.002
Propionate	171 ^a	219 ^b	216.5 ^b	237.0 ^c	3.6	<0.001
Butyrate	133.5 ^c	120.8 ^b	94.0 ^a	90.4 ^a	1.895	<0.001
BCVFA	21.0 ^c	13.9 ^b	14.0 ^b	11.1 ^a	0.291	<0.001
Protozoa (log cells/mL)	4.97 ^c	4.61 ^b	4.26 ^a	4.30 ^a	0.088	<0.001

^{a-d} Means with different superscript differ (n=4).

Conclusion Stevia extract rich in iminosugars had an antiprotozoal effect on its own. The antiprotozoal effect of ivy saponins combined with an iminosugar-rich stevia extract was greater than that of ivy saponins alone, shifting the fermentation towards higher propionate and lower butyrate and ammonia concentrations. Further *in vivo* trials are needed to confirm the observed effects *in vitro*.

Acknowledgements This work has been funded by Innovate UK, Project "Ivy for ruminants", Ref: 101091.

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Impact of soil-enhancers and plant bio-stimulants on the microbial profile of the maize forage: A glasshouse experiment

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Application Increased populations of yeasts and moulds on forage crops can cause many diseases and can lead to silage spoilage via poor aerobic stability and the production of mycotoxins. The application of these products is expected to increase growth and yield of the crops and improve the nutritional value and aerobic stability of silage.

Introduction Poor aerobic stability and mycotoxins cause significant silage spoilage (Wilkinson and Davies, 2013). Environmental conditions (heat, humidity), basal soil fungal communities as well as insect infestation, influence the fungal population of the forage crop, which subsequently will influence aerobic stability of the silage produced and its mycotoxin load. Soil-enhancers and plant bio-stimulants are applied during the growth of the crops in order to promote the growth and health of plant roots, enhance nutrient availability in the soil, contribute to a healthier microbiome, increase the yield and improve nutritional content and digestibility. Their potential to increase nutritional value and aerobic stability of silage warrants further investigation. The aim of the current study was to investigate the impact of a soil-enhancer (SE) derived from microbial fermentation and a plant bio-stimulant (PB) which is a blend of micronutrients and fermentation growth factors, on soil, forage and silage quality under controlled conditions; here we present the microbial profile of the crop.

Material and methods The two products (SE and PB) were tested on forage maize. The experiment was conducted in a glasshouse at Rothamsted Research, North Wyke, Okehampton, Devon, UK (NW) and the soil was collected from NW farm field based on its suitability for growing the crop type (Crediton series, a free draining permeable soil over a soft sandstone). There were 40 pots in total (each treatment contained 10 pots); SE was added at sowing to the soil (2 L/ha) while PB was sprayed directly onto the grown crop (1 L/ha). Experimental treatments consisted of either (i) Untreated control, (ii) Soil treated with SE, (iii) Plants treated with PB, (iv) Soil and plants treated with SE and PB respectively. Half of the plants were ensiled into laboratory-scale mini silos for 60 d. Microflora from the crops was cultivated in growth media with focus on Enterobacteria spp. (ENB), Pseudomonas spp., Lactic acid bacteria (LAB) and yeasts. The following media were used; De Man De Rosa Sharpe (MRS) for LAB (pour plates), Violet Red Bile for ENB (pour plates), Potato Dextrose for yeasts (spread plates) and Pseudomonas-CFC for Pseudomonas spp. (spread plates). All plates were incubated at 30 °C aerobic conditions except for PDA plates which were incubated at 23 °C for 3 d, after which colonies were enumerated. Data were statistically analysed from Log₁₀ transformed means using GenStat18 program (Lawes Agricultural Trust). One-way analysis of variance (ANOVA) was used for the statistical analysis.

Results Mean counts of LAB and Pseudomonas spp. were lower in the control than other treatments, whereas mean counts of ENB and yeasts were higher in control than in the other treatments. The lowest mean counts of yeasts were observed in crops treated with SE while mean counts observed in crops treated with PB and SE+PB are similar. Mean counts of Pseudomonas spp. were the highest in crops treated with PB and SE+PB.

Table 1 Means and standard deviation in counts of ENB, LAB, Pseudomonas Spp. and Yeasts in maize crops (Log₁₀ colony forming units g⁻¹ fresh weight). The dilutions used for colony count were 10⁻⁴, 10⁻⁴, 10⁻⁴ and 10⁻³ respectively. Subscripts are used to indicate the significant difference using ANOVA (a=highest, c=lowest)

Average Log (CFU/g)	Control	SE	PB	SE+PB	S. E. D	P
ENB	6.42 ^a	6.27 ^b	6.20 ^b	6.09 ^c	0.054	P=0.01
LAB	5.96 ^b	6.38 ^a	6.60 ^a	6.62 ^a	0.189	P<0.05
Pseudomonas	7.25 ^c	7.35 ^b	7.49 ^a	7.51 ^a	0.010	P<0.01
Yeasts	6.29 ^a	6.06 ^c	6.17 ^b	6.19 ^b	0.013	P<0.01

Conclusion The increased mean count of LAB along with the decreased mean count of yeast colonies suggests that SE, PB and SE+PB promotes a more desirable microbial community for ensiling maize. This is important as increased LAB numbers indicate a more stable ensiling microbial environment and the production

of high quality silage. Lower mean count of yeasts results in decreased production of ethanol and decreased dry matter loss. Decreased mean count of ENB increases the available nutrients for LAB during the first stage of ensiling. Each of these factors contributes to a rapid decrease in pH and a faster establishment of anaerobic conditions in the silage. However, further research is required in order to monitor if this community change carries through the ensiling process, how the treatments interact with other microorganisms and how the treatments interact with each other.

Acknowledgements The authors acknowledge and thank the funding from Alltech.

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Effect of milk replacer and colostrum alternative on the development of the rumen fungal community

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Application Early life interventions such as milk replacers can achieve similar animal performance at weaning compared to lambs raised with the ewe but can limit the rumen microbial development in terms of fungal diversity. Thus, special care must be taken to maximize feed intake and rumen microbial development before weaning in lambs fed milk replacer in order to improve feed utilization and animal productivity later in life.

Introduction Nutritional manipulation of the rumen microbiome to improve productivity and health are limited by the resilience of the ecosystem once established in the mature rumen. It has been suggested that early life interventions may allow manipulation of the rumen microbial community with the potential to produce lasting effects into adult life. The use of colostrum alternatives and milk replacer has been promoted in recent years to maximize the number of lambs weaned, but their effects on the rumen microbiota and animal productivity beyond weaning are still unknown. Fungi are primary colonizers of plant material in the rumen, thus the establishment of the rumen fungal community is key for efficient feed utilization in pasture based ruminant systems. This study investigated the effect of using colostrum alternative and milk replacer on the development of the rumen fungal community and the implications later in life.

Material and methods A total of 24 pregnant ewes carrying triplets were used. Within each triplet set, lambs were randomly allocated to the experimental treatments: 1) NAT: natural lactation (ewe's). 2) N-ART: ewe's colostrum and milk replacer and 3) ART: colostrum alternative and milk replacer. After birth ART lambs received 50g of colostrum alternative (Lamb Volostrum, Volac[®]), then N-ART and ART were fed with milk replacer *ad libitum* (Lamlac Instant, Volac[®]), while NAT lambs were kept with the ewes. During lactation all lambs had free access to ryegrass hay and creep feed. All lambs were weaned at 45d of age and grouped together in the same ryegrass pasture for fattening. Animal growth was monitored throughout and rumen samples were taken at weaning (45d) and at the end of the fattening (154d). Rumen concentration of anaerobic fungi was determined by qPCR and the structure and diversity of this community in terms of Richness, Shannon, Evenness and Simpson indexes was determined based on the sequence of the D1 region of the large sub unit of rDNA using Ion Torrent Next Generation sequencing (Detheridge *et al.*, 2016). Data were analysed by ANOVA blocking by triplet set.

Results No differences across treatments were observed prior to weaning in terms of animal productivity and anaerobic fungi concentration; however NAT lambs at weaning had a more diverse rumen fungal community than lambs fed milk replacer in terms of Richness, Shannon, Evenness and Simpson indexes. At weaning NAT lambs had a greater abundance of the early forage colonizer Neocallimastix, whilst N-ART and ART lambs had a similar fungal population with higher levels of Buwchfawromyces. When animals were fed on pasture, a substantial increase in the levels of Orpinomyces (long life cycle) was observed across treatments suggesting a longer rumen retention time. NAT lambs maintained the greater fungal diversity during the fattening period and may explain their daily live weight gain during this phase.

Table 1 Animal performance and rumen fungal diversity

	WEANING					FATTENING				
	NAT	N-ART	ART	SED ¹	P	NAT	N-ART	ART	SED ¹	P
Weight (kg)	18.6	18.9	18.3	0.79	ns	39.0 ^a	37.2 ^b	35.6 ^c	0.93	**
Daily gain (g/d)	326	332	318	14.2	ns	179 ^a	153 ^b	151 ^b	5.23	***
Anaerobic fungi ²	5.26	5.36	5.38	0.249	ns	5.93	6.17	6.01	0.129	ns
Fungal diversity										
Richness (OTU)	61.5	47.5	49.6	7.55	†	46.4 ^a	34.2 ^b	40.8 ^{ab}	4.49	*
Shannon	1.61 ^a	0.98 ^b	1.13 ^{ab}	0.253	*	1.24 ^a	0.94 ^b	1.15 ^{ab}	0.127	*
Evenness	0.39 ^a	0.24 ^b	0.27 ^b	0.054	*	0.33	0.27	0.31	0.029	†
Simpson	0.40 ^b	0.63 ^a	0.60 ^a	0.080	**	0.45	0.56	0.48	0.050	†

¹Within a row means without a common superscript differ.

²Rumen concentration of anaerobic fungi (log rDNA fungal copies/mg DM)

*** P<0.001; ** P<0.01, * P<0.05, † P<0.1, ns not significant.

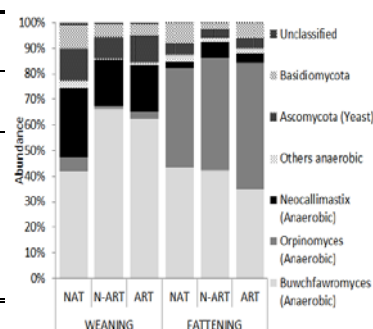


Figure 1 Rumen fungal distribution

Conclusion Use of colostrum alternative and milk replacer facilitated the successful artificial rearing of lambs and gave similar weaning weights to lambs reared with the ewe. However, natural lactation promoted a greater microbiological development of the rumen fungal community at weaning which was maintained later in life and may be associated with higher daily live weight gain during fattening.

Acknowledgements This trial was funded by Volac Ltd., WISE Network (ERDF) and BBSRC (BBS/E/W/10964A01).

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Feed restriction provides a niche environment for metabolic activity of the *Methanobrevibacter gottschalkii* clade in the bovine rumen

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Application Feed restriction directs H₂ utilisation in favour of CH₄ production and leads to a large increase in relative abundance of *mcrA* transcripts from one of the two major methanogen clades in the rumen and a large decrease in the other.

Introduction Methane (CH₄) generated from agriculture is now the dominant source of anthropogenic CH₄ emissions with enteric fermentation from livestock accounting for 40% (Tubiello, *et al.* 2014, Sauniois, *et al.* 2016). A period of feed restriction followed by *ad libitum* feeding is widely used in livestock management to reduce feed costs. Following a period of dietary restriction, resumption of normal feeding leads to accelerated growth known as compensatory growth. Previous work showed that dietary restriction caused a dramatic increase in rumen acetate:propionate ratios which showed strong positive correlations with a large increase in relative abundance of 16S DNA of one of the two major methanogen clades and a large decrease of 16S DNA of an abundant uncultured Proteobacteria species (putatively Succinivibrionaceae) (McCabe, *et al.* 2015). In the present study we looked at RNA from the same feed-restricted rumen samples to determine methanogenic activity at the level of gene transcription. Methyl co-enzyme reductase (MCR) is a multi-subunit enzyme which is unique to methanogenic archaea and catalyses the final step in CH₄ formation and the initial step in anaerobic CH₄ oxidation (Wongnate and Ragsdale 2015). The MCR subunits are coded for by the *mcr* gene operon which usually comprises the genes *mcrA*, B, C, D and G. Transcription of the *mcrA* gene has previously been shown to be correlated with CH₄ production (Freitag and Prosser 2009). To determine the contribution of different bovine rumen methanogens to methanogenesis during dietary restriction, we conducted *mcrA* cDNA amplicon sequencing and analysed the abundance of different *mcrA* cDNA sequences and their phylogenetic origin.

Material and methods Holstein Friesian bulls (n=60) were divided into two groups and subject to one of two dietary regimes (n=30). One group was subjected to an *ad libitum* diet (group A) and the other group was subjected to a restricted diet (group R) for a total of 125 days. After this period, 15 animals from each group (R and A) were slaughtered. The remaining 15 animals from both group R and group A were then offered an *ad libitum* diet for a further 55 days and then slaughtered after this period. These animals were designated RA and AA. All animals were offered the same diet consisting of 70:30 concentrate:forage, with R animals receiving a restricted ration compared to A animals. Rumen digesta samples were collected immediately after slaughter, snap frozen in liquid nitrogen and then stored at -80°C. *mcrA* cDNA libraries were constructed following extraction of RNA from rumen liquid digesta and sequenced on the Illumina MiSeq platform. cDNA sequences were quality assessed and trimmed and then translated into amino acid sequences and clustered at 97% identity. Evolutionary history was inferred by the maximum parsimony method using MEGA7, comparing a reference sequence from each cluster against classified MCRA protein sequences. A non-parametric statistical analysis between treatment groups was conducted with R. Diversity metrics were calculated using PRIMER7.

Results A total of 17 of the 25 MCRA clusters were differentially represented amongst the four treatment groups. Diversity of relative abundance of functionally active methanogens was significantly reduced (P<0.001) in treatment R relative to the three *ad libitum* treatment groups (A, AA, RA). *mcrA* transcripts assigned to the genus *Methanobrevibacter* (Mbr.) were the most abundant in all treatment groups and were significantly more abundant in group R than in groups A, AA and RA (P<0.05). The abundance of *mcrA* transcripts assigned to the Mbr. *gottschalkii* clade was increased in R relative to all other treatments groups (P<0.001) whereas the *mcrA* transcripts assigned to the Mbr. ruminantium clade were dramatically reduced in R compared to groups A, AA and RA (P<0.0001). Four MCRA clusters were classified as the family Methanomassiliicoccaceae (Mss) and relative abundance of *mcrA* transcripts assigned to all four of these clusters was significantly reduced (P<0.01) in the restricted treatment group in comparison to the *ad libitum* treatment groups.

Conclusion In feed-restricted animals, the methanogenic activity of Mbr. *gottschalkii* increased and the methanogenic activity of the Mbr. ruminantium clade and members of the Methanomassiliicoccaceae family decreased. This study shows that there are large changes in the composition of methanogenically active species in the rumen depending on feed intake. This has major implications for targeted CH₄ mitigation approaches such as anti-methanogen vaccines.

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Impact of diet on the diversity of resistance and pathogenicity related genes in the rumen microbial community of apparently healthy cattle

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Application As diet has a major impact on rumen microbial community composition and potentially on the functional genes content, it is possible that certain diets will suppress the abundance of antibiotic resistance (AR) genes or Proteobacteria, thus benefiting human and animal health.

Introduction Diet change is known to impact the composition and functionality of the rumen microbiome, potentially generating a stress on the rumen microbiome community. In addition, it could have a negative repercussion on the overall health and production performance of cattle. This unbalanced microbial community is characterized by a “bloom” of specific microbial populations, mostly Proteobacteria known to be one of the dominant phyla in rumen. Many pathogenic bacteria classified as Proteobacteria, possess a wide range of functional genes to resist and survive host or microbial attacks and colonize the rumen. Therefore, the overall aim of our research was to improve our knowledge on the diversity of resistance and pathogenicity related genes in the rumen microbial community of apparently healthy cattle fed with “forage” diet containing 500 g/kg concentrate (dry matter basis) or “concentrate” diet (900 g/kg concentrate) using metagenomics.

Material and methods Eight animals balanced for breed type (4 Aberdeen Angus sires and 4 Limousin sires) and diet were evaluated using forage or concentrate diets without antibiotic addition (Rooke *et al.* 2014). *Post-mortem* rumen digesta samples were used and total DNA was extracted prior to metagenomics analysis. We used the same set of data obtained as Roehe *et al.* (2016) that identified functional microbial genes based on the KEGG genes database. Canonical variate analysis (CVA) helped to identify the main factor (e.g. diet, breed type, age or live-weight) explaining the difference in functional gene composition. Independent samples t-tests determined the difference in the abundance of the functional genes between treatments. Both analyses were carried out using Gen-Stat (16th edition).

Results Within the 193 genes associated with resistance, colonization or pathogenicity functions identified from the metagenomics data, changes in AR genes (e.g. macrolide efflux protein) and type IV pilus assembly protein Pil genes known to promote adhesion to epithelial cells in Proteobacteria, were significant ($P < 0.05$) between diets. Interestingly, each sample showed a specific pattern of genes. CVA confirmed that diet change was the only factor tested significantly correlated with the functional gene composition (no overlapping between the circles of confidence) and explaining difference observed. No significant host effects were detected.

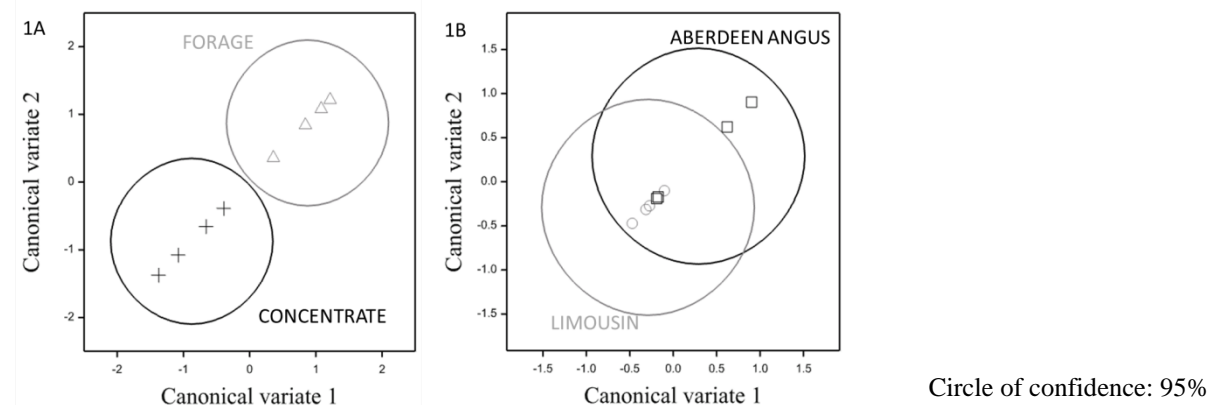


Figure 1 CVA result based on diet (1A) or breeding (1B) effects on the functional gene composition.

Conclusion Diet has an impact on the composition of genes associated with resistance and pathogenicity activities in the rumen microbiome. Concentrate diet mainly increased the abundance of macrolide efflux protein related gene while forage had a broader effect, increasing the abundance of different functional genes. Other factors such as stress should be investigated to explain the variation in the functional gene composition observed between the cattle.

Acknowledgements This project was funded by BBSRC, REF, the Scottish Government, Defra and the UK Agricultural Greenhouse Gas Inventory Research Platform.

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Performance characteristics of broiler chickens fed diets supplemented with phytobiotics as alternative for antibiotics

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Application Dietary supplementation of phytogenic feed additives (garlic and ginger) at the rate of 10g/kg in combination have positive impact on final weight, weight gain and feed conversion ratio of broiler chickens.

Introduction For many years, the control of pathogenic microbiota has solely relied on the continuous use of synthetic antibiotics. However, emergence of resistant gastrointestinal microorganisms and increasing concern of consumers for drug residues in poultry products have led to the production of alternatives like phytobiotics to prevent the gastrointestinal micro-organisms. Some of these phytogenic feed additives have been successfully incorporated into the feeding standard of poultry birds without any deleterious effect or toxic residues (Oyekunle and Owonikoko, 2002). The study is therefore, designed to determine the performance characteristics of broiler chickens fed diet supplemented with phytobiotics as alternative to antibiotics.

Material and methods Two hundred and sixteen day-old broiler chicks were randomly allotted to six dietary treatment groups of 36 birds in a complete randomized design. Each treatment group was further divided into three replicate groups of 12 birds each. The treatments consisted of diets containing no embaceryl and phytobiotics (control); diets with 5% embaceryl (T1); 5% garlic bulb powder (GBP) and ginger rhizome powder (GRP) (T2); 7.5% GBP + GRP (T3); 10% GBP + GRP (T4) and 12.5% GBP + GRP (T5). GBP and GRP were included at ratio 1:1 into a compounded commercial concentrate diet. Performance characteristics were determined through assessment of live weight, feed intake, weight gain and feed conversion ratio. The weight of the birds were taken on their arrival to the site, subsequent weight and weight of feed consumed were taken on weekly and daily basis for eight weeks All data collected were analysed using Analysis of Variance (SAS, 2005), while significant ($P < 0.05$) differences were compared using Duncan's Multiple Range Test (Duncan, 1955).

Results

Table 1 Performance characteristics of broiler chickens fed diet supplemented with phytobiotics (Garlic and Ginger)

Parameters	CONTROL	T1	T2	T3	T4	T5	SEM
F.I (g)	4360 ^c	4361 ^c	4381 ^a	4351 ^d	4372 ^b	4291 ^e	115
FW(g)	1283 ^c	1340 ^b	1360 ^b	1377 ^{ab}	1500 ^a	1307 ^{bc}	86.25
WG(g)	1243 ^c	1300 ^b	1320 ^b	1337 ^b	1460 ^a	1267 ^{bc}	82.25
FCR	3.51 ^a	3.35 ^a	3.39 ^a	3.34 ^a	2.99 ^b	3.40 ^a	0.23

Means with same superscripts on the same row are not significantly different

F.I = Feed Intake; FW = Final Weight; WG = Weight Gain; FCR = Feed Conversion Ratio.

T1= EMB; T2= GBP+GRP at 5%; T3 = GBP+GRP at 7.5%; T4= GBP+GRP at 10%; T5 = GBP+GRP at 12.5%.

EMB=Embaceryl (antibiotic); GBP=Garlic Bulb Powder; GRP=Ginger Rhizomes Powder.

Conclusion Dietary supplementation of garlic bulb powder and ginger rhizomes powder at a rate of 10g/kg in combination has positive impact on performance of broiler chickens. It was concluded that supplementation at this level may replace in-feed antibiotics without any deleterious effect on the general performance of the broiler chickens.

Acknowledgements The authors gratefully acknowledge the funding from Tertiary Education Trust Fund (TETFund).

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Faecal and urinary elimination of antibiotics and antibiotic resistance genes in dairy cows following intramammary infusion

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Application Manure from cows receiving intramammary antibiotic preparations should be segregated and treated to remove antibiotic residues and antimicrobial resistance genes.

Introduction There are several lines of evidence that demonstrate an association between antibiotic use in food animals and antibiotic resistance in bacteria pathogenic to humans. Appropriate risk assessment and development of efficient mitigation strategies are limited, however, due in part to a lack of data on the elimination of antibiotics and associated resistance genes from animals. The objectives of this study were to investigate 1) faecal excretion and urinary disposition kinetics of cephalosporin and lincosamide antibiotics commonly used on dairy farms, 2) faecal excretion of antibiotic resistance genes (ARG), in dairy cows following intramammary infusion.

Material and methods Three end of lactation dairy cows were administered cephalosporin antibiotic (cephapirin; 1200 mg/cow) and 3 lactating cows were administered 2 doses of lincosamide antibiotic (pirlimycin; 50 mg/cow; 24 h apart) intramammary. Three lactating cows received no antibiotic. Faeces and urine were collected immediately before antibiotic infusion (0 h) and at 2, 4, 8, 12, 16, 24, 36, and 72 h post-cephapirin infusion, at 2, 4, 8, 12, 16, and 24 h after the 1st dose of pirlimycin, and at 2, 4, 8, 12, 16, 24, 36, 48, and 72 h after the 2nd dose of pirlimycin. Additional faecal samples were collected on d 1, 3, 5, 7, 14, 21, and 28 following treatment to quantify ARG in faeces. Immediately following collection, faecal and urine samples were stored at -20°C. Samples were extracted and cleaned by solid phase extraction (SPE), and subjected to UPLC-MS/MS quantification of antibiotics. Urine concentration-time data were fitted into a non-compartmental model using PKSolver (Zhang *et al.*, 2010) with extravascular input to estimate kinetic parameters. Elimination rate constant (K_{el}) of antibiotics in urine was calculated using regression analysis (PROC REG in SAS) of antibiotic concentrations in urine after maximum concentration. Abundance of ampC, tetO, tetW, and 16S rRNA genes were determined using qPCR (Aminov *et al.*, 2001; Shi *et al.*, 2013; Suzuki *et al.*, 2000). Elimination kinetic parameters, maximum concentration (C_{max}), and hourly excretion data were reported as means and standard errors of mean estimated using PROC MEANS in SAS. Data on ARG were analysed (PROC GLIMMIX in SAS 9.2) using a mixed model with antibiotic treatment and day as fixed effects, and cows nested within treatments as random effect. Prior to statistical analyses, non-normal data were logarithmically transformed to achieve normality and ARG data for day 0 were used as a covariate.

Results The elimination half-life ($t_{1/2}$), C_{max} in urine, time to reach C_{max} (T_{max}), and K_{el} for cephalosporin were 14.9 h, 156 µg/L, 12.0 h post-infusion, and 0.048 1/h, respectively. For the 1st and 2nd doses of pirlimycin, $t_{1/2}$, C_{max} , T_{max} , and K_{el} were 11.6 and 20.2 h, 221 and 327 µg/L, 12.0 and 4.00 h post-infusion, 0.039 and 0.038 1/h, respectively. Peak faecal concentration of pirlimycin (249 µg/kg) was attained at 16 h post-2nd dose. Pirlimycin-treated cows had a lower abundance of 16S rRNA compared to control or cephalosporin-treated cows. The relative abundance of ampC was influenced by day ($P = 0.04$) with day 3 post-treatment being greater compared to day 5 ($P = 0.06$) and day 28 ($P = 0.09$) post-treatment. The absolute abundance of faecal tetW was not influenced by antibiotic treatment, but tended to increase over time ($P = 0.11$). The relative abundance of tetW was affected by antibiotic treatment ($P = 0.01$) and day ($P = 0.05$). Pirlimycin-treated cows had greater relative abundance of faecal tetW compared to control or cephalosporin-treated cows.

Conclusion Excretion of antibiotic resistance genes increases shortly after antibiotic treatment followed by a gradual return to the baseline level of resistance which seems to be a normal part of core resistome in the cow's digestive tract. Prolonged excretion (days rather than hours) of antibiotics suggests that manure from cows receiving intramammary antibiotic preparations should be segregated and treated to remove antibiotic residues.

Acknowledgements Agriculture & Food Research Initiative Competitive Grant no. 2013-67021-21140 from the USDA National Institute of Food and Agriculture.

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Factors that motivate farmers to invest in automated monitoring of reproductive status in dairy cows: Lessons from France and Scotland

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Application As the importance of precision farming in generating practical solutions for dairy production increases, feedback from farmers on the factors that motivate them to invest in different technologies is vital.

Introduction Monitoring of reproductive performance is an important aspect of dairy cow production. Although the use of electronic oestrus detectors is already widespread, information on what motivates farmers to either use or not to use such devices is conflicting (Borchers and Bewley, 2015). A survey was conducted in France and Scotland to better understand the motivations; farmer satisfaction; impact of the sensors on farm work; doubts concerning use of automated oestrus detection; and elements that farmers who do not have the technologies would like to know prior to investing in such technologies.

Material and methods One-to-one questionnaire interviews were conducted between 2014 and 2015. On average the interviews lasted one and half hours to gain an in-depth understanding in the farmer's responses. A total of 123 randomly selected farmers, with and without technologies, were interviewed in the two countries. Of the respondents, 52 were equipped with different reproductive performance monitoring equipment while 71 were not equipped. In France the survey was conducted in Doubs (east of France, n = 32) and in Brittany (north west of France, n = 51) while in Scotland, the survey was conducted in South West Scotland (n = 40). Data analysis was conducted using frequencies, percentages and analysis of variance where appropriate.

Results In both countries, there was no difference in herd size, milk yield, and number of cows per worker between farmers with- and without technologies (Table 1). For the majority (83%) of farmers equipped with technologies, the initial motivation was to improve reproductive management. Only 17% of the farmers invested in reproductive performance monitoring technologies in order to improve working conditions. The highest satisfaction came from easiness of use, after sales service, and reliability while the highest impact was on farmers' mental load, farm schedule flexibility and remote management. For farmers who had no equipment, the most important aspects that would motivate them to invest in equipment were reliability, price, access to information on the operating principles, and compatibility with other farm equipment. The main doubts to invest were due to price, cost-benefit ratio, lack of need, and reduced human to animal relationship. The majority of farmers indicated interest in having a guided tour of the equipment in operation before investing.

Table 1 Description of surveyed farms in France and Scotland

Survey Location	Number of cows		Milk yield (kg/cow/year)		Cows/worker	
	N.E.	E	N.E.	E	NE.	E
Brittany	30 to 160	30 to 135	4 900 to 9,500	7, 000 to 10,400	28 to 85	22 to 120
Doubs	29 to 143	39 to 192	5600 to 11,000	6,700 to 12,000	19 to 48	24 to 49
Scotland	70 to 1,100	100 to 916	4500 to 10,000	6,800 to 11,000	28 to 193	25 to 131

N.E. = Not equipped, E = Equipped

Conclusion In contrast to previous reports indicating that dairy farmers adopt different technologies predominantly because of increasing herd size, in both the surveyed countries, improving reproductive performance was a much bigger motivation to invest in technology. The highest impact for technology users was on improved farmers' mental work load.

Acknowledgements Automated Monitoring of Reproduction: Innovations and Applications for Dairy Farming (CASDAR MARIAGE) financed by the French Agricultural Ministry and SRUC Strategic Research Programme funded by the Scottish Government.

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A comparison of two cow-side reproductive performance monitoring technologies in dairy cows

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Application Cow-side reproductive performance monitoring technologies in dairy production are important as decision support systems. Introducing a quantitative aspect to otherwise subjective measurements improves the accuracy in the comparison of the technologies

Introduction Reproductive failure remains one of the main reasons for culling in dairy herds. Prolonged inter-calving periods occasioned by failure or delay in conceiving reduces the lifetime productivity of the cows and increases the herd replacement rate. Effective and accurate oestrus detection is necessary to ensure timely service thereby increasing the chances of conception. Visual observation is one of the most used methods of oestrus detection. However, it is time consuming and requires at least three, 30 minute sessions spread out throughout the day (Roelofs *et al.* 2005). With changes in dairy systems and the need to improve fertility, different reproductive performance monitoring technologies are being employed. The choice of a suitable technology is mainly based on accuracy, ease of application, cost, automation, continuous animal monitoring, housing system and available labour (Firk *et al.* 2002). The objective of this study was to compare the effectiveness of two proprietary cow-side oestrus detection technologies. Cow-side oestrus detection technologies are rapid-results test carried out on the farm that aid on-the-spot decision making.

Material and methods Two oestrus detection methods were selected based on their function. P4 Rapid is a lateral flow technique based on milk Progesterone (P4) (Ridgeway science, UK), and EstroTECT™ is a mount detector (Rock way Inc., Spring Valley, WI). This study was carried out at the SRUC Dairy Research and Innovation Centre between April and November 2016. A total of 78 dairy cows between 1st and 7th parity were milk sampled three times weekly from 21 days *post partum* until 15 days into a confirmed pregnancy. An aliquot of the collected milk sample was used to run the P4 rapid test and the rest quantified for progesterone using ELISA as per manufactures instructions. (M-plate, Ridgeway Science, UK). A total of 3000 observations were made and a progesterone threshold of $\leq 1.5\text{ng/ml}$ was set to determine true oestrus periods. P4 Rapid results were analysed by comparing the test line to the control line as per the manufactures instructions. A modified categorisation was used for the results denoting them as darker, same, lighter, faint or absent corresponding with low to high progesterone levels, respectively. EstroTECT™ Heat Detectors were also first applied on day 21 *post-partum* and replaced after each oestrus event. On the day of oestrus the EstroTECT™ Heat Detectors were removed and analysed by noting the degree of exposure of the luminescent colour as per manufactures instructions. These were graded as intact (no exposure), partial (<50% exposure), full (> 50%) or lost (not recovered). Intact and partial were not considered as oestrus events while full and lost were considered as oestrus events. In order to objectively quantify the degree of luminescence, the authors further subdivided the EstroTECT™ Heat Detectors into 12 equal grids (Grid method) which enabled a numerical calculation of degree of exposure. The performance of each technique was determined through analysis of sensitivity, specificity and positive predictive values, while the relationship among the different techniques was determined using Spearman rank correlation analysis.

Results For P4 Rapid, an accuracy of 86.71% was achieved with a sensitivity, specificity and positive predictive value of 87.91%, 86.02% and 78.30% respectively. There was a negative relationship between ELISA P4 levels and P4 rapid outcome ($r = -0.79$, $P < 0.05$). There was a positive relationship between ELISA P4 levels and EstroTECT™ Heat Detectors outcome ($r = 0.054$, $P < 0.05$). For EstroTECT™ an accuracy of 63% was achieved with a sensitivity, specificity and positive predictive value of 11.91%, 92.69% and 46.94% respectively. When the Grid method was used, there was a mean luminescence of $82\% \pm 25.14$ on the day of oestrus. With this method, the sensitivity, specificity and positive predictive value were 86.04%, 24% and 79.56% respectively. The Grid method therefore improves the sensitivity of EstroTECT™ by 70% thereby increasing its robustness as a cow-side oestrus detection test.

Conclusion Based on their performance, P4 rapid was better at detecting oestrus than the EstroTECT™. However, performance of the EstroTECT™ was improved when analysed by the Grid method. This Grid method has potential for use by both the farmer and inseminators in selecting animals to be presented for service

Acknowledgements The Bill and Melinda Gates Foundation for funding, SRUC Dairy Research and Innovation Centre and the University of Edinburgh

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The effect of temperature on the activity budget of dairy cows measured using novel sensor technology

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Application Understanding the response of cattle to potential heat stress situations will enable the development of mitigation strategies. Such strategies in future could be based on sensor monitored changes to behaviours such as activity budget.

Introduction Heat stress impacts on both production and behaviour in dairy cattle (Allen *et al.*, 2015). To date much of the research into heat stress has taken place in countries with hot climates however with increasing global temperatures it is likely that the UK will experience more frequent days which exceed the 21.5°C at 80% RH suggested by Bryant *et al.*, (2007) to be capable of inducing heat stress. This study aimed to monitor the behaviour of a group of high yielding dairy cattle during periods of potential heat stress.

Material and methods Four hot weeks (at least 3 consecutive days at >21.5°C and minimum temperature not <17°C) and four cool weeks (at least 3 consecutive days at >17°C and the maximum temperature not >21.5°C) were identified from the mean daily temperatures logged by four tinytag temperature loggers installed in the cubicle house on a dairy farm in SW England. All cows in the shed were fitted with multi-system sensor which logged position, activity and orientation, of which 55 were present for all weeks. A support vector machine (a machine learning algorithm) was used to classify feeding, standing and lying behaviours. The impact of temperature and stage and level of production on behaviour were analysed using linear modelling (GENSTAT v 18).

Results Feeding time was not influenced by temperature. Daily lying time decreased and standing times increased in hot weeks (Table 1). First parity animals had different lying and standing times to older cows. Cows with high milk yields had reduced standing times and a non-significant trend towards longer lying times.

Table 1 Multivariable linear regression models showing the effect of temperature, days in milk, parity and milk yield on total daily feeding lying and standing times

Variable	Daily feeding time		Daily lying time		Daily standing time	
	Coefficient (s.e.)	P	Coefficient (s.e.)	P	Coefficient (s.e.)	P
Constant	5.86		13.23		2.14	
Temp (hot)						
Cold	-0.011 (0.059)	0.847	0.220 (0.081)	0.007	-0.239 (0.072)	<0.001
Days in milk (0-100d)						
101-200d	-0.050 (0.068)	0.464	-0.052 (0.094)	0.579	0.107 (0.083)	0.197
>200d	-0.262 (0.088)	0.003	0.287 (0.121)	0.019	-0.018 (0.107)	0.864
Parity (1 st)						
2 nd	-0.122 (0.104)	0.240	-0.603 (0.143)	<0.001	0.730 (0.126)	<0.001
3 rd and above	-0.116 (0.093)	0.213	-0.367 (0.129)	0.005	0.482 (0.113)	<0.001
Lactation Yield						
(<10230)						
10230-11766	-0.711 (0.982)	0.469	0.179 (0.135)	0.186	-0.107 (0.119)	0.370
11766-13245	-0.044 (0.101)	0.662	0.249 (0.140)	0.076	-0.295 (0.123)	0.017
>13245	-0.871 (0.981)	0.375	0.228 (0.135)	0.092	-0.319 (0.119)	0.008
Model significance		0.029		<0.001		<0.001

Conclusion The drive to eat for a high yielding dairy cow is significant and may explain why feeding times are not affected by temperatures in this study. It is possible that cows modify the time at which they feed to allow them to maintain feed intake. Lying and standing behaviour were affected by temperature, parity and milk yield.

Acknowledgements Our thanks go to all the staff at the study farm and to Omnisense for the technology support.

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Evaluation of a 7 day fixed timed artificial insemination protocol for UK dairy heifers. Is a second insemination beneficial?

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Application Inseminating heifers twice following a 7 day progesterone based controlled internal drug release (CIDR) synchronisation protocol (at 48 and 72 hours after CIDR withdrawal) is a commonly used practice which is believed to lead to an increased number of pregnancies per synchronised heifers. Results presented here suggest that inseminating heifers once (56 hours after CIDR withdrawal) will lead to similar results while saving the farmer the cost associated with conducting a second insemination (labour, second dose of semen).

Introduction Fixed timed artificial insemination (FTAI) programmes for dairy heifers can be economically efficient, especially in herds where heat detection is suboptimal. The 7 day CIDR synch programme is commonly used in the UK with several farmers inseminating heifers twice (at 48 and 72 hours after CIDR withdrawal) in order to achieve higher numbers of pregnancies per synchronised heifers. Our objective was to determine if serving heifers using FTAI twice (at 48 and 72 hours after CIDR withdrawal) after a 7 day CIDR synchronisation protocol would result in more pregnancies per synchronisation (P/S) comparing to serving them only once (56 hours after CIDR withdrawal).

Material and methods Holstein and Holstein-Friesian heifers (n= 237) from 5 farms in the South West of England were randomly assigned, within each farm, to one of two treatment groups. Both groups received a CIDR (Eazi-Breed CIDR Cattle, Zoetis) on day 0, an intra-muscular injection of 0.15 mg d-cloprostenol (2ml Prellim, Zoetis) on day 6, and the CIDR was withdrawn on day 7. Heifers were then inseminated by the same person and using the same bull, and semen type. Animals in the 2AI Group were served twice on the morning of day 9 and 10 as near to 48 and 72 hours after CIDR withdrawal as possible. Group 1AI animals were served once on the afternoon of day 9 as near to 56 hours after CIDR withdrawal as possible. Measurements of withers height, body condition score and heart girth weight were taken at day 0. The maximum diameters of follicles and corpora lutea were recorded at both day 0 and day 6 using ultrasonography. Multivariable logistic regression modelling was used to assess the associations of herd, treatment group, age, withers height, estimated weight, CL size at day 0 and day 6, dominant follicle size at day 0 and day 6, presence of CL at day 0 and day 6 and BCS category with P/S.

Results There was no statistically significant difference in P/S between the two treatment groups (0.55 ± 0.046 for group 2AI and 0.60 ± 0.045 for group 1AI, $P = 0.510$). Dominant follicle size at D6 ($P < 0.001$) was statistically significantly associated with P/S. Heifers with the largest dominant follicle sizes (16-22mm) were 5.54 (95% CI: 2.43 - 12.63) times less likely to be pregnant than those heifers with the smallest dominant follicles (8-10mm) at day 6. Heifers with the presence of a CL at day 0 were 1.95 (95% CI: 0.917 - 4.150) times more likely to be pregnant than those without.

Conclusion We show here that one insemination (56 hours after CIDR withdrawal) yielded similar results to two inseminations (at 48 and 72 hours after CIDR withdrawal). Given the increased cost associated with a second insemination we could suggest that dairy heifers should only be inseminated once following a CIDR synchronisation programme (56 hours after CIDR withdrawal).

Acknowledgements This work was carried out by J.P. Walsh in partial fulfilment of the requirements for the Diploma in Bovine Reproduction, University of Liverpool. Farmers (Clients of the Delaware Veterinary Group) are gratefully acknowledged for providing the heifers and time needed to carry out this study. Zoetis, UK Limited is gratefully acknowledged for providing the drugs used in this study.

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Reduction in daily milk yield associated with sub-clinical bovine herpes virus-1 infection- the importance of proactive herd health and production management

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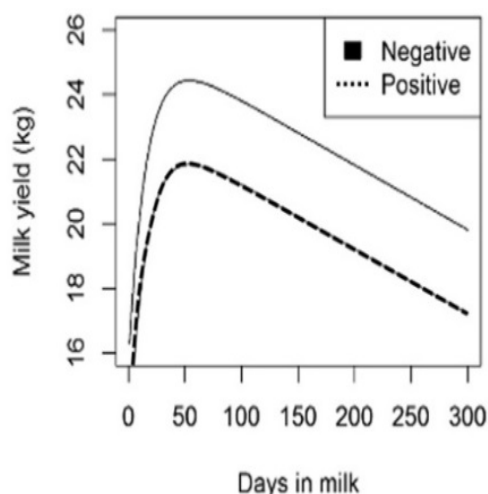
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Application BoHV-1 causes a number of clinical effects and syndromes in cattle including the respiratory complex of signs that is known as 'Infectious bovine rhinotracheitis' (IBR). It is estimated that IBR costs £4 million per year to the UK farming industry.

Introduction In England and Wales, the prevalence of dairy herds endemically infected with BoHV-1 has increased in recent decades based on the presence of specific antibody in bulk milk. Bovine Herpesvirus 1 (BoHV-1) incursion into a naïve population of adult dairy cows typically leads to a variety of clinical syndromes described as Infectious Bovine Rhinotracheitis (IBR). These may include respiratory, ocular and nervous signs, accompanied by pyrexia, infertility and abortions and associated sudden decrease in milk yield. However, BoHV-1 may instead persist and lead to sub-clinical disease with insidious production losses, rather than overt clinical signs. Typically, data on milk production impacts of BoHV-1 are described in herds with clinical IBR outbreaks over five to nine weeks duration, but the effects of sub-clinical BoHV-1 infection on milk production over a longer period have not been studied. BoHV-1 contributes to potentially serious economic consequences including trade restrictions and an adverse impact on animal welfare. The aim of this study was to investigate the potential effect of subclinical BoHV-1 infection on milk production over a 2 year period in a commercial UK dairy herd.

Material and methods Infection status of cows (93 infected or 36 not infected) in a closed autumn-calving dairy herd was assigned from serology on a single occasion in May 2010. We infer nothing about the temporal dynamics of virus circulation in the herd, other than the observed change in herd classification based on bulk milk serology. The herd comprised of 129 pedigree Holstein cows with approximate annual milk yield of 9,000 kg per cow. A multi-level linear model was used to evaluate the impact of infection status on milk production, using milk records that were routinely collected over two years from January 2009 to December 2010. Based on bulk milk serology and intermittent blood samples from young stock up to February 2010, the herd was assumed previously uninfected with BoHV-1.

Results Seventy two per cent of cows were seropositive to BoHV-1 on individual blood samples taken in May 2010. Risk of sero-conversion varied with parity; both parity 1 and parity ≥ 4 cows were at higher risk. These are two potentially vulnerable groups in this herd, with social stress factors for parity 1 animals entering the herd but higher yield and energy deficit for parity ≥ 4 cows; both potentially compromising immune function. Importantly, cows that were seropositive to BoHV-1 in May 2010 produced 2.6 (95% CI 2.0 to 3.2; $p < 0.05$) kg/day less milk throughout lactation compared to those that were seronegative (Figure 1).



Conclusion BoHV-1 seropositive cows produced 2.6 kg/day less milk throughout lactation compared with cows that were seronegative. Those cows with antibodies to BoHV-1 on average therefore failed to produce almost 1,000 kg of milk per year. The mean estimate of potential milk loss in this study is larger and predicted to last longer than previous estimates. Herd health and production management includes prioritisation of management interventions; however sub-clinical disease may be inapparent without an effective monitoring strategy. The large potential losses in milk production in this study highlights the importance of effective risk management such as through biosecurity and vaccination in infectious disease control, even in the absence of clinical signs

Acknowledgements - We are grateful to MSD Animal Health and in particular Paul Williams MRCVS for funding the original study. We are also grateful to the study farm J.H. Nicholson & Partners for their co-operation throughout and in funding the serological investigation.

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Technical feasibility and experimental development of sand recycling options for dairy cattle bedding

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Application A system by which used cattle bedding sand can be recycled, reducing finite resource consumption and the need for waste disposal, but reducing mastitis causing pathogens and retaining the sand's physical properties is desirable.

Introduction Sand is generally considered the 'gold standard' material for dairy cow bedding in the UK, due to its comfort, absorbency and inorganic nature which intrinsically inhibits pathogen growth. The overall aims and desired outcomes of this pilot study were as follows:

- 1) To characterise cattle bedding sand (physically, chemically and biologically) before and after its use.
- 2) To assess the efficiency & effectiveness of six potential cattle bedding treatment methods for the recycling of used sand bedding materials

Material and methods This investigation was carried out using single composite grab samples of pre-use (PU), & in-use (IU) sand bedding material from one farm in North Yorkshire.. Pre-use Recycled Manure Solids (RMS) bedding from another farm was also sampled comparatively. The materials were characterised based on chemical (Carbon, Nitrogen, Phosphorous, Calorific value), physical (qualitative analysis of material structure) and biological characteristics (baseline respiration, substrate induced respiration), of the bedding microcosm. Bedding samples were then treated at lab scale with processes which could potentially be implemented in the field: washing (WS), furnace heat treatment of the sand at 550°C in an oven (FS) or autoclaving or double-autoclaving (AC, or DAC), Following treatment, re-characterisation occurred in triplicate. Microcosm headspace gas was analysed for carbon dioxide at 0, 21, 24 and 192 hours before and after treatment without glucose addition to indicate basal respiration, and with glucose addition to indicate stimulated respiration rate. The mean result of each triplicate result from every test, for each sample was calculated and the standard error of this mean was calculated. One-way analysis of variance (ANOVA) tests were conducted between all triplicate samples for each characteristic, between the rates of carbon dioxide production at 0-24 hours from all microcosms, and between carbon dioxide concentrations at each measurement over the 192 hour period, significance was set at a P value of 0.05.

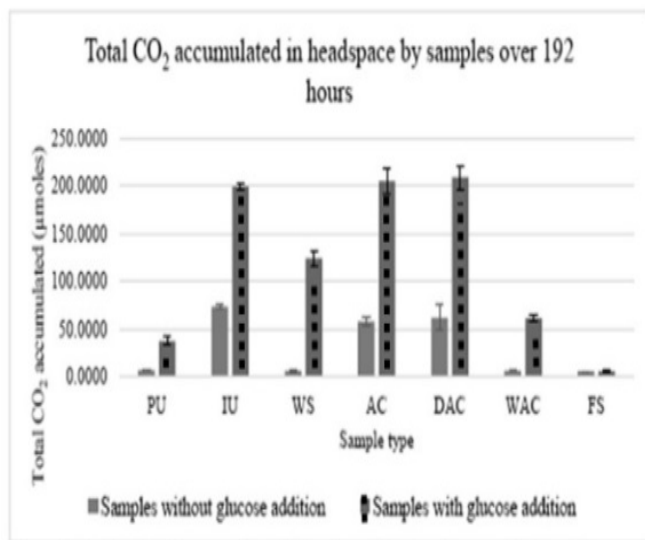


Figure 1 Total CO₂ accumulation in headspace over 192h for sand treatments (error bars show SEM).

Results PU & IU sand were physically, chemically and biologically distinct. The structure of sand was fine to medium grained, sub-rounded homogenous silicate sand of grain size of (100 – 500 µm), after use approximately 80% of the material was as pre-use, with the addition of organic matter collections of 1-5cm diameter. Significantly higher respiration potential were noted in IU sand when compared to PU sand. RMS, displayed distinctly different qualitative and quantitative qualities to the pre-use and post-use sand, appearing as a very low density, fibrous heterogeneous material, with fibres of up to 3cm in length. Furthermore, RMS respiration potential was significantly higher than either PU or IU bedding sand.

Post-treatment there were no significant differences in the physical properties of the sand based on microscopy. FS reduced respiration potential to below that of clean, PU sand. FS & WS also significantly lowered respiration potential relative to IU sand. Autoclaving (AC & DAC) did not decrease respiration potential relative to IU sand.

Conclusion Washing samples effectively reduced sand to the respiration potential of pre-use sand. Autoclaving samples increased respiration potential. The most effective treatment method was FS dry heat treatment at 550°C. This returned sand to its original physical properties and respiration potential was decreased to below that of clean pre-use sand.

Acknowledgements We are grateful to the study farm J.G. Houseman & Sons for their co-operation.

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The effect of light or dark environments on the growth, consumption and mortality in yellow mealworms (*Tenebrio molitor*)

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Application The alternative feed ingredient *Tenebrio molitor* (yellow mealworm) grows better in dark than a natural light/dark environment thereby allowing cost savings associated with the environment they are reared in.

Introduction Interest in the use of insects as alternative feedstuffs to promote global food security has grown substantially since a key FAO publication in 2013 (van Huis, 2013). The yellow mealworm contains all essential amino acids and has been highlighted as having the potential to replace fishmeal and soy meal as a protein source for feeding production animals (Ghaly & Alkoik, 2009). Mealworms may also be used as an intermediary in feeding animals, as they can be fed on lower-grade feedstuff than traditional production animals and then potentially used as a feed (Tetlow *et al*, 2016). Factors that influence mealworm production efficiency will ultimately determine their applicability as feed and food. This study investigates how lighting conditions affect mealworm yields.

Material and methods Mealworms aged 6 weeks were acclimatised for 3 days in a common container at 28°C in natural light/dark cycles (UK, April), with wheat bran (WB) (crude protein 167.4g/kg, crude fat 30.1g/kg, gross energy 17.91MJ/kg), supplemented with carrots as a water source, used as a feed source through the experiment. Two hundred larvae were then randomly allocated to one of two treatment groups (n=6), placed in Petri dishes: 'Light', larvae were exposed to natural light cycles; 'Dark', larvae were kept in complete darkness by placing the Petri dishes within an opaque box for 14 days. The total biomass and number of mealworms in each replicate was assessed on days 4, 7 and 11 and 14, and on intermediate days of the trial any dead individuals were removed, then new feed and carrot was provided. Data was analysed using IBM SPSS 22, data were checked for normality and homogeneity. One-way ANOVA was used to determine difference between treatment groups; Kruskal-Wallis test was used to analyse count data and average weight gain per larvae and death rate was calculated using a linear regression. Significance was accepted at $P \leq 0.05$.

Results Mealworms kept in complete darkness achieved greater total weight gain ($P < 0.001$, Fig. 1A) and their average weight gain (growth rate) throughout the trial was greater per larvae, per replicate ($P = 0.002$, Fig. 1B). There was no overall difference in WB or carrot consumption over the 14 day trial period. There was no significant effect of light environment on total deaths after 14 days or on the death rate throughout the trial (Fig. 1C).

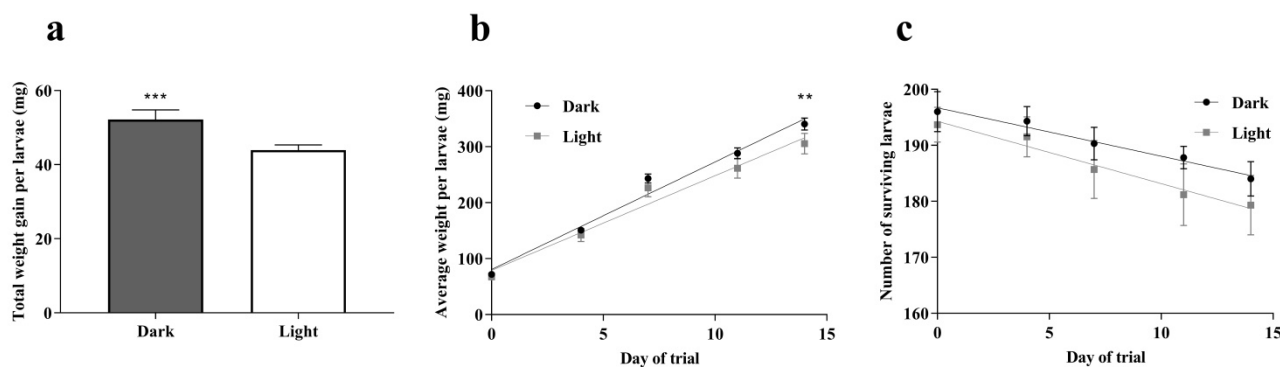


Figure 1 The effect of 14 day treatment of continuous dark (Dark) or natural light cycle (Light) on mealworms. A. Total weight gain per larvae over (***, $P < 0.001$). B. Effects on average growth rate (**, $P < 0.01$). C. Effects on average number surviving. Error bars are SD and n=6 per treatment group.

Conclusion We conclude that mealworms do not appear to require a natural light/dark cycle to grow and may grow better in a dark environment.

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Evaluation of calf coats on the performance and health of artificially reared winter born beef calves to 12 weeks

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Application Artificially reared purchased winter born dairy-bred beef calves should be fitted with a calf coat for the first week in the rearing unit at which point on removal of the coat they would be approximately 24 days of age.

Introduction When ambient temperatures drop below the calf's lower critical temperature (8 to 15° C) it uses energy for heat production, at the expense of growth (Davis and Drackley, 1998). During prolonged periods of low ambient temperatures immune function can be indirectly affected as the nutrition and energy available for support is limited. This can then lead on to the animal being predisposed to ill health (Rawson *et al.*, 1989, Nonneck *et al.*, 2009). Calf coats have been produced as a means to provide calves protection from low ambient temperatures. It is suggested that these coats can reduce heat loss up to 52% (Rawson *et al.*, 1989). However there has been little investigation in to their effects on the performance and health of calves. The objective of this study was to investigate the effect of calf coats on the performance of artificially reared winter born dairy-bred beef calves in the UK.

Material and methods Forty January-February 2013-14 born Holstein (n = 30) and Continental x Holstein (n = 10) bull calves were purchased at a mean age of 17.3 days. This would be similar to commercial practice with purchasing calves from markets. The calves were randomized according to breed, age and weight to treatment groups; Control and Calf Coat with calves fitted with a coat on arrival (Calf Jacket, Holm & Laue). They were housed in individual straw bedded pens in an enclosed naturally ventilated building in Shropshire, UK. The calves were fed warm whey based milk replacer (Wynngold Bloom [23% CP, 20% Oil], Wynnstay Group Plc) fed at 39° C at 187.5g per 812.5ml of water and fed at 2 litres twice per day via a teat from a Wydale feeder offered at head height. Calves were offered ad lib early weaning concentrates (Start 'n' Wean, Wynnstay Group Plc) and gradually weaned at day 42. The calves were moved into a group pen at weaning until 12 weeks. The mean temperatures in the building for January, February, March and April were 6.6° C, 7.3° C, 9.5° C and 11.6° C respectively. The data were analysed using ANOVA.

Results The mean calf liveweight at start was 48.0g±1.79kg (mean±s.e.d). The calves with coats recorded numerically higher (P=0.078) daily liveweight gains (DLWG) from start to 1 week and higher last rib girth measurement at weaning (P=0.021). Last rib girth measurement is an indication of rumen development. Feed intakes to weaning were 27.9kg of CMR per calf and 17.7kg and 13.4kg concentrates (s.e.d ± 1.43; P=0.179) for the Control and Coat calves respectively.

Table 1 DLWG excluding coat weight (g) and last rib girth measurement at 12 weeks

	Control	Coat	s.e.d	Sig
Start – 1 week	486	610	62.4	0.078
1– 3 weeks	543	611	66.7	0.307
3 - 6 weeks (weaning)	765	816	71.45	0.475
Start - weaning	644	713	43.7	0.122
Weaning – 12 weeks	1101	1161	53.5	0.267
Last rib girth at weaning (cm)	103.3	107.6	2.52	0.021

Coat bloom score was significantly improved (3.9 vs 3.5 s.e.d ± 0.2, coats vs control respectively) effect of treatment P=0.007; effect of time P<0.01; treatment x time interaction P=0.245) in calves with coats and faecal score (1.2 vs 1.4 s.e.d ± 0.1, coats vs control respectively) also improved (P = 0.003) concurring with a lower incidence of scours in calves with coats (25.0% vs. 57.1% χ -squared test P=0.04). There were no significant effects of calf coats on measures associated with respiratory disease (cough score, nasal discharge, ear score and eye discharge score) or the incidence of treatment for respiratory disease (45.0% vs. 47.6% χ -squared test P=0.86).

Conclusions Artificially reared winter born calves with coats recorded higher DLWGs from start to 1 week and overall from start to 12 weeks gained an extra 5.6kg with an improvement in last rib girth measurement at weaning and a reduced incidence of scours. The control calves having recorded lower DLWGs to weaning did not exhibit compensatory growth.

Acknowledgements The authors gratefully acknowledge the financial support from Wynnstay Group Plc.

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Potential alternative protein rich feed resources for the provision of rumen un-degradable protein and amino acids

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Application New processing techniques facilitate the extraction of specific protein (SP) from conventional distiller's dried grains and solubles (DDGs) that result in a greater rumen un-degradable amino acid (RUAA) supply. These SP can be applied to increase ruminant productivity, feed conversion efficiency and reduce environmental impact.

Introduction Providing adequate amounts of rumen degradable and undegradable protein increases nitrogen capture into food products, thus creating greater food security while lowering environmental impact. Fast growing animals and higher yielding dairy cattle are likely to benefit from RUAA increasing feed conversion efficiency. This research aimed to assess the degradation and residual feed RUAA content of two SP extracted from corn (cDDGS) and compare these with cDDGS, soya bean meal (SBM) and RUAA processed from SBM and RSM.

Material and methods Applying the model developed at Cornell, a total of thirty-three samples of either; cDDGS, SBM, RUAA SBM, RUAA RSM, and two SPs (SP2 and SP50) were dried and ground (1 mm) and combined with a partial mixed ration (PMR), placed in a buffered mineral solution, flushed with CO₂ and incubated for 16 h at 39°C for the measurement of *in vitro* gas production (IVGP) using the Ankom-RFS system. Chemical analysis of feeds was completed pre and post incubation and RUAA profile was estimated using gas liquid chromatography. Means were analysed using a general linear command in Minitab, applying feed resource as a fixed effect and all other factors as random effects in the model. A confidence interval of 0.95 was applied and significant differences were tested using Tukey's and stated at a P value <0.05.

Results Gas production from feed degradation was highest from cDDGS and lowest from Hiprosoya, but similar in RUAA from SBM and RSM, SP2 and SP50. Residual protein was greatest from RUAA SBM, but similar from RUAA from Hiprosoya, SP2 and SP50, but lowest from RUAA RSM. Liquor pH did not differ. VFA and ammonia concentrations varied, most notably SP50 had the highest total VFA and lowest A:P ratio, while SP2 was similar to RUAA from SBM and/or RSM.

Table 1 Gas production, residue protein, liquor pH and fatty acid concentrations after 16 h of incubation of Hiprosoya, cDDGS, RUAA RSM, RUAA SBM, SP2 and SP50

	Hiprosoya	cDDGS	RUAA RSM	RUAA SBM	SP2	SP50	SEM	P value
Gas, ml	62.8 ^b	75.5 ^a	68.9 ^{a,b}	69.9 ^{a,b}	64.8 ^{a,b}	68.4 ^{a,b}	2.91	0.025
Liquor pH	6.4	6.4	6.4	6.4	6.5	6.4	0.01	0.084
Ammonia, mmol/l	17.5 ^a	16.7 ^{a,b}	17.3 ^a	15.0 ^b	16.0 ^{a,b}	16.6 ^{a,b}	0.53	0.020
Total VFA, mmol/l	59.8 ^b	56.5 ^b	42.1 ^b	70.5 ^{a,b}	42.2 ^b	99.4 ^a	7.42	0.001
A:P ratio †	2.1 ^{c,d}	2.1 ^{b,c}	2.4 ^{a,b}	2.2 ^{b,c}	2.4 ^{a,b}	1.8 ^d	0.06	<0.001
CP %	45.3 ^b	40.0 ^c	37.9 ^c	53.8 ^a	47.7 ^b	47.7 ^b	0.72	<0.001
Lysine %	1.30 ^b	1.50 ^b	1.60 ^b	2.00 ^{a,b}	2.10 ^{a,b}	2.00 ^{a,b}	0.50	0.020
Methionine %	0.45 ^b	0.42 ^b	0.45 ^b	0.60 ^a	0.65 ^a	0.60 ^a	0.11	0.010
Cystine %	0.26 ^b	0.28 ^b	0.28 ^b	0.29 ^b	0.60 ^a	0.50 ^a	0.18	<0.001

^{a,b,c} - Means in rows with different superscript letters differ significantly

† - Acetic to propionic acid ratio

Conclusions Gas production was greatest from cDDGS, but similar in Hiprosoya, RUAA SBM and RSM, SP2 and SP50. Residual feed RUAA content of SP2 and 50 were similar to that of RUAA SBM, demonstrating the potential of SP2 and SP50 to increase the RUPAA from cDDGS.

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The effect of cross inoculation of cattle rumen fluid on *in vitro* dry matter digestibility

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Application Improving the efficiency with which cattle digest fibre could increase profitability and reduce negative environmental impacts as well as reduce reliance upon supplementary feeding of concentrates.

Introduction Variability in an animal's ability to digest fibre is observed within herds of the same breed of animals. Differences in rumen microbial ecology have previously been linked to cattle that differ in residual feed intake (Guan *et al.*, 2008). Attempts to manipulate the microbiota through cross inoculation of rumen fluid *in vivo* (Weimer *et al.*, 2010; Griffith *et al.*, 2016) have proven unsuccessful apparently due to host 'control' of its microbiota. The aim of this study was to determine the effect of mixing rumen fluid from cattle of differing fibre digesting ability on the digestion of a high fibre diet, in the absence of host 'control', via the use of an *in vitro* rumen model.

Material and methods Rumen fluid was collected at time of slaughter from 10 Holstein-Friesian steers (ABP York; Experiment 1) and 11 Charolais cross steers (Dawn Meats, Cornwall; Experiment 2). A 24-hour fermentation was performed with 0.5 g dried grass as a substrate (Graze-On, Northern Crop Driers Ltd, UK; 94% dry matter), 45 ml of salivary buffer (Mould *et al.*, 2005) and 5 ml of rumen fluid to identify the two animals at the extremes ('Good' and 'Bad') of *in vitro* dry matter digestibility (IVDMD; Udén, 2006) for each group of animals (0.37 vs 0.21 for Experiment 1; 0.40 vs 0.36 for Experiment 2). In Experiment 1, a consecutive batch culture (CBC) technique was used to culture the Good, Bad and a 1:1 Mix of both fluids (2.5 ml of each fluid, n = 6 for each group) over a 16-day period. An initial 24-hour fermentation was followed by seven 48 hour fermentations and finishing with a final 24-hour fermentation. Experiment 2 consisted of four 48 hour CBCs with samples removed at predefined intervals. Three bottles for each group (n = 9) were removed at each time point. Data was analysed by a general linear model with Tukey's post-hoc test (IBM SPSS Statistics 21). For Experiment 2, a Gompertz curve was fitted to the data for CBC1 and 2 (GenStat 12th Edition).

Results For Experiment 1, IVDMD significantly improved ($p < 0.001$) between the start (end of Day 1) and end of the experimental period (Day 16), with an average increase of 45, 142 and 63% for the Good, Bad and 1:1 Mix fluids. Cross inoculating rumen fluid (1:1 Mix) provided an intermediary IVDMD response at 24 hours only (0.34 vs 0.20 vs 0.29 for Good, Bad and 1:1 Mix respectively). Differences between the fluids were lost with consecutive cultures. IVDMD increased with each CBC up to Day 9. For Experiment 2, the fitted curve of the Good fluid was significantly different to both the Bad and the 1:1 Mix with a higher IVDMD for both CBC1 and 2 ($p < 0.001$). There was no significant difference between the curves of the Bad and 1:1 Mix ($p > 0.05$). By day 8 (CBC4), there was no overall effect of rumen fluid ($p > 0.05$). The IVDMD values at the end of each CBC are shown in Table 1.

Table 1 IVDMD values at the end point of each consecutive batch culture of Experiment 2

	CBC1	CBC2	CBC3	CBC4	SEM	p value		
						Fluid	Time	Fluid*Time
Good	0.601 ^a	0.718 ^a	0.700 ^a	0.717 ^a				
Bad	0.570 ^b	0.701 ^a	0.672 ^b	0.712 ^{ab}	3.41E-05	< 0.001	< 0.001	< 0.001
1:1 Mix	0.580 ^b	0.677 ^b	0.677 ^b	0.706 ^b				

^{a,b,c} Different superscript letters denote significant differences within a column

Conclusion Despite removing the effect of the host animal on the rumen microbial community, no benefit of cross inoculation was observed for either experiment beyond an initial intermediary improvement at 24 hours of Experiment 1, suggesting that the community associated with the Good rumen fluid was unable to establish when mixed with another rumen fluid. Bacterial community structure will be explored through future sequencing work.

Acknowledgements BBSRC (BB/J004308/1) North Wyke Farm Platform (NWFP) National Capability who supplied the rumen fluid of forage fed animals

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Effect of levels of intake on rumen fermentation profile, nutrient digestibility, methane emissions and feeding behaviour

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Application Improving our understanding of animal-pasture interactions in the uplands is a key component for future sustainable intensification of ruminant livestock systems. Changes in the level of intake without changes in diet composition have a measureable impact on nutrient digestibility and methane emissions.

Introduction Differences in rumen fermentation profile (including methane emissions) underlie variations in feed efficiency and residual feed intake (Jami *et al.*, 2014). Therefore, understanding the relationship between feed intake and rumen fermentation is key to understanding the biological basis for animal differences in productivity, health and efficiency. The objective of this experiment was to examine the effect of differing levels of intake on rumen fermentation profile, nutrient digestibility, methane emissions, and feeding behaviour in sheep.

Material and methods Six Aberdale cross Texel ewes (90.2 ± 1.89 kg BW) fitted with rumen fistula were used in a duplicated 3×3 Latin Square with three 21-d experimental periods. Treatments comprised dried grass nuts fed to meet one (1M), 1.5 times (1.5M), or two times (2M) maintenance energy requirements (NRC, 2007). During each of the three experimental periods, sheep were weighed at d 1, 7, 14, and 17 to calculate average daily gain and feed efficiency. Feed offered and refusals were recorded daily to measure feed intake. From d 15 to 17 of each period, sheep were housed in individual metabolic crates, where total faecal and urine production were recorded daily to measure nutrient digestibility. From d 18 to 20, ewes were housed in individual methane chambers to determine gas production. On d 16 of each period, a video camera recorded feeding behaviour. On d 17 of each period, a total of 5 mL of blood were taken from jugular venipuncture immediately before feeding and 4 h later for haematology analysis. Also, 50 mL of ruminal content were collected from the ruminal cannula immediately before feeding, and 2 and 4 h after feeding to measure pH, volatile fatty acid (VFA) and ammonia concentration, and protozoa count. Data were analysed using the MIXED procedure of SAS (University Edition 2.3, SAS Institute, Inc., Cary, NC). The model included as fixed effects the square of the Latin Square arrangement, period, animal nested within square, treatment and, when necessary, hour after feeding, and the interaction treatment \times hour.

Results The average daily gain was greater ($P = 0.01$) in 2M than 1M ewes (0.49 vs -0.09 ± 0.079 kg BW/d). The 1M ewes had a lower ($P < 0.01$) VFA concentration than 1.5M and 2M (69.9 vs 101.8 and 111.4 ± 6.99 mM), and a greater ($P = 0.04$) acetic-to-propionic ratio than 1.5M (2.40 vs 1.97 ± 0.108). The ammonia concentration was greater ($P = 0.01$) in 1.5M than in 2M ewes (14.14 vs 8.89 ± 1.024 mM). Dry and organic matter digestibility per unit of metabolic weight were greater ($P < 0.05$) in 1M than 1.5M and 2M ewes (2.61 vs 2.47 and 2.43 ± 0.029 %/BW^{0.75}; 2.68 vs 2.55 and 2.50 ± 0.027 %/BW^{0.75}, respectively). Emissions of methane per unit of DM intake were greater ($P < 0.05$) in 1M than 1.5M and 2M ewes (18.33 vs 15.08 and 13.53 ± 0.772 g/d \times kg DMI). No differences ($P > 0.05$) were found in protozoa count, feeding behaviour or haematology.

Conclusion Results show that increased levels of intake have a measureable impact on diet fermentation and digestibility, reducing the acetate-to-propionate ratio, ammonia production, digestibility rate and methane emissions. These changes could be attributed to the different nutrient fermentation pattern and the higher flow rate from the rumen to the lower digestive tract.

Acknowledgements The authors acknowledge the financial support provided by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment.

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Mitigating feed nutrient losses in the rumen through dietary inclusion of cashew nut shell liquid: An *in vitro* and *in vivo* evaluation

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Application Dietary inclusion of cashew nut shell liquid (CNSL) inhibited rumen degradation of nutrients leading to lower methane and ammonia production. Efficiency of protein utilization was better in animals fed CNSL compared to those on control diet.

Introduction Rumen microbial feed degradation leads to dietary energy and protein losses in the form of methane and ammonia. Ruminant nutritionists have advocated the use of plant-based additives in ruminant diets to protect such losses and improve nutrient efficiency. However, much of the evidence that these plant substances improve nutrient efficiency is laboratory based. The aim of this study was to evaluate the potential of CNSL in mitigating nutrient losses in the rumen for improved nutrient efficiency *in vitro* and *in vivo*.

Material and methods Dietary treatments were Panicum maximum leaves supplemented with concentrate pellet containing CNSL at four levels of 0 (control diet), 5, 10 and 15 ml/kg. Panicum and pellets were at a ratio of 70: 30 of the required dry matter (DM). For *in vitro* studies, 500 mg (n=12 per diet) DM basis was weighed into dacron bags and incubated in 100 ml glass syringes for 24 h following the procedure of Menke and Steingass (1988) with slight modifications. At the end of 24 h, crude protein (CP) and organic matter (OM) degradation were determined by recovering the residue. Methane (CH₄) production was determined by sodium hydroxide infusion. For *in vivo* studies, 24 goats were divided into four groups balanced for body weight and each group fed one of the four diets at 5% body weight (DM basis) for 60 days. Nutrient intake and weight gain were measured in animals. The CP and OM content in feeds and *in vitro* residues were determined according to AOAC (2000) while protein efficiency ratio (PER) was estimated by relating weight gain to protein intake. Samples of rumen liquor were recovered after *in vitro* 24 h incubation while collection from goats *in vivo* was done using suction tube three hours after feeding on day 60 of feeding. Rumen ammonia nitrogen (NH₃-N) *in vitro* and *in vivo* was determined by Kjeldahl method (AOAC, 2000). Data were analysed using one way analysis of variance procedure with mean differences separated using the Duncan's multiple range test (SAS, 1999).

Results As shown in Table 1, *in vitro* CP, OM degradation and CH₄ production were lower (P < 0.05) with diets containing 10 - 15 ml/kg CNSL relative to the control diet. *In vitro* and *in vivo* NH₃-N production reduced. Protein utilization improved and daily weight gain in animals was significantly higher in CNSL fed animals compared to the control group.

Table 1 Effect of dietary inclusion of CNSL on *in vitro* and *in vivo* nutrient utilization

	Level of CNSL inclusion in supplemental diet				s.e.m	P
	0 ml/kg	5 ml/kg	10 ml/kg	15 ml/kg		
<i>In vitro</i>						
OM degradation, OMD (%)	70.50 ^a	68.88 ^a	66.90 ^b	65.48 ^b	0.41	<0.001
CP degradation, CPD (%)	73.39 ^a	70.66 ^b	67.74 ^c	65.83 ^c	0.53	<0.001
NH ₃ -N (ml/dL)	20.83 ^a	16.53 ^b	14.92 ^{bc}	11.88 ^c	0.89	<0.001
CH ₄ production (ml/g OMD)	34.88 ^a	27.32 ^{ab}	21.07 ^{bc}	15.18 ^c	2.48	0.013
<i>In vivo</i>						
CP intake (g/day)	61.67 ^a	54.95 ^b	51.00 ^c	49.42 ^d	0.30	<0.001
Rumen NH ₃ -N (ml/dL)	38.92 ^a	26.26 ^b	20.12 ^b	18.07 ^b	3.20	0.011
Animal weight gain (g/day)	23.38 ^c	29.20 ^b	30.05 ^{ab}	30.95 ^a	0.66	<0.001
Protein efficiency ratio (PER)	0.381 ^c	0.533 ^b	0.595 ^{ab}	0.639 ^a	0.02	<0.001

^{a,b,c,d} Means within same row with different superscripts are significantly (P<0.05) different

Conclusion The reduced OMD, CPD, NH₃-N and CH₄ in the rumen particularly at 10 - 15 ml/kg CNSL suggests inhibition in microbial degradation of dietary protein and carbohydrate, reduced nutrient losses and a possible increase in by-pass nutrients. Improved PER and higher animal weight gain reflects better utilization of dietary proteins at post-ruminal site.

Acknowledgements The authors acknowledge the laboratory assistance of the Animal Nutrition technologists, FUNAAB.

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The effects of field bean (*Vicia Faba*) inclusion level in the diet of freshly calved dairy cows

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Application Including 9.0 kg/day of field beans in the diet of freshly calved dairy cows reduced milk protein content and fat plus protein yield. This may be acceptable if the cost of the concentrate is substantially reduced.

Introduction As the UK dairy sector seeks to reduce its reliance on imported protein feeds; interest in locally-grown protein crops is increasing. Field bean (*Vicia Faba*) is a grain legume of particular interest as it can be successfully grown in many parts of the UK. In a previous study (Johnston *et al.*, 2016) field beans were included in the diet of mid lactation dairy cows at up to 5 kg/day without any loss in performance. However, information on optimum field bean inclusion levels in the diets of freshly calved cows is limited. This experiment examined the performance of freshly calved dairy cows when field beans were included in the diet at three inclusion levels.

Material and methods Seventy Holstein-Friesian dairy cows (31 primiparous and 39 multiparous, mean parity 2.14, s.d., 1.23) were used in a continuous design 20-week experiment involving three treatments. Cows were allocated to treatments within 24 hours of calving. Treatments examined (FB0, FB-Low, FB-High) differed in concentrate type offered, with concentrates containing either zero, 368 or 735 g field bean (*Vicia Faba*, Var. *Fuego*)/kg fresh weight, respectively, with the field beans either partially or totally replacing the 'normal concentrate protein' ingredients with the latter two treatments. The main ingredient composition (g/kg, fresh basis) of each of the concentrates offered was as follows: 'FB0' - maize meal, 245; soya hulls, 171; wheat, 171; soya-Hipro 147; rapeseed meal, 147; maize gluten feed, 74; minerals, 25; molasses 20 field beans 0; 'FB-Low' - maize meal, 183; soya hulls, 135; wheat, 86; soya-Hipro 74; rapeseed meal, 74; maize gluten feed, 37; minerals, 25; molasses 20; field beans 368; 'FB-High' - maize meal, 122; soya hulls, 98; minerals, 25; molasses, 20; field beans, 735. These concentrates were included within a total mixed ration comprising 45% grass silage and 55% concentrate, on a dry matter basis. Fresh feed was offered daily at approximately 09.00 h (at 1.1 of the previous days intake), with uneaten feed removed the following day at approximately 08.00 h. The complete diet was offered via a series of boxes, which cows accessed via feed gates linked to an electronic identification system, thus enabling individual cow intakes to be recorded daily. Cows also received 0.5 kg of soya hulls twice daily during milking. Cows were milked twice daily, with milk yields recorded automatically at each milking, and the total daily milk yield for each cow for each 24-hour period calculated. Milk samples were taken during two consecutive milkings each week and analysed for fat, protein and lactose content using an infrared milk analyser. Mean milk production data were calculated for the 20-week experimental period. Data were analysed in GenStat using an ANOVA.

Results Actual intakes of field beans with treatments FB-Low and FB-High were 4.4 and 9.0 kg/day (fresh basis). Including field beans in the diet of freshly calved dairy cow had no effect on total feed intake, milk yield or milk fat content ($P>0.05$) (Table 1). However, milk protein content was reduced with increasing levels of field bean inclusion ($P<0.005$), while milk fat + protein yield with FB-Low and FB-High tended ($P=0.051$) to be lower than for FB0. Neither average cow live weight nor body condition was affected by treatment ($P>0.05$) (Table 1).

Table 1 Effects of field bean inclusion level in dairy cow concentrates on mean cow performance over a 20 week period

Treatment				S.E.M	P-Value
	FB0	FB-Low	FB-High		
Total DMI (kg/day)	22.1	21.8	22.6	0.56	0.624
Milk yield (kg/day)	35.7	33.2	34.5	1.04	0.232
Milk fat (g/kg)	42.6	42.4	41.2	0.75	0.366
Milk protein (g/kg)	33.6 ^a	33.4 ^{ab}	31.8 ^b	0.41	0.005
Milk fat + protein yield (kg/day)	2.706	2.562	2.470	0.070	0.051
Average body condition score	2.49	2.53	2.49	0.035	0.654
Average live weight (kg)	598	601	598	8.3	0.967

Means with the same superscript within a row do not differ significantly ($P>0.05$)

Conclusion Including up to 9.0 kg/day of field beans in the diet of early lactation cows had no effect on total DMI, milk yield or milk fat content, but reduced milk protein content, and tended to reduce milk fat + protein yield. This loss in performance may be acceptable if significant savings are made in the cost of the concentrates offered.

Acknowledgements This work was co-funded by DAERA and AgriSearch.

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A description of interoestrus and interservice intervals and associated fertility in sixteen UK dairy herds

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Application This study investigated the intervals between oestrus events, finding interservice intervals to be longer than interoestrus intervals. This indicates a need for further work to elucidate the mechanisms behind this, whilst also highlighting the limitations of current methods of analysing oestrus detection data.

Introduction With marked differences between the reproductive traits of the modern dairy cow and her genetically distant predecessors (Royal, 2000), it seems plausible that parameters established previously may no longer describe the dairy cow's oestrous cycle. The objective of this study was to describe the current interoestrus and interservice intervals in cows from United Kingdom dairy herds. Associations between these intervals and fertility performance were also examined.

Material and methods Sixteen dairy herds located in Cheshire, Shropshire, Staffordshire and Wrexham County were recruited. All herds employed Reproductive Management Systems (RMS, Genus PLC) for breeding management, with this selection criterion ensuring that the same protocol for oestrus detection and timing of insemination was used. This standardised approach meant that the interservice interval reflects the interval between the associated oestrus events. Oestrus events were defined as observed signs of behavioural oestrus. Interoestrus intervals and interservice intervals from a five year period (1/1/2009 to 31/12/2013) were examined. Interoestrus intervals were defined as periods between two recorded oestrus events of eleven to thirty-one days apart in a lactating animal with no insemination at the first oestrus event. The event terminating an interoestrus interval may be a service, as the act of insemination cannot exert an effect on the previous interval. Interservice intervals were defined as periods between two recorded oestrus events of eleven to thirty-one days apart in a lactating animal with an insemination at both the first and second oestrus event. Censoring for eleven to thirty-one day intervals includes the greatest range of interval possible with minimal risk of bias from oestrus detection inaccuracies. Pregnancy data were examined for 100 days after the last oestrus event to ensure all animals with oestrus events had reasonable opportunity for pregnancy diagnosis. 1747 interoestrus intervals and 8667 interservice intervals were included. 2953 (28%) of the intervals that culminated in a service resulted in a successful pregnancy. Multivariable analysis was conducted using STATA 13 (StataCorp LP) to fit random effects models with random effects at herd level and cow level. Model One had the outcome variable of length of the interval (days) between two oestrus events, both service and non-service. Model Two was a logistic regression model with the outcome variable being the odds of an insemination resulting in a pregnancy. Variables were considered for inclusion in initial models based on biological plausibility and results of univariable analysis, with a backwards stepwise methodology was used to select variables for final models.

Results Modal interoestrus interval was twenty-one days, conforming to established descriptions. Modal interservice interval was twenty-two days, with a higher upper range (twenty-eight days) than previously reported. Interval length increased with both days in milk and parity. In contrast, each subsequent interval within an individual lactation resulted in a decrease in interval of 0.17 days (95% CI -0.22, -0.13) after adjusting for days in milk. For each day increase in interservice interval there is a two per cent increase in the odds of a service resulting in pregnancy (OR 1.023, 95%CI 1.010, 1.036).

Conclusion This work supports recent work (Remnant *et al*, 2015) that indicates the current interservice interval is longer than the previously established modal value. However, where previous work has extrapolated this finding to suggest the modern dairy cow has a longer oestrous cycle, this study's analysis of interoestrus intervals distinct from interservice intervals shows that interoestrus intervals have not increased. It appears therefore that the basic physiology of the oestrous cycle remains unchanged and that insemination exerts an effect to lengthen the subsequent oestrous cycle. Intervals were also affected by parity, days in milk and number of oestrus events within the same lactation. Identifying mechanisms for these effects will require further investigation. Length of interservice interval had a positive association with subsequent pregnancy. A longer interval increased the odds of conception to the second insemination of the interval. This may be explained by follicular physiology and oocyte age at ovulation. Further work is needed to explore these theories.

Acknowledgements This work was carried out by Thomas Greenham in partial fulfilment of the requirements of the Diploma of Bovine Reproduction, University of Liverpool. The dataset was provided by Genus PLC, with special thanks going to Peter Jackson, Huw Lloyd and Jenny Hildon for their support, technical help and engagement with the project.

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Associations between age at first calving and subsequent performance in UK Holstein and Holstein-Friesian dairy heifers

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Application This study describes unfavourable associations between increased age at first calving and future reproductive performance, udder health and lifetime milk production and highlights the importance of optimal replacement dairy heifer management.

Introduction Second only to feeding costs, heifer rearing costs represent a large expense for a dairy farm (Heinrichs *et al.*, 1993). Age at first calving (AFC) largely affects rearing costs with most published studies suggesting an optimal AFC of 23 to 24 months (Pirlo *et al.*, 2000). Here we use a large dataset and describe the AFC of UK milk recorded herds and its relationships with subsequent lactation performance (focusing on production, fertility, and udder health).

Material and methods Data were provided by the Centre for Dairy information and derived from lactation records compiled by the following major UK milk recording organisations: National Milk Records, Cattle Information Service and United Dairy Farmers. An initial database comprised first and successive lactation production records for 446,523 pedigree Holstein/ Holstein-Friesian heifers calving in milk recorded herds in the UK during the period between January 1st 2006 and 31st December 2008. Information relating to identification, origin, and ancestry were available for each heifer alongside lactation number, age at calving (months) and date of calving. Available production data included total milk yield (kg), total fat and protein (kg), fat and protein percentages, days in milk and end dates for both 305 day and complete lactation periods respectively. A mean somatic cell count (SCC) was included for each complete lactation. To explore the associations between AFC and performance in successive lactations, lactations terminated up until the 31st of December 2012 were included in the dataset. Mixed effects regression modelling was performed using STATA 13 (@StataCorp LP) to explore the associations between AFC and production (305 day lactation yield, 305 day fat yield, 305 day protein yield and lifetime milk yield), reproductive performance (calving interval between lactation 1 and 2), and logSCC. The random effect of sire nested within season, year and farm was fitted in all the models.

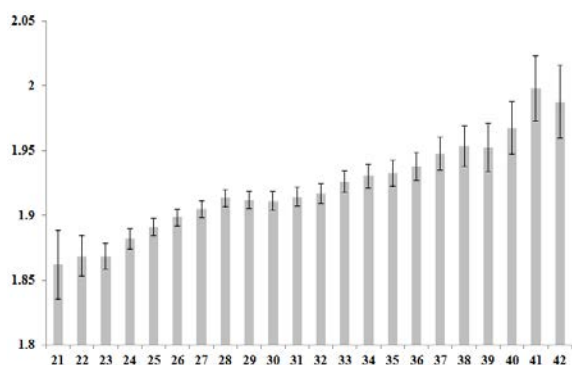


Figure 1 logSCC (\pm confidence intervals) by age at first calving

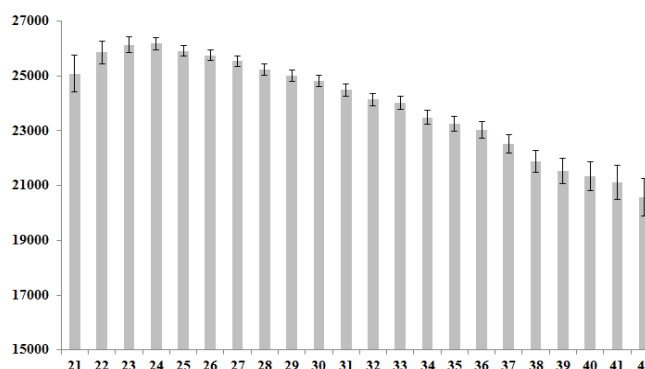


Figure 2 Lifetime milk yield (\pm confidence intervals) by age at first calving

Results The mean and median AFC across all heifers was 29.1 and 28 months respectively. Of the 396,534 heifers included in the study 48,567 heifers (12.3%) calved for the first time at 24 months of age or younger. 162,157 heifers (40.9%) were 30 months or older when calving for the first time. Increased AFC was associated with increased first lactation milk, fat and protein yields. However, it was also associated with increased calving interval, increased first lactation SCC (Figure 1), and decreased lifetime milk yield (Figure 2).

Conclusion A lower age at first calving was shown here to be associated with better reproductive performance and decreased SCC. First lactation milk production was lower among younger heifers but lifetime milk production was significantly increased. AFC distribution highlights the need for improvements in youngstock management among UK dairy farms.

Acknowledgements This work was carried out by N.T. Eastham in partial fulfilment of the requirements for the Diploma in Bovine Reproduction, University of Liverpool.

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Improving female fertility and calf survival in the UK Limousin beef cattle

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Application Fertile suckler beef cows and low calf mortality are essential to a sustainable beef industry. The development of GEBVs for fertility and calf survival traits will provide accurate breeding values to be used as tools for selection.

Introduction Industry wide, little genetic improvement has been made for female fertility or calf survival. This project uses national datasets and genomics to improve existing fertility traits and calf survival through delivery of genomic breeding values to all users of Limousin bred cattle. Currently these traits are reliant on data collection and pedigree information meaning EBVs aren't available with good accuracy until later in life. The inclusion of genomics will enable accurate breeding values to be produced from birth making them available at the time of selection. Making informed selection decisions based on accurate information is crucial to increasing genetic gain. In turn, this will improve the efficiency and sustainability of the beef industry.

Material and methods The project includes the evaluation of three female fertility traits and calf survival. Age at first calf (AFC) was defined as the age (days) of the cow at her first parity. Calving interval 1 (CI1) was defined as the time (days) between parities. Lifespan (LS) was defined as the number of parities before 6.5 years of age or death, whichever is first. Survival (SV) was defined as survival beyond 10 months of age. The evaluation includes all animals which are more than 45% Limousin and have valid phenotype records (1,747,355 records). A number of strict data edits were applied to the dataset to ensure only valid phenotypes were included in the evaluations. A pedigree was then built from a super-pedigree which includes information from BCMS, herd books and national milk records. This data was combined with a reference population of more than 4000 genotypes to form a SNP key. Genomic Estimated Breeding Values (GEBVs) were produced using this SNP key by applying the single step process which enables the output of breeding values for both genotyped and non-genotyped animals simultaneously. Reliabilities were calculated using GBLUP. Parameter estimation for the evaluation was carried out on a subset of this dataset in ASREML.

Results Heritabilities were found to be comparable with literature estimates. A negative correlation was found between lifespan with calving interval 1 of -0.46 meaning that a shorter calving interval is associated with a longer lifespan. Correlations between age at first calf with lifespan and age at first calf with calving interval 1 were found to be not significantly different from zero. The new GEBVs provide increased accuracy compared with the current pedigree evaluation EBVs which will improve the accuracy of selection.

Trait	Number of records	h^2
AFC	58,148	0.134 (0.0137)
CI1	27,861	0.045 (0.0156)
LS	34,307	0.049 (0.0125)
SV	55,149	0.13 (0.02)

Figure 1 Heritability estimates (standard error)

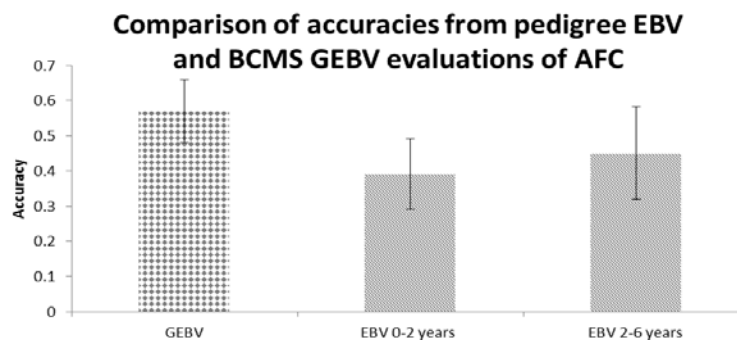


Figure 2 Improvement in accuracy with the use of GEBVs

Conclusion GEBVs have been successfully produced from commercial data and have improved accuracy. The GEBVs are expected to be published in 2017.

Acknowledgements Thanks to British Limousin Cattle Society, Innovate UK and BBSRC.

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Immunoglobulin G glycans in milk in early pregnancy and as potential early biomarkers of pregnancy in dairy cattle

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Application The results of this study show that while there are significant changes in concentrations of glycans in pregnant cows during early pregnancy, the differences recorded are of insufficient magnitude to be used as an early pregnancy biomarker. An earlier pregnancy biomarker would find widespread application and increase herd reproductive efficiency.

Introduction Glycans are carbohydrate structures that may be bound on proteins as post-translational changes. In the case of immunoglobulin G (IgG), glycans modulate the immune response. It has been shown in humans that glycosylation of IgG changes with pregnancy (Hayes *et al.*, 2014) and IgG desialylation has also been observed in cows in milk throughout early lactation (Takimori *et al.*, 2011). Changes in IgG have been described during early lactation, but not during early pregnancy. The aims of this study were to identify and describe the changes on the IgG glycans during the oestrous cycle and early pregnancy, as well as exploring their usefulness as pregnancy biomarkers.

Material and methods A total of 45 spring-calving Holstein-Friesian and dairy cross-bred cows were sampled during the oestrous cycle preceding AI, and again following AI. They were confirmed to be pregnant at day 35 after AI. Milk samples were collected daily on days 11-21 (day of oestrus = day 0). The milk whey was separated and preserved at -20°C until analysed. Whey was filtered before analysis through a 1µm filter. The IgG was purified from the sample using protein G affinity columns. Afterwards, the protein was reduced and alkylated to allow Peptide-N-Glycosidase (PNGase) to cleave the N-glycans, which were finally washed and separated from the protein. Glycans were tagged with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and Ultra-high Performance Liquid Chromatography (UPLC) was performed on a hydrophilic interaction column. The chromatogram from each sample was manually integrated and the relative abundances for each of the 23 peaks were calculated. A Wilcoxon statistical test was performed for all time points and peaks, pairing the data for the same cow and day relative to oestrus between the non-pregnant and the pregnant days. All peak areas were log transformed and a 10-fold cross validation was performed. Differences between peak areas were considered significantly different at $P < 0.05$.

Results Table 1 shows significant peaks from Figure 1 per day of the cycle and their increase or decrease in response to pregnancy status.

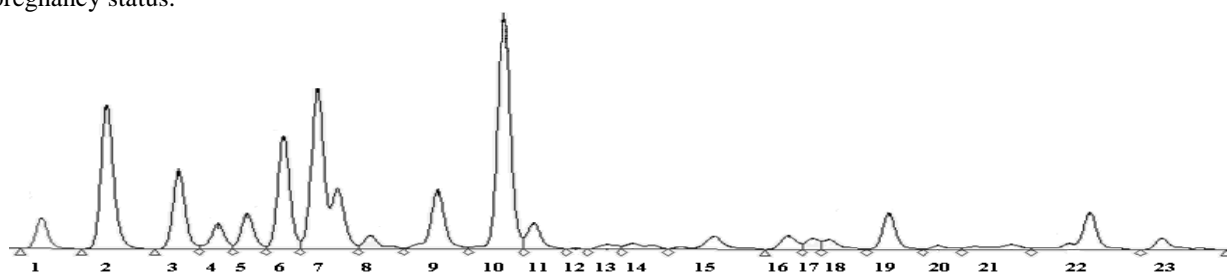


Figure 1 Typical chromatogram with the peak areas 1 to 23.

Table 1 Significantly different peaks per day of the cycle

Day of the cycle	11	12	13	14	15	16	17	18	19	20	21
Decreased peaks on pregnant cows	-	-	-	19	-	-	-	-	-	-	-
Increased peaks on pregnant cows	23	16,23	-	-	8	13,21	-	14	23	-	-

Conclusion. Glycan peaks were identified that differed between pregnant and non-pregnant cows on days 11-21 after oestrus. Despite the statistical differences in peaks between pregnant and non-pregnant cows, the difference was insufficient to allow any glycan peak be used as a possible biomarker of pregnancy.

Acknowledgements The authors gratefully acknowledge funding from SFI (SFI13/IA/2025).

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Identification of circulating miRNA biomarkers of pregnancy status in dairy cattle

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Application A miRNA gene (miR-25) has been identified as a potential biomarker of early pregnancy in dairy cows.

Introduction Current bovine pregnancy detection methods include rectal palpation, ultrasound scanning, Pregnancy Associated Glycoprotein ELISA, Pregnancy Specific Protein B ELISA, progesterone immunoassays, in-line progesterone testing and visual observation of oestrus (often with the use of aids to detection; e.g. tail paint/heat pads). However, these assays suffer from limited sensitivity and results from these pregnancy test methods are not reliable until at least day 28 post AI. Therefore, there is a need for the development of an early, reliable, pregnancy test that would allow producers the opportunity to rebreed at the next oestrous event. Expression levels of miRNA genes in plasma have been suggested to differ between pregnant and non-pregnant cows and therefore, may be used as markers of early pregnancy (Ioannidis and Donadeu, 2016).

Material and methods The oestrous cycles of 20 dairy cows were synchronised, they went through one (control) oestrous cycle and these cows were artificially inseminated during the following oestrus. Blood was collected by jugular venepuncture on day 21 of the control oestrous cycle and on day 21 post AI. All cows were confirmed pregnant by ultrasound scanning on day 35 post AI. Total RNA was extracted from whole blood. The RNA yields and quality were examined using a Nanodrop spectrometer and Agilent bioanalyser, respectively. RNA was reverse transcribed to cDNA for each specific miRNA gene of interest. TaqMan real-time qPCR was used to measure the expression of selected target and reference miRNA genes. The Cq values were imported into GenEx software and the Cq values from the miRNAs of interest were normalised to the two most optimal reference genes (ranked in GeNorm within GenEx), miR-93 and miR-191. Relative quantities of gene expression were calculated to the highest Cq value. Relative gene expression data were logged and analysed using a paired t test in GraphPad Prism.

Results Relative gene expression levels (expression levels of the target genes versus the reference genes) were higher ($P < 0.05$) on day 21 of pregnancy compared with day 21 of the control (oestrous) cycle for miR-25 (Figure 1). There were no differences in expression levels between day 21 of pregnancy compared with day 21 of the control cycle for any other miRNA genes analysed ($P > 0.05$) (Figure 1).

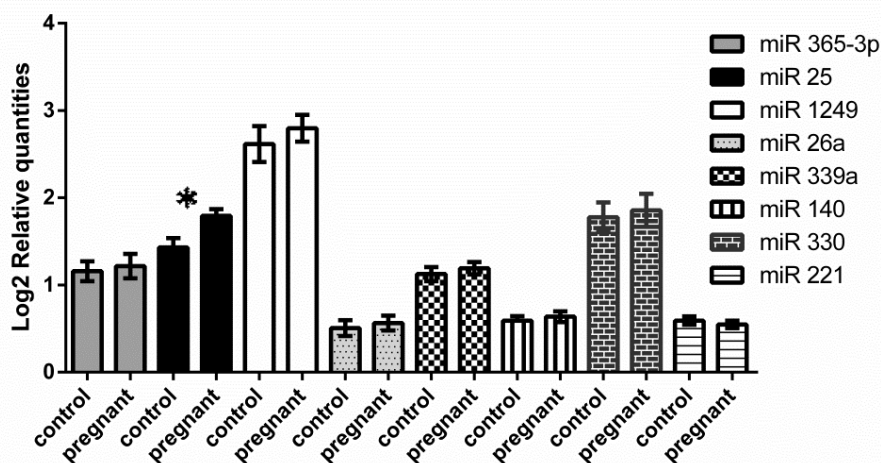


Figure 1 Relative expression of miRNA genes during day 21 of pregnancy and day 21 of the control cycle. * = $P < 0.05$

Conclusion As expression of miR-25 was greater on day 21 of pregnancy compared with day 21 of the control oestrous cycle, it has been identified as a potential biomarker of early pregnancy. Therefore, it may be used, in combination with additional markers, for the development of an early pregnancy test for dairy cows.

Acknowledgements The authors gratefully acknowledge funding from Science Foundation Ireland (SFI 13/IA/2025).

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Do weaner pigs (*Sus scrofa domesticus*) prefer olfactory enrichment?

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Application Pigs in this study preferred rope soaked in garlic oil, the pigs interacted with the garlic scented rope more often than an unscented rope.

Introduction In the UK it is a legal requirement for pig producers to provide environmental enrichment to encourage investigative and manipulative behaviors of their stock (EU directive 2001/93/EC). Preventing enrichment from losing its novelty and decreasing the rate at which animals become habituated to a stimulus is currently a concern. The use of garlic (*Allium sativum*) is highly prevalent in the livestock sector due to many beneficial natural properties (Augusti, 1996). The aim of this study was to see if weaner pigs displayed a preference for olfactory enrichment (rope soaked in garlic oil) compared to non-scented enrichment.

Material and methods A total of 146 (Landrace × Large White) weaner pigs divided by two batches, the first containing 76 and the second 70, were used for this study. The pigs were 4 weeks old at weaning and were housed in pens (25 pigs per pen, 6 pens over 2 replicates) according to weight. All pens measured 250cm by 350cm and had slatted plastic flooring. Each pen had two close-knit chains at 6mm diameter and 1.8m in length, attached to a metal bracket. With an equal distance of 45cm apart, attached to these chains were either a control rope (unscented) or treatment rope (garlic) that was soaked in a concentrated garlic oil extract (Pegasus Health) with purified water. This solution was used in a ratio of 30ml (oil): 1litre (water). Ropes were soaked individually for a 4-hour period and dried overnight. All ropes were then attached 2 hours before the pigs initially entered the pen. Pig had a standard piece of enrichment in addition, which was a porcichew attached to a chain. Garlic ropes were re-sprayed with a concentration of 10ml: 500ml on day 8 of the trial. Measurements of pigs interacting with ropes were collated by recording length of interaction, number of individuals interacting and the frequency of interaction with each rope; measurements were taken daily for 2 weeks. Time budgets and behaviours were statistically analysed using Kruskal-Wallis to identify the most performed behaviour alongside a Mann-Whitney U test as a post hoc test. All statistical analyses were completed through the use of GenStat version 17.

Results Figure 1 and Figure 2 below give the results. Arrow indicates when the ropes were re-sprayed with garlic oil

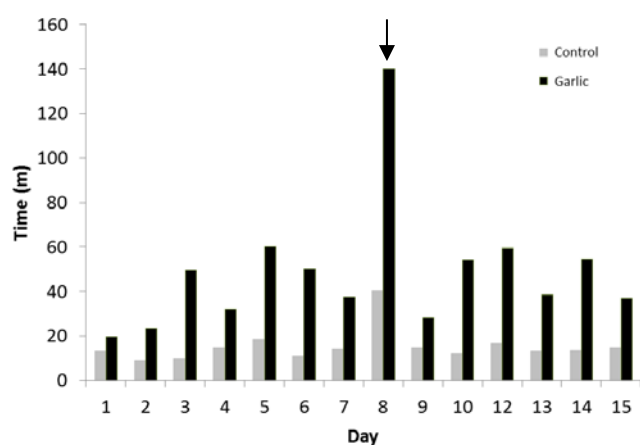


Figure 1 Time pigs spent interacting with the ropes

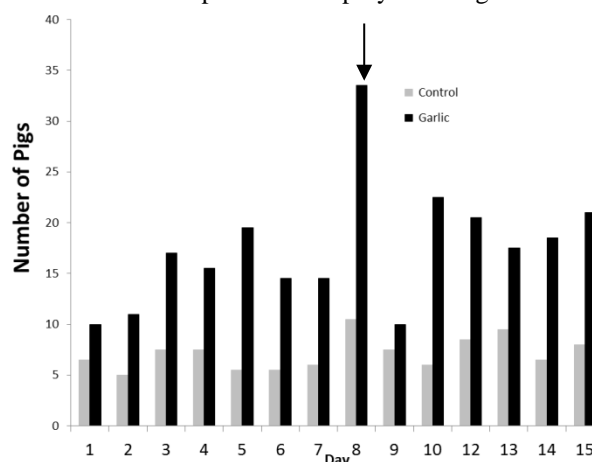


Figure 2 Number of pigs using the ropes

The garlic enrichment device significantly increased the length of time pigs spent interacting with the rope ($P < 0.05$) compared to the control (unscented) enrichment device. Significantly more pigs were interacting with the garlic enrichment rope than with the control rope ($p < 0.02$). Interactions with the garlic rope increased following re-spraying, i.e. increased from 40 mins/day to 140 mins/day.

Conclusion Weaner pigs have a preference towards olfactory enrichment. When the ropes were re-sprayed the interaction increased suggesting the addition of scent reduces habituation to an enrichment device. Garlic scented ropes may be useful to distract pigs when a tail-biting outbreak occurs.

Acknowledgements The authors acknowledge help and support from Sturgeons farm and technical staff.

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Effect of oral progesterone and caffeine at the end of gestation on piglet growth and survival

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Application Adding caffeine (4.8 g / day) to the diets of sows receiving oral progesterone (Regumate Porcine) at the end of gestation increased stillbirths and reduced post-partum piglet survival.

Introduction High incidences of piglet mortality, either during parturition or prior to weaning, reduce the efficiency and profitability of pig production. One of the causes of piglet mortality is intra-partum hypoxia, and this is higher in sows which farrow prematurely and, or have extended farrowing durations. When provided separately at the end of gestation, oral progesterone prevented premature farrowing (Vanderhaughe *et al.*, 2011), and caffeine increased gestation length and reduced stillbirths (Dearlove *et al.*, 2015). However, elevated progesterone at the end of gestation may impair colostrum production and piglet viability at birth. Therefore, the aim of the current study was to determine whether oral caffeine supplementation would increase the growth and survival of piglets born to sows receiving oral progesterone to prevent premature farrowing.

Material and methods Twenty Large White x Landrace pregnant sows (parity 3.0 ± 0.45 (Mean \pm SEM); range 1 - 8) were moved to farrowing crates 7.1 ± 0.34 days (range 6 - 11 days) prior to predicted farrowing date (gestation day 115). From day 111 to 113 of gestation, all sows received 5 ml of Regumate Porcine (0.4% w/v oral solution; MSD Animal Health) daily on top of their morning ration. Sows were stratified according to parity and predicted farrowing date, and allocated at random to receive a diet supplemented with either 0 g caffeine / kg diet (CONT) or 2.4 g of caffeine / kg diet (CAFF) from day 113 of gestation until parturition (n = 10 sows / treatment). The total number of piglets born, born alive and dead, and the number of mummified fetuses produced were recorded for all sows. Piglets born alive and dead were weighed on the day of birth (birthweight; BW). Piglets were weighed individually on day 5.2 ± 0.39 post-partum and at weaning (25.2 ± 0.39 days post-partum). Piglet mortality was recorded throughout lactation. Treatment effects on all measures were determined using a one-way ANOVA (Genstat version 15; VSN International Ltd., Hemel Hempstead, UK), with total litter size included as a co-variate.

Results Total litter size and gestation length were unaffected by treatment (15.1 ± 0.62 and 115.8 ± 0.23). The number of still born piglets was lower ($P < 0.05$) and the number of live born piglets higher ($P = 0.054$) for CONT compared with CAFF sows (0.7 ± 0.58 vs 3.2 ± 0.58 , and 14.5 ± 0.85 vs 11.7 ± 0.85 , respectively). There were no treatment effects (CONT versus CAFF) on litter weight at birth (21.9 ± 1.557 and 20.2 ± 1.57) or piglet birthweight (BW): mean (1.52 ± 0.07 and 1.37 ± 0.06 kg; $P = 0.124$); minimum (1.03 ± 0.10 and 0.80 ± 0.09 kg; $P = 0.107$) or maximum (1.89 ± 0.08 and 1.80 ± 0.10 kg; $P = 0.399$). CONT litters contained a lower proportion of piglets with a BW < 1 kg (0.05 ± 0.02 versus 0.16 ± 0.05 ; $P = 0.072$); and CAFF reduced the proportion of < 1 kg BW piglets which survived parturition (0.51 versus 0.81). There was a tendency for CONT piglets to be heavier on day five post-partum ($P = 0.063$) and at weaning ($P = 0.098$) (2.17 ± 0.11 versus 1.96 ± 0.13 kg and 6.98 ± 0.31 versus 6.01 ± 0.56 kg, respectively). However, piglet growth rate from birth to weaning was similar ($P = 0.118$) for CONT and CAFF litters (0.218 ± 0.01 and 0.183 ± 0.02 kg / day). CONT sows weaned more piglets than CAFF sows (12.9 ± 0.53 versus 8.7 ± 0.96 ; $P < 0.05$), and the proportion of liveborn piglets surviving to weaning was higher for CONT compared with CAFF litters 0.90 ± 0.04 versus 0.74 ± 0.05 , respectively.

Conclusion Surprisingly, the current data demonstrated that oral caffeine supplementation at the end of gestation increased piglet mortality, both during and soon after parturition, when provided to sows receiving progesterone to prevent premature farrowing. This finding contradicts previous evidence that caffeine supplementation at the end of gestation reduced stillbirths (Dearlove *et al.*, 2015). Supplementation with two inhibitors of uterine contractility in close succession may have increased farrowing duration, thus causing the currently observed increase in piglet mortality. Progesterone also inhibits caffeine metabolism (Partosch *et al.*, 2015) and, as a result fetuses may have been exposed to higher levels of caffeine than in previous studies (Dearlove *et al.*, 2015), potentially explaining the increased incidence, and reduced survival, of low birthweight piglets. Regardless of the mechanism, these data provide the first evidence that using progesterone in combination with caffeine at the end of gestation has negative outcomes for the piglet.

Acknowledgements The authors gratefully acknowledge the research and technical support staff of Spen Farm.

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Use of electrostatic particle ionisation (EPI) equipment in pig buildings: what is the potential to improve pig and worker health (in and outside the pig environment)?

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Application Use of EPI equipment reduced respirable dust and Gram negative bacteria in some samples in pig buildings, this led to a significant reduction in enzootic pneumonia like lesions.

Introduction Air quality in modern slatted pig buildings can be affected by a number of contaminants which have been known to have an adverse effect on pig and staff health. These include ammonia, dust, and gram negative bacteria. It was claimed that EPI air treatment could be effective in reducing these pathogens in the air by both pigs and pig unit staff.

Material and methods Two fully slatted pigs rooms (of C.360 pigs) on a university unit, representative of commercial UK production housing one batch of pigs each (identical for genotype and management, feed etc. but 3 weeks apart in time) were used. One room was fitted with EPI equipment and one room was not and was used as the control. Ventilation, temperature, relative humidity, dust, atmospheric (via single stage Andersen sampling) and settle gram negative bacteria and ammonia (both at fan height and at pig height) were measured across 10 weeks of the finishing period (from transfer into the building at 9 weeks of age). Pig health was assessed via the same methodology used for the BPHS health scoring system used in UK abattoirs. Pig performance was measured via individual identification and weighing at routine intervals. Feed delivery was quantified via use of a Roxell multifast feed system to allow feed delivered/gain (FCR) to be calculated. Replication of dust data was by repeated measures over time. Replication of BPHS data was by individual animal. Replication of FCR and growth data was by pen (13 pens treatment, 13 control). For ammonia and dust autoregressive moving-average models were used with a seasonal component where appropriate to account for the diurnal cycle. Where a significant relationship was determined ventilation rate was use as a covariate in statistical analysis of air quality (dust, ammonia and bacteria) parameters. Microbial results were analysed via a linear mixed effects model. To correct for the fact that the SD of the difference between treatment and control varied between days the measured values were divided by an estimate of within-day SD. Replication was by multiple samples over time.

Number of replicates for each variable are outlined in the table below under “N”.

Results

Parameter	N	Mean	Difference (treatment-control)	P Value
NH3 at fan height	263	3.53		<0.001
NH3 at pig height	263	3.99		<0.001
Dust <PM1	83	-0.00502		<0.001
Dust PM1-PM2.5	83	-0.0017		0.001
Dust PM2.5-Resp	83	-0.0047		<0.001
Dust Resp-PM10	143	-0.0267		NS
Dust <PM10	143	-0.0308		NS
Settle E-coli	40	-0.62		NS
Settle G-ve	40	-88.63		0.003
Andersen E-coli	36	-0.32		NS
Andersen G-ve	40	-2214.96		NS

There was a highly significant reduction in enzootic pneumonia-like lesions in the treatment pigs at slaughter ($P < 0.001$) with EP-like lesions estimated to be 4.5 times more likely to occur in the control pigs. No significant impact on any other health parameters or pig performance measurements was seen.

Conclusion Electrostatic particle ionisation has had a significant impact on a number of air quality parameters within modern slatted pig housing. Most notable the EPI significantly reduced “Respirable” dust (all factions below Respirable) and gram negative bacteria, detected by means of settle plates. It is likely that this has contributed to the reduction in EP-like lesions in these pigs as reductions in particulates has been significantly linked to reductions in pneumonia-like lesions in other work.

Acknowledgements The AHDB acknowledges Warkups ltd. as part funders of this project. Thanks to Select Statistics for analysis of the data. Thanks to ADAS for the loan of equipment and assistance with the project. Thanks to HAU pig unit staff for collecting samples.

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Relationship between piglet behaviour and vitality after farrowing with colostrum intake and early survival

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Application The colostrum intake of small birth weight pigs, showing little movement (i.e. low vitality), is likely sub optimal and therefore these pigs should be prioritised for cross-fostering to optimise their chance of survival.

Introduction In addition to birth weight, neonatal vitality is an important piglet characteristic influencing both pre-weaning mortality and growth. Piglet vitality at birth has long been used as an indirect measure of piglet viability and both sow's parity and farrowing performance seem to influence colostrum intake of piglets (Muns *et al.*, 2016). However, producers might further benefit from a vitality measure that is easy to perform by visual assessment at a more convenient moment, such as at the end of farrowing or before cross-fostering. Therefore, the objective of the present study was to study the relationship between a vitality score determined from newborn piglets' behaviour after farrowing and their colostrum intake.

Material and methods The study was performed on a commercial farm during May-April, 2015. From 79 sows (parities 1-8), 952 piglets were used. After farrowing, all piglets were individually evaluated in a 30-second test for two behavioural traits (adapted from Muns *et al.*, 2013) in a circular enclosure (55 cm diam.), i.e. rooting stimulation (R), and number of completed circles around the enclosure (NCC). The final vitality score was the sum of R and NCC. Birth order and birth weight were recorded as was heart rate, and blood oxygen saturation at birth using a portable Oxygen meter. Rectal temperature was recorded at birth and at 24 h (day 1). Colostrum intake was estimated from piglet's weight gain over the first day and mortality at day 1 was recorded. Multiple regression analyses for colostrum intake and survival at day 1 were performed following a stepwise procedure. Sow was introduced as random effect.

Results Piglets' behaviour parameters score and vitality score frequency distribution after farrowing are presented in Table 1. Vitality score was independent of birth weight ($P > 0.05$). In the final model, colostrum intake was positively influenced by birth weight, sow parity ($P < 0.001$, respectively), vitality score ($P = 0.015$) and negatively influenced by number of piglets born alive. In the final model, piglet survival at d 1 was positively influenced by birth weight ($P = 0.052$) and vitality score ($P = 0.082$). However, vitality score had no relation with birth order, heart rate or blood oxygen saturation ($P > 0.05$, respectively).

Table 1 Description of the two different parameters evaluated in a 30-s test to obtain piglet's vitality score (modified after Muns *et al.* 2013) and piglet scoring frequency distribution for the different vitality parameters evaluated after farrowing.

Item	Frequency	Percentage
Rooting Stimulation (R)		
0: Shows no rooting, searching or udder stimulation behaviour	25	2.6%
1: Shows searching or udder stimulation behaviour	927	97.4%
Number of completed circles around enclosure (NCC)		
0: Not able to turn 360° nor walk within 30 s	496	52.1%
1: Able to turn 360° or walk along the enclosure within 30 s	456	47.9%
Vitality Score (sum of R and NCC)		
0	22	2.3%
1	477	50.1%
2	453	47.6%

Conclusion According to our results, a visual assessment of the piglet's vitality (sum of R and NCC) after farrowing complements body weight and sow characteristics on estimating colostrum intake, thus becoming a promising tool to decide the best management action to perform on the animals and to identify non-viable pigs early in life within normal farm conditions.

Acknowledgements The authors gratefully acknowledge funding from the Rachadapisek Sompote Fund for Postdoctoral Fellowship, and from the Ratchadaphiseksomphot Endowment Fund (Chulalongkorn University, Bangkok, Thailand).

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The use of processed soy to reduce fishmeal in newly weaned piglet diets

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Application Fishmeal is a finite resource and under both supply and economic pressure, the current study has evaluated two soy based proteins and concluded that fishmeal can be partially replaced effectively.

Introduction Soy is commonly used in livestock diets with inclusions of up to 250g/kg in the early diets for pigs. However, it is widely documented that soy anti-nutritional factors (such as Trypsin inhibitor activity (TIA) and allergenic proteins) negatively impact piglet performance especially around the time of weaning. Processing of soy can reduce these compounds by methods such as heat, enzyme treatment or extrusion. Two soy proteins were evaluated in this study and were chosen to be of similar total protein content but derived by differing processing conditions; AlphaSoy 530 (AS) with 530g/kg crude protein (CP) (AS) produced by an extrusion based process and a commercially available soy protein concentrate, 560g/kg CP (SPC). Fishmeal (FM) is an accepted high quality protein used in piglet creep and weaning diets to maximise voluntary intakes and performance. Our study was designed to evaluate the performance of pigs fed a reduced fishmeal diet supplemented with one of two soy products in the first 3 weeks post weaning.

Material and methods 135 piglets (genetics: JSR Genpacker 90 (LW×LR) and PIC Camborough ((LW×LR) × White Duroc)) were allocated to one of three dietary treatments from weaning for 22 days, treatments were balanced for sex and litter origin in a random block design (5 pigs per pen, 9 replicates). Diets were formulated to contain appropriate levels of vitamins and minerals; phase 1 diet (day 0-9) was formulated to have 10.30MJ/kg NE and 1.35% SID lysine (approx. 210g/kg CP); phase 2 diet (day 9-22) was formulated to have 9.30MJ/kg NE and 1.28% SID lysine (approx. 200 g/kg CP). FM was included in phase 1 at 60g/kg in the control and 30g/kg in the treatment diets, hipro soy was included at 220g/kg in the control and 145g/kg in the treatment diets; phase 2 contained 30g/kg fishmeal in the control and zero in the treatment diets, hipro soy was included at 240g/kg in the control and 165g/kg in the treatment diets. The soy based proteins were included at 75g/kg replacing 30g/kg FM and balanced against hipro soy and wheat in both phase 1 and 2. Pigs were weighed on day 0,7,14 and 22. Pen feed intake was measured on the same days as weighing and FCR calculated using this data. Feeds were formulated and ingredients sourced by AB Agri Ltd; AS530 was purchased from Agrokorn A/S while FM and SPC were commercially available. Data was analysed by ANOVA in JMP Pro 13(JMP.inc).

Results Whilst daily live weight gain (DLWG) was significantly increased when fed phase 1 ($P=0.004$) for those piglets fed FM compared to soy based protein DLWG for the whole feeding period (figure 1) was not significantly different with performance for AS (449g/d) the same as FM (441g/d) and SPC (402g/d) $P=0.144$. Final weight did not significantly differ for pigs fed on AS (18.45kg) compared to FM (18.25kg) and SPC (17.41kg) $P=0.155$. Overall feed conversion efficiency (figure 2) was not significantly affected by treatment, AS was lowest (1.18) compared to FM (1.19) and SPC (1.25) $P=0.208$.

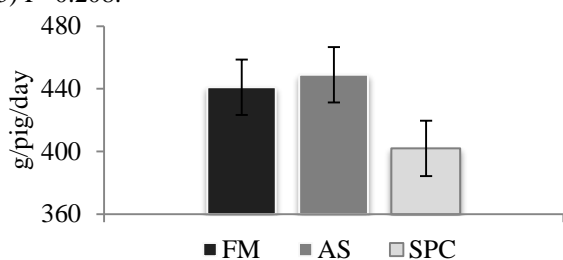


Figure 1 Overall daily live weight gain from day 0-22

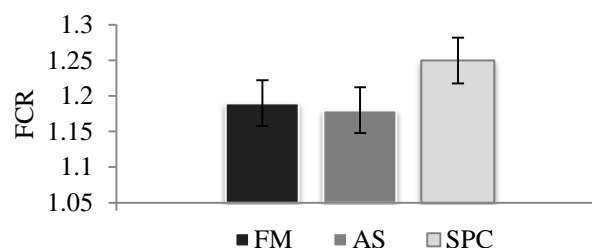


Figure 2 Overall feed conversion efficiency day 0-22

Conclusion Processed soy proteins can be an effective fishmeal replacer in piglet weaning diets without compromising growth performance or efficiency when formulated to maintain dietary net energy and SID lysine.

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Effect of cereal fermentation and carbohydrase supplementation to grow-finisher pig diets on growth and carcass quality

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Application Fermenting the cereal fraction of the diet prior to feeding increased pig growth rate, while xylanase and β -glucanase (XB) supplementation in either fresh or fermented liquid feed was effective in improving pig feed efficiency.

Introduction Fermenting liquid feed prior to feeding can improve diet quality due to a reduced pH, proliferation of lactic acid bacteria and reduced enterobacteria counts (Lawlor *et al.* 2002; Canibe *et al.* 2014). However, effects on growth performance are inconsistent in the literature. Fermenting only the cereal fraction of the diet has been suggested as a good practice to avoid degradation of free amino acids (Niven *et al.* 2006). Furthermore, XB supplementation to pig diets may improve feed efficiency in pigs by increasing the digestibility of non-starch polysaccharides and this effect could be increased where diets are liquid fed. The aim of this study was to assess the effect of fermenting the cereal fraction of the diet with or without enzyme supplementation during fermentation on growth and carcass quality of liquid fed finisher pigs.

Material and methods A total of 252 pigs (31.0 kg; ± 0.65 SEM) housed in same sex pens of 7 pigs/pen (n=9 pens/treatment) were allocated to 1 of 4 dietary treatments in a 2x2 factorial arrangement: (1) Fresh liquid diet (Fresh) where the diet was mixed with water immediately prior to feeding; (2) Fresh+XB (2200 units xylanase/kg and 100 units β -glucanase/kg of feed; Rovabio Exel AP, Adisseo, France); (3) Fermented liquid diet (Ferm) where the cereal fraction (35% wheat, 38% barley and 11% pollard) of the diet was fermented prior to feeding the diet and (4) Ferm+XB. The experiment lasted 55 days during which growth and feed intake were recorded. The fermented liquid cereal was prepared by an initial fermentation for 52 hours using a starter culture (Sweetsile, Agway, UK) in 2 tonne tanks which were replenished daily with a water:cereal ratio of 2.5:1. At slaughter, carcass weight, muscle depth and fat depth were recorded. The data was analysed by the MIXED procedure of SAS 9.1.

Results Pigs fed the fermented cereal diets had increased ADG ($P < 0.01$). At slaughter pigs fed the fermented cereal diets had higher final live weight ($P < 0.001$) and carcass weight ($P < 0.01$) but a reduced lean meat percentage ($P < 0.05$). Pigs fed the diets supplemented with XB had improved feed conversion ratio ($P < 0.05$) while other growth and performance parameters remained unchanged ($P > 0.05$).

Table 1 Effect of cereal fermentation and enzyme supplementation on growth and carcass quality

Item	Treatment				SEM	P-value		
	Fresh	Fresh+XB	Ferm	Ferm+XB		ferm	XB	ferm*XB
Initial live weight, kg	40.7	40.6	40.6	40.7	0.50	0.922	0.994	0.999
Final live weight, kg	95.1 ^b	96.8 ^{a,b}	98.1 ^a	97.5 ^a	0.50	<0.01	0.233	<0.01
Average daily gain, g/d	963	993	1024	1012	13.2	0.004	0.482	0.084
Average daily feed intake, g/d	2608	2583	2659	2594	35.5	0.368	0.195	0.564
Feed conversion ratio, g/g	2.81	2.67	2.71	2.65	0.047	0.171	0.043	0.445
Carcass weight, kg	71.8	72.8	74.5	74.1	0.61	0.002	0.616	0.220
Kill out, %	76.1	75.7	76.1	76.4	0.23	0.092	0.772	0.105
Muscle depth, mm	47.5	49.0	47.5	47.2	0.46	0.057	0.183	0.062
Fat depth, mm	11.2	11.9	11.9	12.1	0.27	0.105	0.118	0.392
Lean meat %	57.8	57.5	57.2	57.0	0.24	0.039	0.254	0.786

Conclusion Fermenting the cereal fraction of the diet improved growth, final live weight and carcass weight but reduced lean meat percentage. Supplementing XB to fresh or fermented diets improved the feed efficiency of pigs.

Acknowledgements Project funded by European Union's Seventh Framework Programme for research (ECO-FCE). A. Torres-Pitarch's PhD funded by the Teagasc Walsh fellowship programme

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Variability in chemical composition and apparent ileal digestibility of amino acids in rapeseed meals available for use in pig feed in the UK

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Application The variation in AID of lysine, methionine and threonine was found to range from 10 to 11% for RSM sources available to the UK feed industry. This should be considered when formulating and incorporating RSM into pig diets.

Introduction Rapeseed meal (RSM) is a highly available home-produced (UK) protein source, which could replace much of the soyabean currently used in UK finisher pig diets. With the UK's two major crushing plants sourcing rapeseed from across the UK and Europe, there may be considerable, but as yet poorly quantified variation in the nutritive value of the RSM products, making appropriate and efficient use of RSM difficult for feed formulators. This project aimed to quantify the variation in composition and, specifically, in apparent ileal digestibility (AID) of amino acids in UK-produced RSM.

Material and methods Over an 18 month period (spanning three harvests), 92 samples of RSM were randomly collected from crushing plants in Hull and Liverpool (Cargill Plc., Weybridge, UK) and were formulated into semi-synthetic diets containing RSM, maize starch, sucrose, soya oil, DCP 20, salt, TiO₂, a minerals and vitamins mixture and limestone flour at 500, 256, 160, 60, 12, 4, 4, 3.3 and 1.2 g/kg, respectively. A nitrogen-free diet contained maize starch, sucrose, soya oil, Solka Floc (International Fiber, Neuss, Germany), DCP 20, salt, potassium carbonate, TiO₂, minerals and vitamins mixture, limestone flour and calcined magnesite at 718.5, 160, 40, 40, 22, 4, 4, 4, 3.3, 3.2 and 1 g/kg respectively. Ninety-two pigs were PVTC cannulated at approximately 25kg live weight. Each RSM diet was offered to eight PVTC cannulated pigs (one in each of 8 time period replicates). The nitrogen-free diet was offered to a total of 16 pigs (2 pigs in each of the 8 time period replicates). Allocations of diets to pigs in each time period was statistically balanced. Pigs were individually housed and fed to appetite. An introduction period of 4 d was followed by a 2-d faecal collection and then two 12 hr ileal digesta collections over the following 48 hours. All samples were immediately frozen at -20°C and subsequently dried at 80°C for 48 hrs. Feed and ileal digesta samples were analysed for concentrations of dry matter (DM), amino acids (AA), and TiO₂. Feed samples were also analysed for neutral detergent fibre (NDF) and crude protein (CP) concentrations. Endogenous losses of N and the AID and standardised ileal digestibility (SID) of AA were calculated. Simple regressions were conducted to establish relationships between the chemical composition and the AID of AAs in the RSMs.

Results The CP and NDF concentrations of the 92 RSMs ranged from 351 to 425 g/kg DM (mean 389 g/kg; SD 14.1) and from 234 to 596 g/kg DM (mean 411 g/kg; SD 73.5) respectively. Mean concentrations of lysine (Lys), methionine (Met) and threonine (Thr) were 18.5 (SD 1.9); 7.3 (SD 0.4) and 16.5 (SD 0.7) g/kg DM respectively. Mean AID of Lys, Met and Thr were 0.660 (SD 0.071); 0.777 (SD 0.091) and 0.598 (SD 0.752) respectively, while mean SIDs for Lys, Met and Thr were 0.774 (SD 0.097), 0.842 (SD 0.116) and 0.755 (SD 0.128) respectively. Mean endogenous losses of Lys, Met and Thr were 1.064 (SD 0.492), 0.226 (SD 0.112) and 1.330 (SD 0.547) g/kg of intake respectively. Coefficients of variation for CP, NDF, Lys, Met and Thr contents were 3.6, 17.9, 10.3, 5.5 and 4.2% respectively, while those for AID of Lys, Met and Thr were 10.8, 11.7 and 11.6% respectively. The AID of Lys, Thr, arginine (Arg), isoleucine (Ile), leucine (Leu) and valine (Val) were each significantly but weakly correlated with CP, NDF and lysine contents (Table 1). The AID of Met was not significantly correlated with either CP or NDF content or with the concentrations of any of the three main AA (Table 1).

Table 1 Correlations (and P values) between protein, fibre and amino acid content and the AID of amino acids and DM

	CP	NDF	Lys	Met	Thr
AID of DM	0.06 (0.014)	0.01 (0.495)	0.01 (0.640)	0.01 (0.387)	0.04 (0.030)
AID of Lys	0.18 (<0.001)	0.23 (<0.001)	0.22 (<0.001)	0.01 (0.978)	0.02 (0.117)
AID of Met	0.01 (0.998)	0.01 (0.146)	0.01 (0.724)	0.01 (0.842)	0.01 (0.694)
AID of Thr	0.18 (<0.001)	0.23 (<0.001)	0.18 (<0.001)	0.01 (0.979)	0.09 (0.177)
AID of Arg	0.02 (0.081)	0.17 (<0.001)	0.08 (0.004)	0.01 (0.864)	0.01 (0.165)
AID of Ile	0.07 (0.006)	0.20 (<0.001)	0.08 (0.004)	0.01 (0.413)	0.01 (0.475)
AID of Leu	0.15 (<0.001)	0.26 (<0.001)	0.19 (<0.001)	0.01 (0.908)	0.02 (0.107)
AID of Val	0.10 (0.001)	0.20 (<0.001)	0.14 (<0.001)	0.01 (0.380)	0.09 (0.179)

Conclusion The degree of variation in the AID of individual amino acids in this study should be considered when including RSM, especially at high levels, into pig diets. Significant but weak correlations between the AID of essential AAs and CP, NDF and AA contents indicated that the chemical constituents of RSM are not good predictors of nutritive value for pigs.

Acknowledgements The authors thank the Department of Agriculture, Environment and Rural Affairs for NI (DAERA) and AB Vista for funding. Cargill and Technology Crops are gratefully acknowledged for the collection of RSM samples.

Quantitative analyses of DNA in pig faeces: a non-invasive measure of feed efficiency?

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Application Pigs fed a xylanase supplemented diet had significantly reduced levels of pig DNA in their faeces compared to control pigs but did not differ in relative bacterial DNA levels. Quantifying animal DNA levels in faeces could be a non-invasive measure of gut cell loss and thereby possibly feed efficiency.

Introduction Gut cell turnover contributes to overall feed efficiency (Cant *et al.*, 1996). Currently all methods to assess gut turnover are invasive and costly, such as stable isotope infusion (Danicke *et al.*, 2000). The overall hypothesis is that detection of pig genomic DNA in faecal samples could be a non-invasive marker of gut cell losses. Xylanase supplementation has been shown to influence intestinal morphology and may therefore effect gut cell turnover (Luo *et al.*, 2009). The present study aimed to assess DNA composition in pig faeces taken from a xylanase supplementation trial.

Material and methods Sixteen newly weaned female Camb12 (Landrace x Duroc x Large White) piglets (8.8±1.38 kg) were allocated to one of two dietary treatment groups, control (n=8) or xylanase (n=8). The xylanase group received the same diet but with Econase® XT 25 (AB Vista) included at 17800-26000 BXU/kg. Faecal samples were collected at week 2, 4 and 6 of the 6-week feed trial. DNA was then extracted from the faecal samples using a modified phenol-chloroform method, this involved 30mg of faeces being homogenised in Nuclei Lysis Solution (Promega) using a hand-held homogeniser prior to phenol extraction. DNA concentration (ng/µl) and quality (260nm/280nm) were assessed using NanoDrop 2000 (Thermo Scientific™) spectrophotometer. DNA yield was calculated as µg/mg of faecal material. Comparison of faecal DNA composition was made using SYBR Green quantitative PCR (qPCR) methodology using a LightCycler® 480 (Roche) instrument. Detection of pig DNA was by quantitative PCR using primers designed specifically for the pig CYTB (mitochondrial) gene, while bacterial DNA was detected using published 16S primers (Mieszkin *et al.*, 2009). PCR products were verified using Sanger Sequencing (Source BioScience, Nottingham, UK). Data was analysed by two-way ANOVA (time x diet) using Genstat 16th Edition, with significance at P<0.05.

Results Faecal samples taken from the xylanase group tended (P=0.062) to have higher extracted total DNA concentration (µg/mg faeces) compared to the control group. Quantitative PCR results for pig CYTB DNA showed there was no interaction between time and diet (P = 0.225), but there was a significant effect of diet (P = 0.039) and a weak trend for an effect of time (P = 0.087) (Figure 1). Conversely, results for bacterial 16S DNA detection indicated no interaction between time and diet (P=0.698), as well as no significant independent effects of time (P=0.145) or diet (P=0.974) (Figure 2).

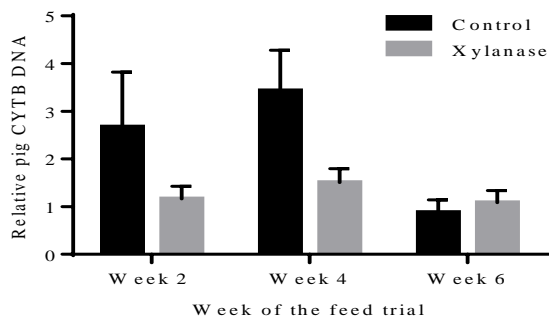


Figure 1: Effect of time and diet on the relative pig CYTB DNA in pig faecal samples. Error bars are ± S.E.M. n=8 for all groups, except Control Week 6 where n=7.

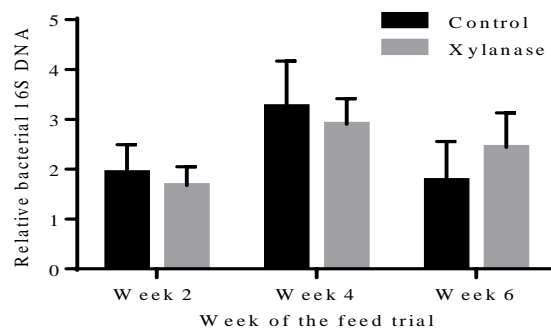


Figure 2: Effect of time and diet on the relative bacterial 16S DNA in pig faecal samples. Error bars are ± S.E.M. n=8 for all groups.

Conclusion A diet containing xylanase appeared to affect the composition of DNA present in the faeces compared to the control. There was significantly less pig CYTB DNA present in the xylanase faecal samples but no significant difference in the bacterial DNA content, potentially indicating that xylanase decreases gut cell losses.

Acknowledgements The authors gratefully acknowledge funding from AB Vista and the University of Nottingham.

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Improving the lifetime performance of small pigs at weaning through the use of nurse sows and/or starter diet allowance

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Application Small pigs (<6kg) nursed until 7 weeks of age had improved feed use efficiency and similar growth performance in the finish period compared to pigs weaned at 4 weeks of age and offered a high specification dietary regime.

Introduction Pigs with a low weight at weaning present a problem for efficient pig production (Douglas *et al.*, 2014). The aim of this work was to identify management and nutritional practices to improve the lifetime performance of low wean weight pigs, in terms of feed conversion ratio (FCR) and growth rate by evaluating the use of nurse sows for rearing low weight pigs at weaning and the effect of high and low levels of starter diets. Effects of these treatments on post weaning pig performance were reported by Craig *et al.* (2016) and this abstract focuses on the finishing pig performance.

Material and methods Over 9 time periods, 266 pigs (PIC 337 x (Large White x Landrace)) were used. In each time period, 30 piglets were selected at weaning (28 days) based on weight. Ten pigs represented a normal weaning weight (8.9kg; NORM) whilst 20 piglets represented a low wean weight (<6kg). Ten low weight piglets were placed on a nurse sow for a further 3 weeks (NURSE) and the other 10 were weaned and placed on a special starter dietary regime which delivered 1kg/pig of a very high specification starter diet (Special Starter), 4kg/pig of a Starter 1 diet and 8kg/pig of a Starter 2 diet (WEAN). The NORM and NURSE pigs were split into groups of 5 per pen at weaning (4 and 7 weeks respectively) balanced for weight and gender. One pen of each treatment was offered a dietary regime which delivered 4kg/pig of Starter 1 diet and 8kg/pig of Starter 2 diet (High). The other pen was offered 4kg/pig of Starter 2 diet (Low). Diets were offered *ad libitum* and when pens had consumed their starter allowances, they were offered grower diets until they reached 12 weeks of age, or 15 weeks of age for the NURSE treatments, then a finishing diet was offered to 21 weeks. On transfer to the finishing house at 10 weeks of age, or 13 weeks for NURSE treatments, the 'Low and High' treatments were combined into one pen of 10 pigs for the NORM and NURSE treatments respectively. Pigs were individually weighed and pen feed intakes recorded at 4, 7, 10, 12, 15, 18, and 21 weeks of age. Average daily gain (ADG), average daily feed intake and FCR were calculated. Mortality was calculated as the percentage of pigs that died in each pen. Individual pig performance was analysed using the linear mixed model methodology with time period fitted as a random effect and a factorial arrangement of Sow*Diet+WEAN fitted as fixed effects (Sow=NORM, NURSE or WEAN; Diet = High, Low or Special), using individual pig as the experimental unit (n=266). A post-hoc t-test was used to assess the effect of NURSE vs. WEAN. Finish data was analysed with pen as the experimental unit (n=9/treatment) using a one-way ANOVA with three treatments: WEAN, NURSE and NORM, and blocked for time period. Analyses were carried out using Genstat Version 10.1.

Results At 10 weeks of age, the weight of pigs on the 'WEAN', 'NURSE High', 'NURSE Low', 'NORM High' and 'NORM Low' treatments was 23.9, 22.2, 19.8, 31.6 and 27.3 kg respectively. Pigs that were offered a 'High' starter diet allocation were heavier throughout the finishing period and had a 7kg advantage at 21wks (P<0.001) due to greater ADG from 12-21wks (P=0.034) (784 g/day and 737g/day for 'high' and 'low' starter diet allocations respectively). WEAN pigs were 3kg heavier than NURSE pigs at 12 wks of age (33.8kg and 30.8kg respectively; P<0.001), but there was no significant difference at 15, 18 or 21wks (46.3, 60.3 and 77.4kg respectively). ADG of WEAN and NURSE pigs were not significantly different from 12-21wks (743, 734 and 701g/day respectively; P>0.05) but the ADG of NORM pigs was significantly higher (803 g/day) than that of NURSE pigs (P<0.001). NURSE pigs had a lower intake from 10-21 weeks (P<0.001) eating 1.58kg/d compared to 1.90 and 1.99kg/d on the NORM and WEAN treatments. From 10-21 weeks, NURSE pigs had a consistently better FCR than WEAN pigs (P<0.003). The FCRs were 2.79, 2.20 and 2.40 for WEAN, NURSE and NORM pigs respectively (P<0.001). Although lifetime mortality (4 to 21 weeks of age) was not statistically different, NURSE High pigs had less mortality (6.7%) than WEAN (12.1%) and NURSE Low (15.6%) pigs. WEAN mortality mostly occurred between 15-21wks while NURSE pig mortality mainly occurred between 7 and 10 weeks of age, and was higher when pigs were offered the 'low' starter diet allowance.

Conclusion Small pigs (<6kg) nursed until 7 weeks of age had improved FCR from 10-21weeks compared to those weaned at 4 weeks of age and offered a high specification dietary regime. Although the weight of pigs at weaning from NURSE sows (7 weeks) was increased, their lifetime performance was enhanced when they were offered the 'high' starter diet regime. Overall, whilst rearing low wean weight pigs on a nurse sow for an additional 3 weeks and then offering them a 'high' starter diet regime had no significant impact on finisher growth performance it improved the feed conversion ratio of these pigs by 0.59 compared to low wean weight pigs which were offered a high specification starter allowance.

Acknowledgements The authors gratefully acknowledge funding from DEARA and Pig Regen.

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Reproductive benefits and trade-offs with increasing sow live-weight and back-fat depth in late gestation

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Application Fatter sows had fewer piglets born alive but the piglets were heavier at birth and at weaning while heavier sows, regardless of back-fat depth, had more total born and born alive and weaning weight was unaffected.

Introduction With many EU countries still experiencing sub-optimal sow productivity (etc UK 26.2 pigs/sow/year vs. Denmark 30.1 pigs/sow/year) (AHDB, 2016), it is important to identify sow factors that affect reproductive output. Recent recommendations and research has focused on the weight and condition of gilts but rarely attention is given to that of animals in later parities. Indeed no recent research on sows is available to establish if recommendations for sows are appropriate for the modern hyper-prolific animal. The objective of this study was to identify the reproductive benefits and/or losses associated with sow live-weight and back-fat depth during gestation by an association analysis.

Material and methods Individual sow data, a total 1058 sow records, were available from feeding experiments conducted at Teagasc Moorepark, Co. Cork and AFBI, Hillsborough, Co. Down, between 2005 and 2015. Animals originated from 10 studies which investigated gestation and lactation diet composition, feed allowance and timing during gestation. Phenotypes available included total born (TB), born alive (BA), piglet birth weight (BWT), percentage pre-weaning mortality (PWM), pre-weaning growth rate per litter (PWG), number weaned (WN), piglet weaning weight (WWT) lactation intake (LACT) and weaning to service interval (WSI). Sow live-weight and P₂ back-fat depth were taken at service, day 25, 50, 80,110 of gestation and at weaning. The association of dependent variables with both sow live-weight and back-fat depth were determined separately using mixed models; parity, month of farrowing and year of farrowing were all included as fixed effects while sow was included as a repeated effect with the appropriate covariance structure among records within sow. In the analysis of BWT, litter size was included as a fixed effect; WN and WSI, litter reared was included as a fixed effect; WM and PWG; litter reared and lactation diet were also included as fixed effects, and when analysing WWT and LACT; litter reared, lactation diet and lactation length were included as fixed effects in the model. In a separate analysis, the association of dependent variables with sow live-weight were quantified with back-fat depth included as a fixed effect in the model. Results presented are for day 110 only as it was the main factor significantly associated with phenotypic outputs.

Results At day 110 mean sow live-weight and back-fat depth were 246.9kg (SD= 34.2) and 15.8mm (SD=4.4), respectively. Table 1 reports a 10kg increase in sow live-weight was associated with increased TB, BA, BWT and PWG, but also an increase in PWM and decreased WN and LACT. When adjusted for back-fat depth, significance was lost for WN. A 1mm increase in back-fat depth was associated with a decrease in BA but an increase in BWT and WWT. WSI was not associated with either sow live-weight or back-fat depth (P>0.05).

Table 1 Linear and quadratic regression coefficients (standard errors) of the association of sow live-weight and back-fat depth at day110 of gestation with reproductive output

Variable	Sow measures		
	Live-weight ¹	Adjusted live_weight ²	Back-fat depth ³
TB	0.14(0.04)***	0.30(0.05)***	-0.09(0.03)**
BA	0.09(0.04)*	0.22(0.05)**	-0.08(0.03)**
BWT	0.02(0.002)-0.0002(0.00004)***	0.02(0.003)**	0.01(0.002)**
PWM	0.42(0.13)+0.01(0.002)***	0.61(0.19)**	0.04(0.10)
PWG	1.68(0.46)***	1.40(0.57)*	0.45(0.29)
WN	-0.06(0.02)-0.001(0.0004)***	0.14(0.16)	-0.003(0.01)
WWT	0.06(0.01)***	0.04(0.02)**	0.02(0.01)*
LACT	-2.60(0.65)***	-1.692(0.52)**	-2.155(0.27)***
WSI	-0.01(0.04)	-0.01(0.05)	-0.02(0.02)

*P<0.05**P<0.01***P<0.001

¹ 10kg increase; ² 10kg increase adjusted for back-fat depth; ³ 1mm increase in back-fat depth

Conclusion Independent of sow parity, improved reproductive performance can be seen with greater sow live-weight and back-fat depth in late gestation. Every 10kg increase in sow-live-weight and 1 mm increase in sow back-fat depth, increased piglet weaning weight by 0.06kg and 0.02kg, respectively, with a reduction in lactation intake of 2.60kg and 2.15kg, respectively, suggesting sows mobilised body reserves to meet the demands of the litter. This study highlights the need to consider both live-weight and back-fat depth in tandem as important indicators of farrowing and pre-weaning productivity.

Acknowledgements The authors gratefully acknowledge funding and support from Department of Agriculture, Food and the Marine (Ireland) and the University of Leeds

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Whole animal system calorimetric estimates of greenhouse gas emissions from finishing pigs of contrasting feed use efficiency

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Application The RFI of pigs in the growing and early finish period was shown not to influence GHG emissions measured using respiration calorimetry of the whole pig system (pig + slurry) during late finish. An unexpected and unexplained lack of difference in late finish FCR may underlie this finding. However low RFI may reduce ammonia emissions

Introduction Pig production is responsible for 13% of all livestock green house gas (GHG) emissions (Philippe and Nicks 2015), with excreta generating 70% of pig CH₄ emissions (Monteny *et al.*, 2006). A goal of livestock breeding is improved feed use efficiency. Low residual feed intake (RFI) (i.e. high efficiency) cattle emit 15-30% less CH₄ than high RFI cattle (Hegarty *et al.*, 2007). The aim of this study was to determine if pig CH₄ emissions are also associated with RFI.

Material and methods Over three time periods, three litters of pigs with 10 or more boars (terminal sire PIC337) were identified at birth and remained intact until 8 weeks of age. In each time period 10 boars were individually housed between 8 and 17 weeks of age and offered a typical grower diet to 12 weeks of age and then a typical finisher diet. Pig weight and intake was recorded weekly. The average daily gain (ADG) (g/day), average daily feed intake (ADFI) (g/day), feed conversion ratio (FCR) and RFI of the animals was calculated between 9 and 17 weeks of age. At 17 weeks of age 6 boars per time period (n = 18 in total) were selected for calorimetry based on their RFI (3 representing 'low' RFI and 3 representing 'high' RFI). Selected pigs were then kept in individual calorimeter chambers for 33 days at 16±1°C and relative humidity 60±10% and offered the same finisher diet. Feed was replenished daily and water was available ad lib. Excreta were allowed to accumulate throughout the 33d confinement. Live weight and feed intake were recorded only at the start and end to minimise disturbance to the pigs and a recording of pig sounds was played to promote normal behaviours. Inlet and exhaust gas concentrations were recorded every 14 minutes and CH₄ recovery was 97 - 103%. Mean emissions of CH₄, CO₂, N₂O and NH₃, and heat production (calculated from O₂ consumption), were reported for pigs and their excreta for the final 3 d only. Data were subjected to analysis of variance with time period as block.

Results Table 1 shows that pigs selected for differences in RFI (P < 0.001) differed also in FCR (P < 0.01) but not in ADG or ADFI during the grower/early finish period. However, the significant difference in pre-chamber FCR was not retained through the 33d period (late finish) in the chambers. Pre-chamber RFI did not affect CH₄, CO₂ or N₂O emissions but NH₃ emissions, O₂ consumption and the associated HP tended to be lower for the low RFI pigs

Table 1 Effect of RFI status on gaseous emissions and heat production

	Low RFI	High RFI	s.e.m.	P value
Grow/early finish period (9 to 17 wks of age)				
ADG (g/d)	887	863	42.6	0.690
ADFI (g/d)	1737	1903	70.6	0.119
FCR	1.99	2.24	0.058	0.008
RFI	-82.5	71.3	16.88	<0.001
Calorimetry study (Late finish)				
ADG (g/d)	1073	1113	48.1	0.565
ADFI (g/d)	2437	2544	92.8	0.427
FCR	2.29	2.30	0.070	0.907
CH ₄ emissions (L/d)	84.1	78.1	7.10	0.565
CO ₂ emissions (L/d)	1081	1147	34.0	0.186
O ₂ consumption (L/d)	907	970	23.8	0.084
N ₂ O emissions (L/d)	34.2	35.7	1.54	0.457
NH ₃ emissions (L/d)	163	294	42.0	0.054
Heat Production (MJ/d)	18.6	19.9	0.496	0.074

Conclusion Pre chamber performance did not affect GHG emissions from the animal system (i.e. pig + slurry) but tended to reduce ammonia emissions, despite similar ADFI and thus N intakes. The unexpected similar in-chamber FCR between high and low RFI pigs may underlie the lack of effect on their GHG emissions.

Acknowledgements This study was part of the ECO-FCE project which received funding from EU 7th Framework Programme for research, technological development and demonstration under grant agreement No. 311794.

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Porcine feed efficiency (FE)-associated intestinal microbiota and physiological traits: finding consistent cross-localation biomarkers for residual feed intake (RFI)

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Application Intestinal bacteria associated with improved feed efficiency (FE) found to be consistent across geographic locations can potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs of similar genetics. Alternatively, they could be exploited as predictive biomarkers for porcine FE.

Introduction Feed efficiency is critical in the pig industry, as feed accounts for ~70% of production costs (Teagasc, 2015). The gut microbiota may influence FE in pigs, considering its role in host metabolism and immunity (McCormack *et al.*, 2016, Ramayo-Caldas *et al.*, 2016, Vigors *et al.*, 2016). The aim here was to investigate the intestinal microbiota composition of pigs ranked on residual feed intake (RFI; as a metric for FE) reared at three geographical locations [Republic of Ireland (ROI), Northern Ireland (NI), Austria (AT)], using common genetics and management protocols.

Material and methods Four trials, comprising 369 pigs were conducted across three geographic locations; ROI [(two batches; one medium herd health [ROI1; n=80] and one high herd health [ROI2; n=138]); NI (one batch; n=87) and AT (one batch; n=64)]. Pigs were ranked as either high or low RFI between day 42 and 91 post weaning (pw), and a total of 100 pigs (50 high RFI and 50 low RFI) were selected for sampling (ROI1; 20, ROI2; 40, AT; 16, NI; 24). Microbiota profiling was determined by high-throughput 16S rRNA gene sequencing performed on faeces, collected at day 42 and 105 pw, as well as ileal and caecal digesta collected at slaughter, and functionality predicted using PICRUSt software. Other potentially FE-associated physiological parameters (salivary cortisol, haematology, serum biochemistry and intestinal immunology) were measured. Growth performance and physiological parameter data were analysed using a mixed linear model in SAS 9.3 and microbiota composition at phylum and genus level was analysed in R using the Kruskal-Wallis test for independent samples and the Wilcoxon-Rank test for paired samples.

Results Intestinal microbial diversity varied with geographic location, health status and intestinal site, but not by RFI. For the high health batch of pigs (ROI2), low RFI (more feed efficient) animals had higher species diversity in the ileal and caecal digesta, as measured by the Shannon and Simpson indices (high RFI=0.90, low RFI=0.95 and high RFI=5.38, low RFI=6.78, respectively; $P<0.05$). In addition, some bacterial taxa varied consistently in relative abundance between high and low RFI pigs across two geographical locations and/or across the two ROI batches. Lentisphaerae (high RFI: ROI1=0.59%; ROI2=0.007%; low RFI: ROI1=1.44%; ROI2=0.013%), Mucispirillum (high RFI: NI=0.004%; AT=0.002%; low RFI: NI=0.008%; AT=0.030%), Methanobrevibacter (high RFI: ROI1=0.006%; ROI2=0.003%; low RFI: ROI1=0.014%; ROI2=0.010%), Ruminococcaceae (high RFI: ROI2=9.5%; AT=6.0%; low RFI: ROI2=11.1%; AT=6.65%), RF16 (high RFI: ROI1=0.05%; ROI2=0.12%; low RFI: ROI1=1.13%; ROI2=0.56%), and two unknown bacterial species were more abundant within the faecal/caecal microbiota of low RFI pigs ($P<0.05$). All of these taxa are major contributors to carbohydrate metabolism, which was also reflected in functional predictions. A low RFI value was also correlated with Lentisphaerae ($R=-0.757$; $P<0.05$). Of the other FE-associated physiological parameters measured, only salivary cortisol differed, tending to be lower in low versus high RFI pigs (1.34 versus 1.76 ng/mL, respectively; $P=0.06$).

Conclusion The rearing environment, health status and intestinal site greatly impacted the pig gut microbiome, which in turn presents challenges when identifying consistent reliable microbial biomarkers for FE in pigs. However, some FE-associated enterotypes (Lentisphaerae, Mucispirillum, Methanobrevibacter, Ruminococcaceae, and RF16) were common across trials and related to a potentially 'healthier' and metabolically more capable microbiota. These taxa could therefore potentially be used as probiotics or targeted by dietary means as a strategy for improving FE in pigs in the future.

Acknowledgements The authors would like to thank the farm staff and technicians for assistance with this work. This project (ECO-FCE) has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration. U.M. McCormack is funded by the Teagasc Walsh Fellowship programme.

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The effect of dietary supplementation with omega-3 polyunsaturated fatty acids from salmon oil on reproductive performance of sows

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Application Dietary supplementation of sows with 10g omega3 PUFA/kg diet throughout one reproductive cycle could be used to increase sow fatness in the subsequent parity.

Introduction It is widely recognised in nutritional research of both humans and farm animals, that inclusion of omega-3 polyunsaturated fatty acids (PUFA), particularly those of marine origin (i.e. the long chain PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), in the diet can have beneficial effects on many physiological processes (Millet & Delezie, 2013; Tanghe & deSmet, 2013). Omega-3 PUFA play a role in the molecular events that underpin reproduction, and although the exact mechanism(s) is/are unknown, it is believed that the mechanism(s) may involve regulation of prostaglandin or cholesterol synthesis and among other mechanisms, endometrial gene expression (Wathes *et al.*, 2007). The aim of this study was to investigate the effect of supplementing sow diets with 10g salmon oil/kg throughout one reproductive cycle on sow performance in this parity and the following one. 'It was hypothesised that supplementation of the sow diet with 10g salmon oil/kg during one reproductive cycle would .increase numbers born alive per litter and reduce preweaning mortality'

Material and methods Two hundred multiparous Large White x Landrace sows (n=100), comprising 7 batches each of between 27 and 31 sows placed on trial at successive 3 week intervals, were fed either a diet supplemented with 10g omega-3 PUFA derived from salmon oil/kg diet or a control diet containing 10mg soya oil in the same carrier matrix/kg diet, from service, through to the next service i.e. approximately 22 weeks. Both diets met all nutrient requirements (NRC, 2012). Sows were fed individually throughout, housed in straw yards in groups of 8 -10 during gestation and from weaning to service and in conventional farrowing crates from 3 days prior to farrowing until weaning. Sows were fed their allocated gestation ration until farrowing and then fed twice daily according to a step up programme from farrowing to weaning. Sows were then followed through a second parity during which all received the same standard commercial diets and normal farm management. Litter size born, born alive and weaned, litterweight born and weaned, sow liveweight and fatness at service, pre-farrowing and weaning and wean to oestrus interval were recorded in both parities. Data were analysed by general linear model in Minitab 16 using the model Diet x Batch with Parity as covariate

Results Both dietary treatments produced similar sow and litter performance throughout both trial parities. Sows averaged 13.5 piglets born alive and 10.8 piglets weaned in trial parity 1, 13.1 born alive and 10.7 weaned in trial parity 2. Litter weight weaned averaged 74.6 kg in the first trial parity and 78.6 kg in the second trial parity. Omega3 PUFA fed sows were fatter in their second parity (when omega 3 PUFA were no longer being fed) than control sows ($P < 0.05$; see Table 1).

Table 1 Sow backfat thickness in mm at 6.5cm from the midline in line with the last rib (P2) at starting service and at weaning for trial parities 1 and 2 when fed diets supplemented with 1% salmon oil (Omega 3 PUFA) or soya oil (Control)

Sow P2 at service	Omega3PUFA	s.e.m.	Control	s.e.m.	P-value
At start	12.6	0.48	12.8	0.44	0.786
After parity1	9.9	0.32	9.4	0.30	0.314
After parity 2	15.6	0.42	14.2	0.39	0.012

Conclusion There were no reproductive performance benefits from adding 10g omega 3 PUFAs/kg to the sow diet. Sows which had received the omega 3 PUFA diet in the first trial parity were significantly fatter than control sows in their second parity. This may be due to changes in metabolism as a result of the omega3 PUFA which had perhaps caused different prostaglandins to be produced compared to those in the control sows. Further work would be required to determine if this were the case.

Acknowledgements The author gratefully acknowledges funding from AHDB

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Genome-wide association studies of telomere length in dairy cattle

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Application Genomic markers and candidate genes identified may inform genomic prediction and selection within a breeding programme aiming to enhance cattle longevity based on animal telomere length.

Introduction Telomeres are repetitive base sequences at the end of chromosomes which they protect from deterioration or fusion with neighboring chromosomes thus enabling the complete replication of genomic DNA. Age, psychological stress, diet and disease have all been associated with telomere length (TL) dynamics. Variability of TL among individual cows is heritable (Ilska-Warner *et al.*, 2017) and has been associated with survival (Brown *et al.*, 2012; Ilska-Warner *et al.*, 2017), indicating a potential use of TL as a correlated trait and/or biomarker for longevity. Genome-wide association studies (GWAS) for TL will reveal its genomic architecture but have only been performed in humans. Our aim was to perform the first GWAS of TL in dairy cattle.

Material and methods The study was based on 246 Holstein-Friesian cows raised at the SRUC Dairy Research Centre at Crichton Royal Farm, Dumfries, Scotland. Cows were equally split into two groups of genetically selected for milk fat and protein yield and control animals. DNA was extracted from blood using a modified protocol based on silica spin columns (Seeker *et al.*, 2016). The leukocyte relative TL was measured with a quantitative PCR assay (Seeker *et al.*, 2016). Individual animal phenotypes derived were telomere length at birth (TLB) and telomere length at first calving (TLC). All phenotypes were first analysed using univariate linear models to identify factors with a significant effect. All animals were genotyped with the the Illumina Ovine SNP50 BeadChip array. Multidimensional scaling analysis (MDS) was performed to detect population substructure. The quality control (QC) of the data considered the following thresholds: Minimum Allele Frequency=0.05, Hardy-Weinberg Equilibrium= 10^{-4} , proportion of individuals and markers with missing genotypes=0.05. The GWAS used a mixed model that included the genomic relationship matrix as a random effect and all factors with a significant effect on TL as fixed effects. The analyses were performed with GEMMA algorithm. A genome-wide significance threshold was set at $P=0.05$ and a Bonferroni-correction for multiple testing was applied. A suggestive (one false discovery per genome scan) significant threshold was also applied. The BioMart data mining tool (<http://www.ensembl.org/biomart/martview/>) within the Ensembl database and the Bos_taurus_UMD3.1 assembly were used to map genes in the regions harbouring the significant Single Nucleotide Polymorphisms (SNPs) from GWAS.

Results After QC 39,024 SNP markers remained. MDS analysis showed that the animals were clustered in two groups corresponding to the two different genetic groups; this sub-structure was accounted for by fitting the two components and the genomic matrix in GWAS. Significant fixed effects were year and season, well position on a qPCR plate, age (TLC) and lactation number (TLC). TLC phenotypes were log-transformed to achieve normal distribution. GWAS identified a SNP on chromosome 6 significantly associated with TLC at genome-wide level. Three SNPs, one located on chromosome 1 and two on chromosome 23, were significantly associated with TLB at suggestive level. The SNP on chromosome 6 was located within the syntaxin 18 gene that has been previously associated with telomerase-independent telomere stability in humans. The two SNPs on chromosome 23 were located within the neural precursor cell expressed developmentally down-regulated 9 (NEDD9) gene, which has a role in cell cycle progression and cytoskeletal regulation.

Conclusion The results of these study shed light in the genetic architecture of TL in dairy cattle in different life stages. TL in cattle is a heritable quantitative trait associated with longevity and could be amended through selective breeding. Genomic markers affecting TL can be used to enhance the accuracy of genomic predictions and selection in a breeding programme.

Acknowledgements The study has been funded by BBSRC grant BB/L007312/1.

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Exploring quantitative trait loci mapping for lameness traits in Holstein dairy cows

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Application Lameness is a serious welfare issue raising socioeconomic concerns in the cattle sector. Outcomes of this study may contribute to selection decisions within breeding programmes aiming to reduce lameness incidence.

Introduction Lameness in dairy cows is associated with animal welfare and severe economic losses. Claw horn disease traits and digital cushion thickness (DCT) are lameness-related traits (Bicalho *et al.* 2009), with recent studies showing moderate to high heritabilities (Oikonomou *et al.* 2014). Our objective is to present the first genomic analysis from an ongoing project aiming to detect genomic regions of interest for these traits.

Material and methods Data included 267 Holstein cows from one herd genotyped with a genome-wide 50K Affymetrix DNA array. After quality control, 35,325 Single Nucleotide Polymorphisms (SNPs) were kept. Animals were phenotyped for five claw horn related traits: sole ulcer, sole haemorrhage, white line disease, digital dermatitis and interdigital hyperplasia. Additional phenotypes for DCT were available for a subset of animals (~125) before calving, at calving and 60 days after calving.

Three genomic analyses for each trait were performed with the following models:

$$1) y = W\alpha + u + \varepsilon \quad 2) y = W\alpha + x\beta + u + \varepsilon \quad 3) y = W\alpha + u_{(i)} + u_{(-i)} + \varepsilon$$

1) Variance component estimation³: y = vector of phenotypes, W = incidence matrix, α = vector of fixed effects, u = random polygenic effects vector, and ε = vector of residual errors (random effect distributions $MVN(0, V_G G)$ and $MVN(0, V_E I)$, respectively, with V_G and V_E = genomic and environmental variances, and G = genomic relatedness matrix).

2) Genome-wide association analysis (GWA) (Zhou and Stephens 2012): x = vector of marker genotypes, β = corresponding regression, and other effects as in model 1; population structure was accounted for by fitting the genomic relatedness matrix in the model and a correction with the inflation factor λ was applied.

3) Regional heritability mapping (RHM) (Cebamanos *et al.* 2014) of consecutive genomic regions of 20 SNPs: $u_{(i)}$ = random effects of genomic region i and $u_{(-i)}$ = random effect of the remaining genome; other effects as in model 1.

In models 2 and 3, significant (one false positive in 20 genome scans) and suggestive (one false positive per genome scan) thresholds were computed using a Bonferroni correction for multiple testing. Quantitative trait loci were explored by matching significant outcomes of the above analyses to the bovine reference genome.

Results

Claw horn traits All traits except for sole haemorrhage exhibited genomic heritabilities significantly greater than 0 (0.20–0.42). GWA revealed a significant peak on BTA6 for interdigital hyperplasia. RHM identified two significant regions on BTA6 for this trait, one of which included the significant SNP from GWA; these regions accounted for 66% of the total genomic (SNP) variance (probably an over-estimate due to Beavis effect) and harboured or were close to a gene related to the immune system (CLNK), a gene related to protein-protein interactions (WDR1) and a two-gene cluster related to bone and skeletal development (EVC and EVC2). Furthermore, the increased power of RHM resulted in additional suggestive regions identified for interdigital hyperplasia (BTA10), digital dermatitis (BTA11 and BTA27) and sole ulcer (BTA12).

Digital Cushion traits Only DCT at calving showed significant results, with RHM, revealing a significant region on BTA12 close to a relevant gene related to lipid and hormone metabolism (DHRS12) and two suggestive regions on BTA1 and BTA7. Regional variance estimates were larger than the total genetic variance, potentially indicating an oligogenic architecture for this trait leading to an under-estimate of the total genetic variance when assuming the infinitesimal model in analysis 1.

Conclusion Interdigital hyperplasia, digital dermatitis, sole ulcer and DCT at calving may be improved based on genomic analysis results. The latter can be used to enhance the accuracy of genomic predictions in breeding programmes.

Acknowledgements GB and ES are supported by the BBSRC ISPG1 (Analysis and Prediction in Complex Animal Systems) (BB/J004235/1). VB is gratefully acknowledging support from the Turkish Ministry of Education. GO is gratefully acknowledging support from the Wellcome Trust (Wellcome Trust ISSF non clinical fellowship).

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Relationships of cellular immune-associated traits with health, fertility and production in dairy cows

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Application Immune-associated traits were heritable, repeatable and associated with health and fertility suggesting such traits could lead to a useful tool in monitoring and improving dairy cow health, fitness and fertility.

Introduction Genetic selection for increased milk yield has been highly successful, resulting in unforeseen negative impacts on health, longevity and production (Oltenucu and Broom, 2010; Pritchard *et al.*, 2013). Recently, there has been growing interest in identifying and measuring immune-associated (IA) phenotypes in livestock which could then be associated with disease/health conditions (Banos *et al.*, 2013; Mallard *et al.*, 2015). Previously we identified a number of cellular IA traits within the circulating leukocyte population which were significantly associated with mastitis, lameness and fertility (Banos *et al.*, 2013). The aim of the present study was to extend previous findings by using a larger dataset, including pedigree information, to estimate genetic and phenotypic variance components for various subsets of blood leukocytes. Further, we investigate the genetic and phenotypic associations between these cellular IA traits and health, fertility, production and other functional traits in dairy cows.

Material and methods All animals in the study were Holstein-Friesians (n=546) from the Langhill lines of dairy cattle housed at the SRUC Dairy Research Centre, Dumfries, involved in an on-going selection experiment for genetic line x feeding system study (Pollott and Coffey, 2008). Blood samples were collected at bi-monthly intervals on 12 separate occasions between April 2013 and March 2015 and included summer and winter samplings. Additional data from 5 sampling points previously collected were also available. Immune-associated data were derived via flow cytometry and analysed using a repeated measures mixed model. Fixed effects included diet group; genetic group; lactation week; assay technique; year by month of calving interaction; and lactation number by age at calving interaction. Cow was fitted as a random effect and a permanent environmental effect of cow was also fitted to account for the use of repeated measures.

Results Heritability estimates (Table 1) were obtained for all traits, the highest of which were observed in the T cell subsets % CD4⁺, % CD8⁺, CD4⁺:CD8⁺ ratio and % NKp46⁺ cells (0.46, 0.41, 0.43 and 0.42, respectively) with between-individual variation accounting for 59% to 81% of total phenotypic variance. All estimates of heritability and repeatability were statistically significant (P<0.05). Strong genetic correlations were observed between % NKp46⁺ and stillbirth (0.61, P = 0.04), % CD8⁺ and lameness (-0.51, P = 0.06), and CD4⁺:CD8⁺ ratio and body weight (-0.52, P = 0.004).

Table 1 Genetic analysis of IA traits with health, fertility and production

Trait	h ²	Repeatability	Strongest correlation	
NKp46 ⁺	0.42 (0.09)	0.59 (0.03)	Stillbirth	0.61 (0.28)
CD4 ⁺ :CD8 ⁺	0.43 (0.11)	0.81 (0.02)	Body weight	-0.52 (0.17)
Lymphocytes	0.35 (0.07)	0.35 (0.03)	Fat %	0.36 (0.14)
Neutrophils	0.27 (0.06)	0.32 (0.03)	Fat %	-0.35 (0.15)
Eosinophils	0.17 (0.07)	0.38 (0.03)	Stillbirth ¹	0.67 (0.37)
CD8 ⁺	0.41 (0.10)	0.76 (0.02)	Lameness episodes ¹	-0.51 (0.26)
Monocytes	0.15 (0.05)	0.18 (0.03)	SCC ¹	0.48 (0.26)
CD4 ⁺	0.46 (0.10)	0.70 (0.02)	-	-

Standard errors in parenthesis; P<0.05;

¹P<0.1

Conclusion Results provide evidence that cellular IA traits are heritable and repeatable, and the noticeable variation between animals would permit selection for altered trait values. Moreover, the associations observed between IA, health, fertility and production traits suggest that genetic selection for cellular IA traits could lead to a useful tool in improving animal health, fitness and fertility.

Acknowledgements The authors gratefully acknowledge funding from the Biotechnology and Biological Sciences Research Council (BBSRC, grant number BB/K002260/1) and the Scottish Government Rural Affairs, Food and the Environment (RAFE) Strategic Research Portfolio 2016-2021.

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Genetic parameters of telomere length in dairy cattle

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Application Telomere length (TL) in dairy cattle is heritable, and has a favourable genetic correlation with functional herd life, attesting to its potential use in breeding programmes for enhanced longevity.

Introduction Involuntary culling in dairy herds causes large economic losses to the sector and delays genetic progress. Brown *et al.* (2012) detected that survival of dairy cows is associated with their TL, thus indicating potential use of TL as a biomarker for longevity. However, little is known about the genetic background of TL in cattle. It is possible that associations of TL with diseases have a genetic background thus expanding its utility beyond that of a biomarker alone. This study assesses genetic parameters of TL at birth (TLB) and after first calving (TLC), including heritability and genetic correlation with functional herd life (HL).

Material and methods The study was based on 308 Holstein-Friesian cows kept at the SRUC Dairy Research Centre at Crichton Royal Farm, Dumfries, Scotland. Cows were equally split into a genetically select and control lines. DNA was extracted from whole blood samples using DNeasy spin columns (Seeker *et al.*, 2016). The leukocyte TL was measured with a quantitative PCR, as the total amount of telomeric sequence relative to the amount of a non-variable copy reference gene sequence within the same DNA sample (Cawthon, 2002). The first sample collected within two weeks after birth (TLB) was available for 305 animals and the first sample after first calving was available for 256 cows (TLC). The TL measurements were first analysed using univariate mixed linear models. Significance of the random effects of the cow, her dam, the environment of the dam and the sire of the cow, was tested using log-likelihood ratio test (LRT). Further, bivariate analyses were conducted to estimate correlations between TLB and TLC, and between TLB and HL defined as the number of days between birth and cull of a cow corrected for the average milk production in the first 4 lactations. All HL records available on the farm (N=3,078) were included in the analyses.

Results The fixed effects found to significantly affect TL were year and season of birth (TLB and TLC) and the well position on a qPCR plate (TLB). Genetic group of the cow was included in the model to account for the difference between selected and control lines. Significant random effects identified for TLB were the direct genetic effect of the cow and the maternal environment of the cow's dam. Proportions of phenotypic variance explained by these two parameters were 0.36 (SE 0.15) and 0.33 (SE 0.10) respectively. The former provides the first, moderately high, heritability estimate of TL in dairy cattle. The latter attest to possibly important effect of the prenatal uterine environment on TL. The direct genetic effect of the cow was the only random term identified as significant for TLC, with a heritability of 0.50 (SE 0.17). The genetic correlation between TLB and TLC was 0.82 (SE 0.26), which is not significantly different from unity, suggesting the two TL traits have a common genetic background. The phenotypic correlation was 0.32 (SE 0.06). TLB was found to be significantly and positively correlated to HL, with genetic and phenotypic correlations estimated at 0.58 (SE 0.28) and 0.17 (SE 0.07) respectively. Fitting a bivariate model to TLB and HL resulted in detection of significant genetic variance for the latter trait, with heritability of 0.18 (SE 0.03). These correlation estimates attest to a favourable association between TL and longevity.

Conclusion The results document the heritable nature of TL in dairy cattle, and provide insight into the presence of maternal effects in early life TL. The positive genetic correlation between TLB and HL proves that the genetic associations of TL with other traits may be higher than expected from phenotypic correlations alone. These results suggest that the utility of TL extends beyond that of a biomarker, with high genetic correlations offering the opportunity to improve the response to selection in traits of lower heritability.

Acknowledgements The study has been funded by BBSRC grant BB/L007312/1. We thank Ainsley Bagnall and other staff at Crichton farm involved in animal sampling.

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Genetic relationships between methane emission and production traits in Holstein-Friesian dairy cows

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Application Obtaining genetic parameters between methane emissions and production traits is vital to the use of genetic selection as a long-term mitigation strategy, whilst minimizing adverse effects on production traits.

Introduction One of the biggest challenges facing modern society is climate change. Greenhouse gases, including methane (CH₄), play an important role in global warming. Globally 2% (FAO 2010) of the anthropogenic CH₄ emissions comes from dairy cattle. Breeding for lower CH₄ emissions offers a cumulative and permanent change over generations (De Haas *et al.*, 2011). Little is known, however, about the genetic parameters of directly measured CH₄ emissions and genetic relations with other traits like milk yield and live weight. This knowledge is important in order to get producers to adopt a breeding strategy that includes CH₄ emissions. In this study the feasibility of breeding for lower CH₄ emissions and the effect on production traits is examined.

Material and methods Data from The University of Nottingham research herd (Sutton Bonington, UK) were used. CH₄ emission data (g/d) from 200 Holstein-Friesian cows (5391 observations) were available. CH₄ emissions were measured with an infrared analyser installed in the feed bin of the milking robots (Garnsworthy *et al.* 2012). CH₄ measurements took place from December 2009 to April 2010. Data on milk yield (MY) and live weight (LWT) were available from November 2008 to October 2010. Data were analysed with ASReml 3.0 (Gilmour *et al.*, 2009). Bivariate random regression repeatability models were fitted on combinations of MY, LWT and CH₄ emissions. Fixed effects fitted were lactation number, weeks in milk, calving month, age of cow and record month. A Legendre polynomial was fitted on weeks in milk in order to account for gradual changes within the trait over lactation at population level. Random effects fitted were a genetic animal effect and a permanent environmental effect. On the genetic animal effect an interaction was fitted with a Legendre polynomial on weeks in milk, in order to account for gradual changes at an individual level.

Results Heritabilities (Table 1) ranged from 0.25 to 0.53 for MY, 0.39 to 0.52 for LWT and 0.32 to 0.53 for CH₄ emissions (significant for weeks 37 to 52 of lactation), which is as expected from literature, except for CH₄ emissions which is higher. The genetic correlation between MY and LWT is highly negative (significant for weeks 1 to 3 of lactation), the genetic correlation between MY and CH₄ emissions is moderately positive (significant for weeks 45 to 52 of lactation) and the genetic correlation between LWT and CH₄ emissions is moderately positive (not significant). The phenotypic correlation between MY and LWT is slightly negative (not significant), the phenotypic correlation between MY and CH₄ emissions is moderately positive (significant for weeks 48 to 52 of lactation) and the phenotypic correlation between LWT and CH₄ emissions is moderately positive (not significant). Again results are as expected from literature.

Table 1 Heritabilities (diagonal), genetic correlations (below diagonal), phenotypic correlations (above diagonal) and repeatabilities (column 7) for MY, LWT and CH₄ emissions

	MY	P value	LWT	P value	CH ₄	P value	Rep	P value
MY	0.25 –	0.00 –	-0.13 –	0.35 –	0.27 –	0.03 –	0.77 –	0.00 –
	0.53	0.23	-0.02	0.40	0.41	0.29	0.86	0.05
LWT	-0.62 –	0.04 –	0.39 –	0.01 –	0.18 –	0.29 –	0.89 –	0.01 –
	-0.16	0.33	0.52	0.10	0.22	0.32	0.91	0.03
CH ₄	0.02 –	0.00 –	0.15 –	0.33 –	0.32 –	0.00 –	0.83 –	0.00 –
	0.48	0.40	0.25	0.36	0.53	0.16	0.89	0.04

Conclusion Heritability estimated for CH₄ emissions suggests there is a genetic component to this trait, which indicates there is potential for genetic selection. Correlations between CH₄ emissions and MY and LWT are positive, indicating that selection for lower CH₄ emission might result in lower MY and LWT. However, due to the small size of the dataset, results are only significant for parts of the lactation and should be treated with caution.

Acknowledgements I.S. Breider is supported by a Nottingham University-SRUC joint studentship.

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Effect of adjusting for live weight, rather than age, in the genetic evaluation of measurements in lambs

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Application The use of live weight as a covariate, rather than age, in the genetic evaluation of ultrasonic muscle and fat depth could lead to higher rates of genetic gain for carcass composition if increases in carcass weight are not desired.

Introduction Ultrasound measurements of muscle and fat depth over the loin in lambs are routinely used as indirect measures of carcass composition in breeding programmes, together with a measure of live weight taken at the same time. These measurements are made at one time point in each flock, and lambs vary in both age and live weight. It is common practice to use age as a covariate in the statistical models used in breeding value estimation for muscle and fat depth, however, when the breeding objective is to improve carcass composition at a fixed carcass weight, the use of live weight as a covariate in these statistical models may be more appropriate.

The aim of this study was to evaluate the effect of age and live weight as covariates on the heritability of ultrasonically measured muscle and fat depths, and on estimated breeding values for these traits.

Material and methods Ultrasonic scanning records of 23 449 Meatline and 64 831 Lleyne lambs over the period 1990 to 2015 were analysed. Mean age at scanning was 146 and 151 days, and mean live weight was 39.6 and 36.4 kg in the Meatline and Lleyne respectively.

Significant fixed effects of Flock/Year/Sex group, rearing type (single, twin or multiple) and dam age (ages 1 to 6, and 7 or older) were fitted in all analyses. Genetic and residual components of variation were estimated for ultrasonic muscle depth and ultrasonic fat depth using ASReml3 (Gilmour *et al*, 2006) to fit an animal model using a pedigree of 25 431 individuals for the Meatline breed and a pedigree of 90 137 individuals for the Lleyne breed.

Either age at scanning or live weight at scanning was fitted as a covariate for ultrasonic measurements. Spearman's rank correlation was used to compare the ranking of estimated breeding values (EBVs) for 486 Meatline sires and 902 Lleyne sires, with 20 or more progeny, estimated using either the age or live weight as a covariate.

Results The use of live weight as a covariate in the statistical model resulted in higher estimates of heritability for muscle depth in both breeds studied and in fat depth in the Lleyne breed. Spearman's rank correlations for EBVs estimated using either age or live weight as the covariate were 0.86 and 0.68 for ultrasonic muscle depth and ultrasonic fat depth respectively in the Meatline breed and 0.78 and 0.85 for ultrasonic muscle depth and ultrasonic fat depth respectively in the Lleyne breed.

Table 1 Estimated heritability (h^2) of muscle and fat depth either fitting age or live weight as a covariate

	Meatline		Lleyne	
	h^2	se	h^2	se
Model using age as a covariate				
Ultrasonic Muscle depth (mm)	0.21	0.014	0.31	0.011
Ultrasonic Fat depth (mm)	0.24	0.014	0.26	0.011
Model using live weight as a covariate				
Ultrasonic Muscle depth (mm)	0.32	0.014	0.36	0.011
Ultrasonic Fat depth (mm)	0.25	0.014	0.41	0.011

Conclusion The use of live weight as a covariate in statistical models used in the genetic evaluation of live weight traits may result in higher rates of genetic improvement for carcass composition at a fixed carcass weight.

Acknowledgements The authors gratefully acknowledge funding for this study from AHDB Beef and Lamb.

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Effect of crossbreeding on the performance and survival of dairy cattle in commercial dairy herds in Northern Ireland

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Application Jersey x Holstein crossbred cows can produce similar yields of milk solids as Holstein cows within a moderate concentrate input system, while tending to have improved survival.

Introduction Selection for milk production within the Holstein-Friesian (HF) breed has led to a decline in functional traits, including fertility (Royal *et al.*, 2000), and this has prompted a renewed interest in crossbreeding. While a number of studies have examined the performance of crossbred cows within low-input grazing systems, the performance of crossbred cows within moderate concentrate input systems has received less attention. This study compared the performance of HF and Jersey x Holstein-Friesian crossbred (J x H) dairy cows during their first four lactations within moderate input systems.

Material and methods This study was conducted on eleven Northern Ireland dairy farms. The farms included both spring and autumn calving herds with a range in concentrate inputs (0.7 - 2.2 t/cow/year), but with an average concentrate input of approximately 1.3 t/cow/year ('moderate input'). All farms participated in a formal milk recording scheme, and milk yield data were obtained from test-day records. On each farm 10-20 pairs of HF cows were matched for milk yield and parity. Within pairs, one cow was bred to a HF sire, while the second was bred to a Jersey sire, using artificial insemination. This was repeated in the following two years with cows bred to HF sires in year 1 subsequently bred to Jersey sires in year 2, and vice versa. The offspring of this breeding programme were allocated to the study when they calved for the first time (192 HF cows and 189 J x HF cows). The Holstein cows on the study were sired by a total of 64 Holstein-Friesian sires, while the crossbred cows were sired by a total of eight Jersey sires. Cow performance (milk yield, milk composition, fertility, and survival) was monitored throughout the first four lactations. Data was analysed across all four lactations, with the model taking account of farm and 'birth-year' effects.

Results Average performance across the 11 herds is presented in Table 1. Holstein cows had a significantly higher lactation milk yield than J x HF cows (614 kg higher: $P < 0.001$), while the crossbred cows produced milk with a significantly higher fat and protein content than the HF cows ($P < 0.001$). The overall effect was that fat + protein yield was unaffected by genotype ($P > 0.05$). Somatic cell count was similar for cows of both genotypes (173, 000 and 176, 000 cells/ml, with the HF and J x HF cows, respectively) while somatic cell score was not different between the two genotypes ($P > 0.05$). Conception rate to 1st service and to 1st plus 2nd service did not differ between genotypes ($P > 0.05$), although calving interval was lower for the J x HF cows ($P < 0.01$). While the number of cows culled due to infertility was unaffected by genotype, more HF cows than crossbred cows were culled due to 'feet and leg' problems ($P < 0.05$). There was a trend for more J x HF cows, than HF cows, to survive until the start of the fifth lactation ($P = 0.06$), while the HF and J x HF cows completed a mean of 3.6 and 4.8 lactations, respectively.

Table 1 Effect of cow genotype on mean performance during lactations 1 to 4.

	Genotype		SEM	SIG
	HF	J x HF		
Milk yield (kg/day)	6951	6337	79.9	***
Fat (g/kg)	42.0	47.3	0.46	***
Protein (g/kg)	33.9	35.7	0.19	***
Fat + protein yield (kg/day)	525	524	5.83	NS
Somatic cell score (000/ml, log ^e)	11.48	11.58	0.071	NS
Conception to 1 st and 2 nd service (%)	71	76	2.6	NS
Calving interval (days)	402	388	4.0	**
Cows culled as infertile (%)	30.2	25.0	3.11	NS
Cows culled with feet and leg problems (%)	4.1	0.5	1.02	*
Cows remaining at start of lactation 5 (%)	38.8	47.9	3.46	$P = 0.06$

Conclusions Within the moderate input systems examined within this study, Jersey x Holstein crossbred cows produced less milk, but had a similar yield of fat plus protein as pure bred Holstein cows. With the exception of a shorter calving interval, fertility performance was not improved with crossbreeding, although cow longevity tended to be improved with crossbreeding. The results demonstrate that Jersey crossbred cows can compete with Holstein cows in terms of milk solids yield, and may help improve longevity.

Acknowledgements This work was co-funded by DAERA and AgriSearch.

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Genetic parameters for production and reproduction traits of the dairy Mpwapwa cattle breed performing in a low input system

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Application results can help optimize genetic selection within the breeding programme of this African cattle breed which plays a significant role in milk production in low input areas of Tanzania.

Introduction The Mpwapwa breed in Tanzania is a result of crossbreeding zebu breeds from Asia with East African zebu and *Bos taurus*. The breeding programme for the Mpwapwa breed was established in 1958 and focused on developing a composite breed for milk production in semi-arid low input areas of central Tanzania (Syrstad, 1990). The breeding programme so far has been based on phenotypic selection (Bwire, *et al.* 2005). However, in order to maximize performance, it is essential to perform genetic selection based on estimates of genetic parameters for the breeding goal traits. The current study aims to estimate genetic parameters for production and reproduction traits of Mpwapwa cattle raised under a low input system in Tanzania.

Material and methods A total of 1,003 total lactation milk yield (TLMY) records of 385 cows were retrieved from the Tanzania Livestock Research Institute database (Mpwapwa, Tanzania). Total lactation yield was adjusted to 305 days (ADLMY) to account for incomplete lactations. Records for age at first calving (AFC) and calving interval (CI) were derived from birth and calving dates. Genetic parameters for these traits were estimated with univariate and bivariate animal models using the ASReml software.

Results Genetic parameters for production and reproduction traits are presented in Table 1.

Table 1 Estimate of heritability (on the diagonal), genetic correlation (above the diagonal) and phenotypic correlation (below the diagonal) with their standard errors for production and reproduction traits

Trait	ADLMY	TLMY	CI	AFC
ADLMY	0.44±0.04	0.87 ± 0.02	-0.06±0.01	0.0
TLMY	0.82± 0.01	0.33±0.11	0.15±0.11	0.0
CI	-0.01± 0.001		0.10±0.05	0.0
AFC	-0.11± 0.0			0.13±0.11

1TLMY = total lactation milk yield; ADLMY = adjusted 305 day lactation milk yield; CI= calving interval; AFC = age at first calving.

All heritability estimates were statistically greater than zero ($P < 0.05$) except for AFC, suggesting that the traits could be genetically improved with selection. The two production traits were highly correlated to each other but not to reproduction, which indicates that there is a weak genetic and physiological relationship between the two sets of traits.

Conclusion Genetic parameters for production and reproduction traits estimated in this study suggest that moderate to high increase in milk yield may be achieved with genetic selection. Selection for improved production is not expected to adversely affect reproductive performance. Environment (e.g. nutrition, disease, management) seems to primarily affect reproductive traits more than additive gene action.

Acknowledgements We are very grateful to Bill and Melinda Gates Foundation for funding through the Scotland's Rural College (SRUC) in the Programme for Enhancing the Health and Productivity of Livestock (PEHPL) and the University of Edinburgh. Thanks also to the Director General (TALIRI) for permission to use the dataset for this study.

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Implications of a clinical BHV-1 infection on the economic performance of an Irish spring calving suckler herd

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Application An economic modelling approach to support the development of disease prevention protocols for pasture based suckler beef farms is presented.

Introduction Bovine herpes virus 1 (BHV-1) is a viral pathogen often cited as putatively affecting the reproductive and productive output of suckled beef farms. The virus, which is highly contagious particularly when it presents itself in the form of infectious bovine rhinotracheitis (IBR), has been shown in previous studies to have a high seroprevalence within beef herds throughout Ireland. The objective of this study was to model the effect of herd sero-prevalence to BHV-1 on whole farm biological and economic performance.

Materials and methods As part of a wider epidemiological study of suckler cow disease status, a total of 4240 cows from 134 spring calving beef cow herds across the Republic of Ireland were blood sampled to measure the sero-prevalence to BHV-1 (Parr *et al.*, 2017). Records pertaining to calving interval, calf mortality and average daily gain were extracted from the Irish Cattle Breeding Federation database.

The effect of seropositivity to BHV-1 on cow and calf performance was thus derived and used to parameterise a whole farm bio-economic simulation model, the Grange Beef Systems Model (GBSM; Crosson, 2006).

Table 1 Effect of BHV-1 seroprevalence on productive and reproductive variables in suckler cow herds (Parr *et al.*, 2017)

	Unit change in seropositive herds
Calving Interval (days)	1.8
Calf mortality <28days (%)	0
Calf mortality 28-225 days (%)	0.5
Average daily gain 0-225 days (kg/d)	0
Replacement rate (%)	1.9

The default parameters for calving interval, replacement rate, calf mortality and average daily gain were modified as per the data generated in the epidemiological study. These data were used to generate two scenarios (representing herds that are either seropositive or seronegative to BHV-1) for a 40 hectare spring calving (mean calving date 15 March) suckler herd.

Table 2 Results of the bio-economic model reflecting a seropositive herd for BHV-1

	Sero negative herd (€ha)	Sero positive herd (€ha)
Gross output	1509	1499
Variable costs		
Concentrate	102	103
Grazing/silage	344	347
Vet/med/ AI	141	142
Other	47	51
Gross margin	876	857
Fixed costs	508	510
Net margin	368	347

Results

The change in economic margins between the two scenarios showed little difference in costs or gross output at herd level. Replacement rate was somewhat greater and accordingly there was a modest increase in area farmed. The effect of longer calving intervals was to delay mean calving date and so reduce the length of the grazing season. The higher level of mortality pre-weaning was reflected in lower beef output from the sero-positive herd. Taken together, the economic significance of disease sero-prevalence resulted in an average 6% loss in net margin for infected herds.

Conclusion

The data from this study indicated that the effect of sero-prevalence to BHV-1 has a relatively small impact on the financial performance of a pasture-based suckled calf-weaning system.

Acknowledgements

We gratefully acknowledge support from the Department of Agriculture, Food and the Marine under the Research Stimulus Fund (Project 13/S/515)

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Effect of bovine herpesvirus-1, bovine viral-diarrhoea and leptospirosis sero-positivity status on some key performance indicator traits in Irish spring calving beef cow herds

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Application Sero-prevalence of reproductively important pathogens and their effects on cow reproductive, calf mortality and performance measures in beef cow herds were determined for the first time in the Republic of Ireland.

Introduction Numerous bacterial and viral pathogens have been putatively associated with poor reproductive, growth health status in cattle herds. Leptospirosis (hardjo-bovis and -prajitno genotypes), bovine viral-diarrhoea (caused by bovine viral-diarrhoea virus; BVDV) and infectious bovine rhinotracheitis (IBR) (caused by bovine herpesvirus-1; BHV-1) are all transmissible diseases that are considered to be of economic importance in the international trade of animals and animal products. Each of these pathogens can lead to clinical disease that can have both direct and indirect effects on productive reproductive (Van Leeuwen *et al.*, 2010) and productive (Tiwari *et al.*, 2007) performance. Therefore the aim of this study was to quantify the effects, if any of the aforementioned pathogens on calving interval, calf mortality (≤ 28 days) and calf growth rate (up to 225 days of age) of Irish spring calving beef cow herds.

Material and methods In the months of May through August of 2014 and 2015, a total of 4240 cows from 134 spring calving beef cow herds across the Republic of Ireland were blood sampled to measure the sero-prevalence of the above diseases. All cows were body condition scored (BCS) and were blood sampled from a coccygeal vessel. All serum samples were tested for antibodies against the above listed pathogens using commercially available diagnostic kits by the Irish Department of Agriculture, Food and Marine Central Veterinary Laboratory Service. All cows were ultrasonically scanned to determine pregnancy status at least 30 days after the end of the breeding season. Records for participating herds pertaining to calving interval, calf mortality and estimated calf average daily gain were extracted from the Irish Cattle Breeding Federation database. All farmers in the study were surveyed to determine the vaccination status for the aforementioned pathogens in their herd. Data were analysed using the generalised linear models procedure of SAS (version 9.3) with terms for herd, parity, calf sex, cow breed, calf sire breed, cow movements and all measured pathogens included.

Results Mean BCS \pm s.e.m was 2.57 ± 0.01 for all cows sampled. Sero-prevalence of leptospirosis, BVDV and BHV-1 was 88%, 92% and 33% and 71%, 78% and 44% in vaccinating and non-vaccinating herds, respectively. Overall herd pregnancy rate at the end of the breeding season was 89 (78-100%) and 88% (81-100%), for 2014 and 2015, respectively. There was no effect of sero-positivity status for BHV-1, BVDV or leptospirosis on calving interval, calf mortality (≤ 28 days) or calf average daily gain as shown in Table 1.

Conclusion The results from this study indicate, for the first time, that there is no negative concurrent relationship between the aforementioned pathogens and the three key economically important traits examined in spring calving beef cow herds.

Table 1 Effects of bovine herpesvirus -1 (BHV-1), Bovine viral-diarrhoea (BVDV) and Leptospirosis status on calving interval, calf mortality (≤ 28 days), and calf average daily gain (≤ 225 days)

Trait Pathogen	Sero-positivity Status		P-value
	Negative (\pm SEM)	Positive (\pm SEM)	
<u>Calving interval (days)</u>			
BVDV	388.33 (\pm 2.94)	386.47 (\pm 1.48)	0.54
BHV-1	385.30 (\pm 1.68)	387.10 (\pm 1.97)	0.43
Leptospirosis	386.55 (\pm 3.13)	385.97 (\pm 1.55)	0.86
<u>Calf Mortality % (0-28d)</u>			
BVDV	2.60 (\pm 0.80)	3.20 (\pm 0.40)	0.15
BHV-1	3.10 (\pm 0.50)	3.10 (\pm 0.50)	0.96
Leptospirosis	4.40 (\pm 0.90)	3.20 (\pm 0.50)	0.15
<u>Av. Daily gain (kg/d)</u>			
BVDV	1.15 (\pm 0.02)	1.17 (\pm 0.01)	0.44
BHV-1	1.16 (\pm 0.01)	1.16 (\pm 0.01)	0.85
Leptospirosis	1.17 (\pm 0.02)	1.17 (\pm 0.01)	0.98

Acknowledgements We gratefully acknowledge support from the Department of Agriculture, Food and the Marine under the Research Stimulus Fund (Project 13/S/515)

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A meta-analysis of heterosis for production and fitness traits in tropical cattle

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Application Knowledge acquired may facilitate the design of breeding programmes based on judicious crossbreeding suitable for tropical conditions.

Introduction Crossbreeding is widely used in the tropics as it allows for combination of complementary traits from local and exotic breeds, and leads to heterosis. Heterosis is thought to be higher when breeds used are more genetically distant (Graml and Pirchner, 1984). This suggests great potential in tropical cattle as breeds are highly diverse, from European to zebu breeds. Type of trait is also thought to have an effect, with fitness traits such as health and fertility, showing greater heterosis. These traits are particularly important in the tropics where low fertility and health can be a problem. It is also thought that heterosis may vary depending on the environment. Higher heterozygosity may allow individuals greater resilience under high stress conditions (David, 1984). Therefore heterosis advantage may be higher under these conditions. A meta-analysis of the literature was carried out to investigate how the type of cross, trait and environment affect heterosis in tropical cattle.

Material and methods Literature was found by searching Web of Science (ISI) using “heterosis” and “zebu” or “Bos indicus” or “sanga” or “crillo” or “tropical” and reference lists of the obtained articles were screened to find additional relevant papers. A total of 44 studies with 519 heterosis estimates were found where each study included at least one tropically-adapted breed. Along with each heterosis estimate, the study, trait, breeds, location of study and performance of both purebred breeds were recorded. In order to standardise heterosis estimates across studies and traits, it was expressed as a percentage of the mean performance of the two purebreds. Each estimate was multiplied by either 1 or -1, such that estimates in the desired direction for the trait were expressed as positive. Five cross types were defined based on breed origin and location. Individual traits were grouped into eleven types each including similar traits (e.g. dairy performance, growth, fertility etc.). Data were analysed with a linear model including cross type, trait type, continent and all interactions as fixed effects.

Results Cross type and trait type were found to have significant effects ($p < 0.001$) on heterosis. The greatest heterosis was found in tropical Bos taurus x European Bos taurus followed by zebu x European Bos taurus crosses ($18.1\% \pm 3.3\%$ and $13.6\% \pm 2.7\%$ respectively). As expected, zebu x zebu and European x European crosses between related breeds had the lowest heterosis ($5.1\% \pm 5.5\%$ and $6.2\% \pm 3.4\%$ respectively). Although health and fertility traits showed relatively high heterosis ($24.5\% \pm 7.9\%$ and $7.9\% \pm 3.5\%$ respectively), this was also very high for dairy performance ($25.3\% \pm 3.0\%$). Longevity traits showed the highest heterosis ($41.5\% \pm 13.7\%$) but this was based on only two studies. Significant interactions between cross and trait type and between continent and trait type were also found ($p < 0.01$).

Conclusion We might have expected tropical and European Bos taurus to be less distantly related with each other than to zebu breeds, leading to highest heterosis in the latter cross. However, our results suggest that tropical Bos taurus have evolved to become well adapted to local conditions distancing themselves from the European breeds. These results highlight the great potential to use rotational breeding systems between different breed types to maximise heterosis and increase productivity. There is also potential in using heterosis and crossbreeding to improve the overall productivity of tropical cattle, in fitness but also production traits.

Acknowledgements Harriet Bunning is grateful for the award of an SRUC PhD Scholarship.

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The effect of floor type on the behaviour and hoof growth of finishing beef cattle

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Application Rubber flooring is an alternative solution to fully slatted flooring to improve the welfare of beef cattle.

Introduction Fully slatted flooring is a common housing system used to accommodate beef cattle. However, their use has been criticised as they are perceived to subject cattle to poorer welfare standards compared to bedded systems. Straw may not be economically viable in regions where availability is low. One alternative may be to accommodate beef cattle on fully slatted flooring covered with rubber. Although, concerns have been raised regarding the use of rubber flooring and the incidence of overgrown claws. Overgrown claws can increase the risk of injury and infection, causing pain and discomfort. The aim of the present study was to evaluate the effect of using different floor types to accommodate finishing beef cattle on their behaviour, locomotion and hoof growth.

Material and methods Eighty dairy origin bulls were blocked according to live weight and breed into 20 blocks, each of four animals, and randomly assigned within blocks to one of four treatments. Bulls on treatment one were accommodated on fully slatted flooring following a growing period of 101 days on concrete slats (CS), bulls on treatment two were accommodated on fully slatted flooring covered with rubber strips following a growing period on rubber slats (RS), bulls on treatment three were accommodated on a solid floor covered with straw bedding following a growing period on concrete slats (CS-S), and bulls on treatment four were accommodated on fully slatted flooring covered with rubber strips following a growing period on concrete slats (CS-RS). Pedometers (IceQubes) were attached to the right hind leg of focal animals within each treatment to measure lying and standing activity. All animals were locomotion scored every 14 days using the method of Flower and Weary (2006). Toe length and heel height were measured at the start and end of the finishing period using the method of Vermunt and Greenough (1995). IceQube data were analysed as a one-way ANOVA. Locomotion scores, toe length and heel height were analysed using linear mixed model methodology, with pen as a random effect and treatment as a fixed effect. If the overall effect of treatment was significant then pairwise differences between treatment means were assessed using Fisher's Least Significant Difference Test.

Results The effect of floor type on behaviour is presented in Table 1. Floor type had no significant effect on the locomotion score of the bulls. Bulls accommodated on CS-RS had the greatest front toe growth (14.5mm) compared to those on RS (11.2mm), CS-S (9.2mm) and CS (6.6mm) ($P < 0.001$). Hind toe growth was least in bulls accommodated on CS (6.9mm) with no significant difference between RS (11.8mm), CS-S (11mm) and CS-RS (13.2mm) ($P < 0.05$). Heel height growth was greatest in bulls accommodated on CS-S in both front (16.8mm) and hind feet (14mm) ($P < 0.001$), but there was no significant effect between CS (7.7mm), RS (7.6mm) or CS-RS (7.8mm) in front heel height growth. Bulls accommodated on RS (7.4mm) had significantly greater back heel height growth than those accommodated on CS (4.6mm) ($P < 0.001$).

Table 1 The effect of floor type on the behaviour of finishing bulls

Behaviours	Floor type				sem	P-value
	CS	RS	CS-S	CS-RS		
Number of steps (steps/day)	674 ^a	1266 ^b	1835 ^c	1162 ^{ab}	207.2	0.002
Total lying duration (min/day)	1057 ^b	934 ^a	845 ^a	883 ^a	45.9	0.013
Number of lying bouts (bouts/day)	11.6 ^a	16.5 ^a	20.8 ^b	15.7 ^a	1.86	0.007
Mean duration of standing bouts (min/bout)	33.2	31.7	29.5	36.7	5.31	0.514
Mean duration of lying bouts (min/bout)	91.3 ^c	57.3 ^b	41.7 ^a	58.4 ^b	5.73	<0.001

^{ab}Means on the same row with the same superscript do not differ significantly ($P > 0.05$)

Conclusion The greater number of steps on RS and the shorter duration of lying bouts on RS and CS-RS compared to CS suggest that rubber flooring is a potential compromise between concrete slats and straw bedding. Although toe growth was greater in bulls accommodated on CS-RS, there was no effect of floor type on locomotion score, suggesting that increased toe growth was not associated with pain or discomfort whilst walking.

Acknowledgements The authors greatly acknowledge funding from DAERA.

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Relationship between the oxidative stress index measured in milk and the health and performance of dairy cows

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Application Measuring the balance between pro-oxidants and antioxidants in milk (the oxidative stress index, OSI) is a non-invasive means of assessing the health status of the cow. When done in early lactation, this technique could be used as a means of identifying more vulnerable cows. Cows with a higher OSI incurred lower veterinary costs in the first four months of lactation.

Introduction A recent review by Abuelo *et al.* (2015) highlighted the vulnerability of dairy cows to health disorders during the transition period. They proposed the use of an 'oxidative stress index' (OSI) to identify cows that were vulnerable to a dysfunctional inflammatory response, which was associated with many periparturient disorders. Oxidative stress indicators in milk have also been associated with an-ovulatory oestrous cycles (Talukder *et al.*, 2015). The objective of this study was to determine whether there was a relationship between the milk OSI of recently calved cows and their current health status, and whether this was associated with their health and performance when they were in mid lactation.

Material and methods Cows that were recently calved (mean 22 days in milk) were selected on the basis of whether they were apparently healthy ('Health', n=12) or had suffered some problems during the calving and postpartum period ('Challenged', n=12). A representative sample of milk was taken from each cow on the same day, and was stored at 3C overnight. The following day, a sample of whey was prepared by centrifuging the milk sample (4500 g), treating the supernatant with acetic acid and centrifuging again. The supernatant was analysed for biological antioxidant potential (BAP) and lipoperoxides (LPO) using, respectively the BAP and Lipocell test kits (Diacron International, Grosseto, Italy). OSI was calculated (mEq/mol) from LPO/BAP. A high OSI would indicate a higher level of oxidative stress. Four months later, data on the lactation performance and event costs for that parity were collected for each cow and these data were regressed with OSI as the independent variable. The effect of health status (Healthy and Challenged) on OSI, lactation performance and event costs was determined by analysis of variance.

Results There was a significant difference in the OSI of healthy and challenged cows (Table 1), but surprisingly OSI was higher in healthy cows. Challenged cows incurred higher treatment costs compared with healthy cows, and within four months, three of these cows had left the herd. There was no effect of health status on the performance of the cows. There was also no difference between the two groups in terms of the number of positive pregnancy diagnoses four months later (n=5 in both cases).

Table 1 Effect of health status in early pregnancy on milk OSI and treatment costs

	Healthy	Challenged	SEM	P
OSI (mEq/mol)	16.6	10.5	1.04	<0.001
Event costs in parity (£)	29.81	70.92	8.71	0.002
Predicted lactation yield (kg)	10052	9067	560	0.200
Predicted milk protein yield (kg)	302	301	15.2	0.968
Predicted milk fat yield (kg)	368	381	20.7	0.661

Event costs were negatively but significantly related to OSI (Event costs in parity = 106.3 - 4.51 OSI), $R^2=0.382$, $P=0.003$, while milk yield was positively, but weakly related to OSI (Lactation milk yield = 7614 + 159.6 OSI), $R^2=0.161$, $P=0.072$.

Conclusion Although a relatively high concentration of pro-oxidative stressors compared with antioxidants in the milk might suggest that a cow was more vulnerable to a dysfunctional inflammatory response, the results from this study would suggest that a higher OSI is associated with cows in better health (with a weak association with better performance). Determining OSI in milk is much less invasive than taking a blood sample and analysing that, and with refinement this method may be a means of measuring biomarkers in the milk that would be associated with cows that are more vulnerable and likely to require more veterinary intervention.

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Prevalence of rumen fluke (*Calicophoron daubneyi*) in cattle herds, sheep flocks and in intermediate snail host, *G. truncatula*, on Welsh farms

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Application This study demonstrates that *C. daubneyi* is established on Welsh farms. Observations on its intermediate snail host may be used as a basis for further research into the parasites epidemiology.

Introduction In recent decades, countries across Western Europe have witnessed significant increases in reports of rumen fluke presence, prevalence and disease. In the UK, rumen fluke detection rates by passive veterinary surveillance increased by 57% annually between 2010 and 2015, with cattle and sheep mortality due to paramphistomosis reported during this period (Millar *et al.*, 2012). Molecular analysis has identified *C. daubneyi* as the prominent rumen fluke species in the UK; however, it is unclear if this is the case on all farms with other paramphistome species historically seen in UK livestock. Despite the seemingly increasing threat to livestock health and welfare, there has been no estimate of *C. daubneyi*'s farm, herd, flock or intermediate host level prevalence in any area of the UK. In this study, the prevalence levels of *C. daubneyi* on participating Welsh farms, cattle herds and sheep flocks were recorded. Prevalence of *C. daubneyi* within its intermediate snail host *G. truncatula* in a proportion of the studies farm was also recorded and compared to liver fluke (*F. hepatica*) prevalence and their exposure to respective fluke eggs.

Material and methods Between November 2015 and February 2016, 100 farmers submitted one or two 25 g composite faecal sample attained from 20 cattle and/or 20 sheep from their herd and/or flock to test for fluke infection. Sedimentary faecal egg count was performed using 20 g of each submitted faecal sample, with rumen and liver fluke infection identified and differentiated via egg colour. The presence of rumen fluke species *C. daubneyi* in each rumen fluke positive sample was confirmed via a species specific PCR protocol, with the DNA analysed extracted from rumen fluke identified during FEC. For a full description of FEC and PCR protocols see Jones *et al.* (2016). Ten *C. daubneyi* positive farms were subsequently visited on four occasions between May and October 2016. During each visit, *G. truncatula* snails were collected along with faecal samples from animals grazing corresponding pastures. Snail DNA was extracted using a Chelex protocol (Jones *et al.*, 2015), prior to PCR analysis to detect infections of *C. daubneyi* or *F. hepatica*. Collected faecal samples were subjected to sedimentation FEC as described above, to estimate the exposure of these snails to infective fluke stages. All statistical analysis was performed in SPSS (v.22) and were performed to a significance value equal to $P < 0.05$. A chi-square test was used to compare *C. daubneyi* prevalence at cattle herd and sheep flock level, and to compare *C. daubneyi* and *F. hepatica* prevalence in collected *G. truncatula*'s. A Mann Whitney U-test was used to compare *C. daubneyi* infection intensity between cattle herds and sheep flocks, with a Wilcoxon signed rank test used to compare mean levels of *C. daubneyi* and *F. hepatica* eggs shed onto *G. truncatula* harbouring pastures.

Results Sixty-one percent of studied farms were positive for *C. daubneyi*. Herd-level prevalence for cattle (59%) was significantly higher compared to flock-level prevalence for sheep (42%, $P < 0.05$), however, there was no significant difference in infection intensity ($P > 0.05$). There was no significant difference between *C. daubneyi* (4%) and *F. hepatica* (5.6%) prevalence within 892 collected *G. truncatula* snails ($P > 0.05$) despite significantly larger quantities of *C. daubneyi* eggs (18.5 EPG v. 2.2 EPG) being shed by grazing livestock onto fields harbouring their habitats ($P < 0.001$) (Figure 1).

Conclusion This study suggests *C. daubneyi* has become endemic on Welsh farms. Questions are also raised regarding the suitability of *C. daubneyi* to infect UK *G. truncatula*'s in comparison to *F. hepatica*.

Acknowledgements The project was funded by an Owen Price scholarship and the Coleg Cymraeg Cenedlaethol. The authors would like to thank all farmers participating in this project and YFC Wales for their co-operation in the study.

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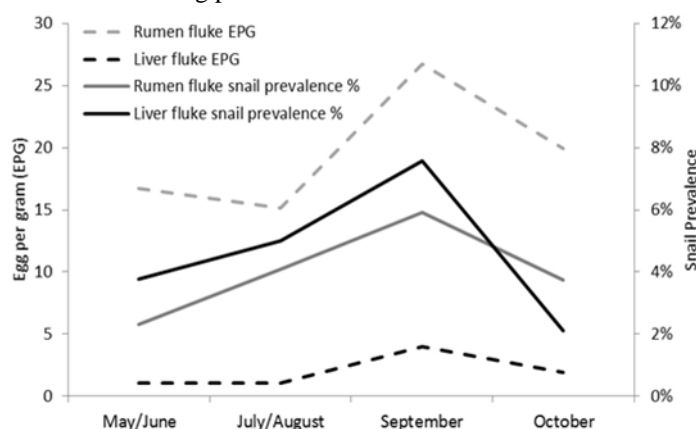


Figure 1 Prevalence of fluke infection within collected *G. truncatula* snails, and the intensity of fluke infection (Egg per gram of faeces shed, EPG) in livestock grazing their habitats

Assessing the effect of early lactation foot trimming on the prevalence of foot lesions at drying off in first and second parity cows

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Application This study aims to establish whether foot trimming of dairy cows early in lactation as a protective measure is preventative against the presence of foot lesions at drying off.

Introduction Lameness prevalence studies have estimated an average prevalence of 20-25% in UK herds, ranging from 8-60% in some studies^{1,2}, with the cause of lameness due to foot lesions reported at over 90%³. Foot trimming is a routine management practice applied to prevent the evolution of foot lesions from subclinical to clinical stages⁴. Studies have shown that subclinical and clinical foot lesions occurred less frequently when animals were routinely trimmed 2 to 3 times per year^{4,5}. In these studies, the timing of trimming was based upon the time of year, not the lactation stage of the animals. The objective of the present study was to evaluate the effect of early lactation foot trimming on the prevalence of lameness causing lesions at drying off.

Material and methods The study population originally comprised 651 first and second parity cows enrolled from 5 Cheshire farms, of which 142 control and 95 treatment cows were correctly trimmed as per the study design. Cows in the treatment group received a routine trim at 50-100 days post calving and at drying off; the control group received a trim at drying off only. Therapeutic foot trimming events were also recorded. Trimming was performed using the Dutch 5 step method and data recorded by technicians from a National Association of Cattle Foot Trimmers (NACFT) approved service. Participating herds were mobility scored by the same trained veterinarian and activity data recorded from IceQube sensors and the CowAlert cloud-based alerting system (IceRobotics, Edinburgh, UK). Data were combined with fertility and lactation data obtained from the milk recording agencies NMR and CIS. Multivariable logistic regression modelling was used in order to explore the associations of multiple explanatory variables with the likelihood of a lesion at drying off.

Results Lesion prevalence at drying off was significantly lower for cows receiving an early trim (P-value = 0.002); second lactation animals showed higher lesion prevalence than first lactation animals (P-value = 0.021). There was a significant difference between treatment and control animals and the prevalence of drying off lesions in first lactation animals only (P-value = 0.001). There was also a significantly greater risk of being culled for animals which did not receive an early trim (P-value < 0.001).

Conclusion This study demonstrates that cows receiving a trim early in lactation are significantly less likely to develop lesions at drying off compared to those which do not. It demonstrates a higher prevalence of foot lesions in second lactation animals compared to first lactation animals, and an increased risk of culling for animals which did not receive an early trim. This supports the adoption of early trimming 50-100 days after calving in heifers to prevent lesions. The utility of early trims in second lactation animals was less clear and further work will be required to determine if early trimming is beneficial in second lactation animals which have previously been protected by an early trim in their first lactation.

Acknowledgements The study was funded by Tesco PLC as part of the Tesco Dairy Centre of Excellence project at University of Liverpool. We would like to thank the farm staff and foot trimmers involved for their co-operation and IceRobotics for loaning activity monitoring systems for each farm.

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Nanoparticles induced oxidative stress and histological changes in gills of *Cyprinus carpio* exposed to water borne engineered Cu nanoparticles

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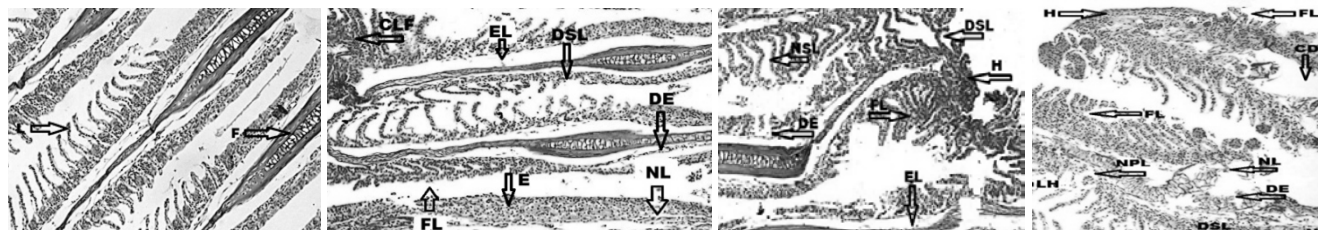
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Application The outcomes of this study will help to create awareness amongst public sector departments, health educators and industries involved in nanotechnology about the potential toxicity of nanoparticles for living organisms.

Introduction Nano-industry is one of the fastest growing industries. Increased production and use of nano-products will inevitably lead to increased levels of discharge of nano-materials into the environment. The aquatic environment is particularly vulnerable as it is likely to act as a sink for many of these particles. Aquatic species are at risk of nano-particles (NPs) exposure and a body of literature is emerging concerning the chemical behaviour of NPs in aquatic systems, including their accumulation and toxicity in aquatic species. Cu-NPs are used in many biotechnological researches and as additives in lubricants, inks, polymers and plastics, as well as metallic coatings. Cu-NPs have also been used in various skin products to enhance healing and prevention of infection. Conversely, the Cu-NPs have the ability to facilitate the direct generation of harmful radical oxygen species (ROS) inside the cell which may be damaged. Therefore, the study assessed the toxic effects of adding engineered Cu-NPs in water being used to raise *Cyprinus carpio*.

Material and methods A fish husbandry system was established prior to the start of experiments to support the health of fish by maintaining the water quality and environment of stock aquariums. *Cyprinus carpio* (*C. carpio*) of same weight (40–45g) were procured from a Fish Seed Hatchery, transported in plastic containers with continuous aeration to the laboratory of GC University Faisalabad, Pakistan. The fish were acclimatized in the tank with 100 L capacity for two weeks prior to the experiment. Un-chlorinated tap water was used and the physicochemical parameters of water were determined by a multi parameter apparatus (HI 9828, HANNA INSTRUMENTS). Total ammonia and water hardness were assessed by titration methods (AOAC, 2005). During acclimatization period, water temperature was maintained at 25°C, while dissolved oxygen and pH were 6.6–7.6 mg/l, and 6.9–7.5, respectively. NH₃ concentration, total hardness and total dissolved solids were 0.4–0.6 ppm, 47–52 ppm and 6.5–7.8 ppt, respectively. Photoperiod was 12 h light and 12 h dark. During the acclimatization period, fish were fed with commercial fish feed. After acclimatization, *C. carpio* from acclimation aquaria were randomly transferred into twelve aerated experimental glass aquaria (10 fish/ aquarium) with the same physicochemical parameters as in the acclimatization period and were acclimated for 48 hours prior to the experiment. Three aquaria per treatment were randomly allocated and fish were exposed in triplicate to 0 (Control) or 0.5 or 1 or 1.5 mg/l Cu as Cu-NPs for 14 days. During this study, water in the aquaria was changed daily and freshly prepared solution was added to maintain the concentration of Cu-NPs at constant level. At the end of the experiment fish from each aquarium were immediately anesthetized into a 200 ppm solution of clove powder. Gills were collected for histological studies and oxidative stress analysis by adopting standard protocols. The data were analysed by Minitab 17 software and the results were expressed as mean \pm SEM. Differences between treatment means were declared significant if $P < 0.05$.

Results The study revealed that with increase in the dose of Cu-NPs the histological alterations including degenerative secondary lamellae (DSL), necrotic lamella (NL), fused lamella (FL), necrosis of primary lamella (NPL), necrosis of secondary lamella (NSL), oedema, complete degeneration, epithelial lifting (EL), degenerative epithelium (DE), and hyperplasia (H) were increased. In the same way Cu concentration in gill tissues increased in dose dependent manner. The highest concentration ($8.29 \pm 0.01 \mu\text{g/kg ww}$) was observed in gills treated with 1.5mg Cu-NPs/l. The decreased level of catalase and elevated levels of lipid peroxidation and glutathione reductase were observed at high dose (1.5mg/l) of NPs (data not shown here).



A= control (normal histology) B - 0.5 mg/l Cu NPs exposure C - 1 mg/l Cu NPs D - 1.5 mg/l Cu NPs

Figure: Microphotograph of *C. carpio* gills in control and treated groups showing histological alteration (H&E, X400).

Conclusion It is concluded that engineered Cu-NPs can induce toxicity in *C. carpio*. So the products of NPs should be handled with care and preventive measures should be adopted about the disposal of NPs in water courses including the river systems.

Characterizing the activity and feed intake of lactating dairy cows during the Peri-oestrus period

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Application Behavioural oestrus can be detected by the number of steps, lying time and dry matter intake in high yielding dairy cows. However, cows in silent oestrus will not be detected by these measurements.

Introduction Over the past 50 years, the percentage of dairy cows in oestrus that stand to be mounted and the duration of oestrus have declined from 80% to 50% and 15h to 5h, respectively (Dobson *et al.*, 2008). The optimal time for insemination is determined by detection of behavioural oestrus on farm, with the start of behavioural oestrus preceding ovulation by approximately 24hrs (Madureira *et al.*, 2015). Thus, accurate detection of oestrus in high yielding cows is essential to maximise conception rate in dairy herds using artificial insemination. The objective of the present study is to determine whether the number of steps, lying time and the amount of dry matter intake can be used as signs of oestrous in high yielding cows in cubicle housing during the oestrus periods.

Materials and methods Thirty Holstein Friesian cows were used for the study with an initial body weight of 637.2 ± 59.9 kg (mean \pm SEM) and 29.6 ± 6.2 days postpartum at the University dairy unit. Cows were housed in a free-stall barn with 34 cubicles (2.7 x 1.2 m). Milk samples (40ml) were collected on Monday, Wednesday and Friday afternoon and stored at 4°C until analysis. Milk progesterone concentrations were analysed by EIA (Ridgeway Science Ltd., UK). A cow was considered in oestrus when progesterone levels were <2 ng/ml, followed by an increase to >15 ng/ml. A cow was defined as in behavioural oestrus when the sum of points scored for observed symptoms at three 30 minutes observation periods (07:30, 12:30 and 19:30) daily was >50 (Van Eerdenburg *et al.*, 2002). A cow was considered to be in silent oestrus when the score was <50 points, during oestrus as defined by milk progesterone profile. IceQubes (IceRobotics Ltd., Edinburgh, UK) were attached to the back left leg of each cow. The IceQube is a 3-axis accelerometer which reports cow activity summarised in 15 minute blocks. The daily dry matter intakes of cows were recorded by a Roughage Intake Control system (Insentec B. V., Marknesse, Netherlands). The datasets were analysed by repeated measures ANOVA (GenStat 17th edition). The Animals Experimental Committee of Harper Adams University gave ethical approval for the study.

Results The number of steps were increased but lying time and amount of dry matter were reduced significantly ($P<0.001$) on the day of oestrus (day 0) compared to 3 days before and 3 day after. During behavioural oestrus the number of steps, lying time and dry matter intake were 2095 ± 217 steps, 7.1 ± 0.34 h/day and 19.8 ± 0.41 kg/day compared to silent oestrus 984 ± 73.5 steps, 9.3 ± 0.45 h/d and 20.5 ± 0.61 kg/day, respectively. There was a significant oestrus x time interaction ($P<0.001$) with steps and lying time, while no significant oestrus x time interaction on the dry matter intake were recorded (Table1).

Table 1 Means of steps, lying time (h/d) and dry matter intake (DM) kg/d 3 days before, on day of oestrus (0) (behavioural and silent) and 3 days. OE = oestrus expression, B = behavioural, S = silent.

Activity	OE	Time/Days							SED	P value		
		-3	-2	-1	0	1+	2+	3+		OE	Days	OE X Days
Steps	B	823	833	891	2095	1195	895	821	152.8	0.163	<0.001	<0.001
	S	851	904	919	984	861	850	835				
Lying time h/d	B	10.2	10.0	10.1	7.1	10.5	10.6	10.3	0.529	0.838	<0.001	<0.001
	S	10.2	9.9	9.9	9.3	10.4	9.3	9.8				
DM intake kg/d	B	22.5	22.1	22.5	19.8	22.5	22.5	22.7	0.906	0.314	<0.001	0.371
	S	21.9	21.8	21.6	20.5	21.8	21.8	22.0				

Conclusion Although the number of steps, lying time and feed intake can all be used to detect behavioural oestrus, none of these parameters are affective in detective silent oestrus.

Acknowledgements The authors gratefully acknowledge funding from HCED-Iraq and special thanks to Carrie Gould and the dairy unit staffs at Harper Adams University.

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Effect of *Fasciola hepatica* infection on the susceptibility to Johne's disease in cattle in UK

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Application The average animal-level apparent prevalence of Johne's disease in cattle farms across England and Wales was 30% during 2013-2015. There was an indication that liver fluke infection can influence the susceptibility to Johne's disease in cattle.

Introduction Fasciolosis caused by *Fasciola hepatica* (liver fluke) is a widespread parasitic disease of ruminants that can result in marked economic losses on cattle farms. Johne's disease is a chronic, degenerative enteritis of cattle and other ruminants, caused by the *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease causes a significant reduction in production efficiency, animal welfare and, also, there is a potential association between exposure to MAP and Crohn's disease in humans. The fact that Johne's disease is mainly subclinical suggests that other contributing factors (e.g., animal co-infections, farm management practices) are important in switching subclinical infection to clinical disease.

Material and methods Sera samples from cattle were collected via the Animal and Plant Health Agency across 3 years (2013-2015) from 49 counties across England and Wales. The exposure to liver fluke was determined by an in-house ELISA that uses excretory/secretory antigens, as described by Salimi-Bejestani *et al.* (2005), with a diagnostic sensitivity of 98% and specificity of 96%, using a cut-off value of 15 per cent positivity. The exposure to MAP was assessed, using a commercial antibody-detection ELISA (LSIVet Ruminant Paratuberculosis Advanced-Serum), with a diagnostic sensitivity of 91-96% and specificity of 98-100%, using a cut-off value of 60 sample to positive ratio. Animal-level apparent prevalence of MAP or liver fluke was measured as the proportion of cattle exhibiting exposure to MAP or liver fluke, respectively. The McNemar test was used to analyse the association between the exposure to liver fluke and MAP and the Spearman's rank-order correlation to determine the relationship between the levels of liver fluke and MAP antibodies in co-infected cattle.

Results In total, 1828 cattle were tested in 2013-2015 for exposure to liver fluke and MAP, with 911, 495, 422 cattle in 2013, 2014, and 2015, respectively. The animal-level apparent prevalence of liver fluke was 33% (n=301) in 2013, 30% (n=150) in 2014, and 31% (n=131) in 2015, with an average prevalence of 32% (582) across 3 years (2013-2015). The animal-level apparent prevalence of MAP was 28% (n=255) in 2013, 31% (n=154) in 2014, and 31% (n=131) in 2015, with an average prevalence of 30% (n=166) across 3 years (2013-2015). The animal-level apparent prevalence of co-infection of liver fluke and MAP was 10% (n=87) in 2013, 9% (n=43) in 2014, and 9% (n=36) in 2015, with an average prevalence of 9% (n=582) across 3 years (2013-2015). Based on the results of the McNemar test, there was a statistically significant association between the exposure to liver fluke and MAP in 2013 (P=0.021), but not in 2014 (P=0.839), in 2015 (P=0.99), and in 2013-2015 (P=0.145). Also, there was not a statistically significant correlation between the levels of liver fluke and MAP antibodies in the co-infected cattle ($r_s=0.07$, P=0.389).

Conclusion These data are a useful source of information on the distribution of these two widespread endemic diseases in UK. The results of the samples collected in 2013 raise the question of whether *F. hepatica* infection can influence the susceptibility to Johne's disease in cattle. Further studies on the interplay between liver fluke and MAP infection in cattle are warranted.

Acknowledgements The authors gratefully acknowledge the Animal and Plant Health Agency (APHA Weybridge, UK) for providing their data.

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Effect of a whey protein gel of rapeseed oil supplement on milk fatty acid composition of Holstein Friesian cows fed maize silage-based diets

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Application Feeding lactating cows a whey protein gel (WPG) of rapeseed oil decreased milk saturated fatty acid (SFA) concentration. This supplemental strategy could be used to replace milk SFA with unsaturated FA.

Introduction Supplementing cow diets with oilseed oils can replace milk SFA with cis-monounsaturated FA (MUFA). However this can also result in increased milk trans FA concentrations. Previous attempts to protect cis-MUFA from rumen biohydrogenation to minimise trans FA production have had varying success. WPG technology has been successfully used to protect unsaturated FA in soyabean and linseed oils from rumen biohydrogenation (Carroll *et al.*, 2006; US Patent Applications 20040058003 and 20050089550). The objective of this study was to determine whether feeding dairy cows a WPG of rapeseed oil was effective at replacing milk SFA with cis-MUFA, whilst minimising increases in milk trans FA.

Material and methods Four multiparous, mid-lactation Holstein-Friesian cows were allocated to one of four treatment diets in a Latin Square design experiment with 14 day periods. Diets consisted of a control total mixed ration (52:48 forage:concentrate, DM basis; forage 3:1 maize silage:grass silage) containing no supplemental lipid, or the same diet with WPG of rapeseed oil incrementally included to provide 300, 600 or 900 g oil/cow/d (WPG300, WPG600, WPG900, respectively) at a predicted DM intake of 25 kg/d. The WPG was prepared as described by Carroll *et al.* (2006) using whey protein isolate (WPI; Volac International Ltd., Royston, UK) and rapeseed oil, replacing wheat and WPI in the concentrate portion. Milk yield and DM intake were measured daily and milk samples from the last two days of each period were analysed for FA composition. Data were analysed using the mixed models procedure of SAS, with period and treatment as fixed effects, and cow as a random effect.

Results WPG inclusion affected ($P=0.025$) DM intake, which was highest for WPG600 (24.6 kg/d). Incremental inclusion of WPG linearly increased ($P=0.002$) milk yield (from 30.6 to 40.9 L/d) and decreased ($P=0.007$) milk fat concentration (from 39.8 to 35.3 g/kg milk). Increasing WPG from 0 to 900 g/d decreased milk SFA concentration (Table 1) due mainly to a linear decline ($P<0.001$) in 16:0 (from 35.1 to 21.7 g/100 g FA for control and WPG900, respectively). This contrasted with linear increases in both cis- and trans-MUFA (Table 1). Milk fat concentrations of both n-3 and n-6 polyunsaturated FA also demonstrated a linear increase ($P<0.01$) with increasing inclusion of WPG supplements, as did total conjugated linoleic acid concentration (Table 1).

Table 1 Effect of incremental amounts of a whey protein gel of rapeseed oil supplement on milk fatty acid composition

Fatty acid group ¹ (g/100 g total FA)	Treatment diet ²				s.e.m.	P ³			
	Control	WPG300	WPG600	WPG900		Diet	Linear	Quadratic	Cubic
Σ SFA	73.3	69.1	62.2	57.9	1.02	<0.001	<0.001	0.936	0.214
Σ cis MUFA	20.6	22.0	27.6	29.7	0.81	0.062	0.032	0.493	0.130
Σ trans MUFA	2.96	3.94	5.76	7.05	0.324	0.027	0.014	0.266	0.137
Σ PUFA	3.60	4.07	4.87	5.48	0.175	0.002	<0.001	0.510	0.317
Σ CLA	0.41	0.47	0.80	0.92	0.055	0.014	0.005	0.512	0.095

¹SFA, MUFA, PUFA and CLA - saturated, monounsaturated and polyunsaturated fatty acids and conjugated linoleic acid.

²WPG300, WPG600 and WPG600 - control diet supplemented with 300, 600 or 900 g oil as whey protein gel supplement.

³Effect of diet and linear, quadratic or cubic relationships for $n = 16$ measurements.

Conclusion Incremental inclusion of the WPG supplement up to 900 g of oil/cow/d resulted in decreased milk SFA and increased unsaturated FA concentrations. This change is similar to that observed in an earlier study whereby a similar amount of lipid as Ca-salts of cis-MUFA was fed (Kliem *et al.*, 2013). Increases in trans-MUFA suggest that rumen protection was not as complete as observed previously (Carroll *et al.*, 2006), although the increased milk cis-MUFA and PUFA concentrations suggest the approach partially protected unsaturated FA from rumen biohydrogenation.

Acknowledgements This study was supported by BBSRC DRINC project BB/1006087/1. The authors gratefully acknowledge staff at the Centre for Dairy Research for their care of the animals.

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The use of high sugar grasses as a strategy to improve nitrogen utilization efficiency: A meta-analysis

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Application Grasses with elevated WSC concentrations may be used to reduce urinary N excretion and improve overall milk N use efficiency of pasture-based dairy cows.

Introduction Pasture-based dairy systems are associated with considerable nitrogen (N) pollution emissions, while the restricted use of concentrate feeds limits the margin of intervention. In this direction, high sugar (HS) grasses, with elevated concentrations of water soluble carbohydrates (WSC), have been reported to increase nitrogen use efficiency, reducing N excretion, particularly in urine. Given the increasing body of studies with HS grasses, our objective was to investigate their effects on production and N excretion of dairy cows using meta-analytical methods.

Material and methods A dataset was created from published studies and 3 unpublished studies (Moorby J., personal communication) conducted in the United Kingdom (UK; $n = 6$), the Netherlands (NL; $n = 7$) and New Zealand (NZ; $n = 5$). Studies with limited information on intake and milk production as well as inadequate methods of calculating N balance (e.g. estimations rather than actual measurements) were excluded from analysis. The dataset was used: (i) to define the chemical composition of HS and control (CTR) forages (all studies), (ii) to analyse effects on production and intake (5 and 6 studies from the UK and NL, respectively), and (iii) to analyse effects on N balance and excretion (5 UK studies). The statistical analysis was performed using a mixed model in JMP (SAS), where study was considered a random effect, and treatment (HS vs CTR), origin (UK, NL and NZ) and their interaction were treated as fixed effects. For the N balance, individual cow data were available and used as such with a similar mixed model (random: study; fixed: treatment).

Results The main difference between HS and CTR grasses was detected as increased WSC concentration of HS at the expense of neutral detergent fibre, but with a considerable variation between countries: HS grasses had on average 44, 27 and 28 g/kg DM higher WSC concentration than CTR grasses for UK, NL and NZ, respectively. In no case was a modification of the crude protein concentration of the forages recorded. Forage and total DM intakes were not different between grass type, but cattle in the NL consumed more forage and total DM compared with those in the UK. Similarly, milk production (on average 24.4 and 25.9 kg/d for UK and NL, respectively) and milk composition were not affected by grass type. In N balance studies, N intake was numerically lower and milk N numerically higher for HS fed cattle resulting into a significantly improved milk N use efficiency for HS fed cattle (0.29 vs 0.26 for HS vs CTR grass fed cattle, respectively; $P = 0.03$). Urine N excretion (g/d) was 26% lower for cattle fed HS grass compared with CTR grasses. Further, urine N:feed N was expressed as a function of dietary WSC:N using a broken-line model with a break point at 9.2 g g⁻¹ WSC:N (Figure 1).

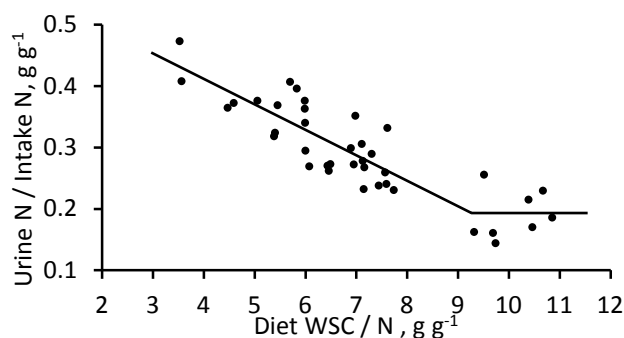


Figure 1 Urine N / Intake N response as a function of dietary WSC / N ($y = 0.58 - 0.04 \times \text{WSC/N}$ and $y = 0.58 - 0.04 \times 9.2$ for the linear and plateau part of the model; RMSE = 0.038)

Conclusion The meta-analysis indicates that feeding dairy cattle HS grasses did not increase milk production but reduced urine N excretion by 26%. A dietary WSC:N ratio of 9.2 g g⁻¹ will minimize excretion of urine N relative to intake.

Acknowledgements Financial support was provided by the Welsh Government and Higher Education Funding Council for Wales through the Sêr Cymru National Research Network for Low Carbon, Energy and Environment. Project: Consequential Life Cycle Assessment of Environmental and Economic Effects of Dairy and Beef Consolidation and Intensification Pathways – ‘Cleaner Cows’.

Dataset available upon request

Particle size and physically effective fibre (peNDF) distribution in a range of grass silage, maize silage and total mixed rations on UK dairy herds

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Application The particle length of maize silage being fed on UK dairy farms was similar to North American recommendations, however, particle length of grass silage and grass silage based total mixed rations (TMR) was considerably longer, which suggests specific recommendations for grass silage based diets are needed.

Introduction The particle size (PS) distribution of dairy cow diets has a large impact on intake, rumen metabolism and performance (Mertens, 1997). Currently, most PS recommendations are based on North American diets and forages, which may not be suitable for the comparatively wetter and longer chop length forages fed in the UK. The objectives of the study were to characterize the range of PS of TMR, grass and maize silages used on UK dairy farms and compare these with current recommendations.

Material and methods Fifty commercial dairy herds located throughout the UK that were feeding a range of grass and maize silage based diets were visited between January and June, 2016. The PS distribution of the fresh forage and TMR samples were analysed using a modified Penn State Particle Separator (Kononoff *et al.*, 2003) with four screens of size 26.9, 19, 8, and 4 mm. The fresh TMR was collected five times across the length of the feed bunk within five minutes of feeding. Following collection, fresh samples of TMR and forages were separated into five fractions; very long (>26.9 mm), long (19 - 26.9 mm), medium (8 -19 mm), short (4 - 8 mm) and fine (<4 mm) and dried at 100°C. The physical effectiveness factor (pef) was calculated as the sum of the dry matter (DM) > 4 mm as a % of total sample DM. The neutral detergent fibre (NDF) content of the TMR, grass and maize silages was analysed as described by Van Soest *et al.* (1991), and the peNDF calculated by multiplying the pef by the NDF concentration (Mertens, 1997).

Results The mean long and very long PS distribution of the TMR was 37% of the DM, approximately 50% greater than that reported (Heinrichs, 2013; Sova *et al.*, 2013) for typical North American diets (Table 1). Despite using a larger sieve size (26.9 mm), the majority (79%) of the grass silage DM was retained on the very long screen, and an even larger sieve may be necessary to appropriately describe grass silage and grass silage based TMRs. In contrast to the grass silage, only 7% of the maize silage DM was retained on the >19 mm screen, and the PS distribution was similar to North American recommendations, although the proportion of DM that was 4-8 mm material was less. The content of peNDF of the TMR sampled was more than double that reported for North American rations.

Table 1 The particle size distribution (% of DM \pm SD) and physical characteristics of TMR (n=50), grass silage (n=49) and maize silage (n=33) fed on UK dairy farms compared to North American recommendations

Fractions	TMR		Grass silage		Maize silage	
	Current study	Sova <i>et al.</i> , (2013)	Current study	Heinrichs (2013)	Current study	Heinrichs (2013)
>26.9 mm	32.8 \pm 23.71	-	78.7 \pm 13.22		---	
19-26.9 mm	4.4 \pm 3.50	19.8	2.7 \pm 2.44	10-20	6.9 \pm 4.71	3-8
8-19 mm	35.6 \pm 15.24	34.3	14.3 \pm 9.68	45-75	73.2 \pm 8.85	45-65
4-8 mm	12.2 \pm 5.67	35.5	2.6 \pm 1.18	20-30	13.1 \pm 5.06	20-30
<4 mm	15.0 \pm 9.52	10.5	1.7 \pm 1.61	<5	6.8 \pm 4.21	<10
s.e.d	2.72		1.44		1.17	
P-value	<0.001		<0.001		<0.001	
Physical effectiveness factor (pef) and physical effective NDF (% of DM \pm SD)						
pef	85 \pm 9.4	54	98 \pm 1.5		93 \pm 4.1	
peNDF	40 \pm 8.1	17	56 \pm 7.3		47 \pm 8.2	

Conclusions The TMR and GS being fed on UK dairy herds had a considerably higher proportion of very long and long particles than recommended for North American diets. This difference in PS distribution was principally due to the inclusion of grass silage in UK diets. Current guidelines for PS distribution are therefore not appropriate for UK dairy rations, and PS distribution guidelines specific for UK GS and GS/MS based TMR diets are therefore needed.

Acknowledgements This study was funded by AHDB Dairy.

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A meta-analysis of supplementing lactating dairy cow diet with soybean oil on milk production, fat, and linoleic acid in milk

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Application Producers are likely to continue to supplement dairy cow feeds with soybean oil to increase milk yield and C18:2 and C18:3 fatty acids concentrations as long as they understand that DMI and milk fat percentage may fall.

Introduction The past few years have witnessed increased interest in producing milk with lower saturated fatty acid (SFA) and higher unsaturated fatty acid (UFA) concentration given its impact on improved nutritional value of milk and enhanced human health. One strategy to enhance UFA-to-SFA ratio of milk is to use oilseeds. Soybean is an oilseed rich in linoleic acid commonly used as a supplement for dairy cattle diet in the form of extruded or oil to increase UFA content in milk. Studies that examined soybean oil effects on dry matter intake (DMI), milk yield (MY), milk fat percentage (FAT%), and concentrations of linoleic (C18:2) and linolenic (C18:3) fatty acids have produced mixed results (indicating positive and negative effects) in lactating dairy cows. Therefore, the present study makes use of a meta-analytic approach to identifying effects of soybean oil on DMI, MY, FAT%, and concentrations of C18:2 and C18:3 fatty acids in dairy cattle milk. The meta-analysis consists of statistical techniques to statistically combine and summarize the findings of different studies (Borenstein *et al.* 2009).

Material and methods This meta-analysis covers peer-reviewed English articles published on reputable academic journals from 1990 to 2016 and indexed on Thomson Reuters. Selection (inclusion) and exclusion criteria were defined in a way that included those studies which consisted of a control group and group(s) receiving soybean oil in free form (not in the form of processed seed). Studies on dairy cows fed through grazing were excluded from the database. Furthermore, we also excluded studies in which dairy cows received oils from resources other than soybean or soybean oil mixed with other oils. Also excluded were studies that did not report SD and/or SEM for the parameters as well as reviews or articles that did not use soybean oil in any of the treatments. Statistical analysis was carried out using Comprehensive Meta-Analysis software version 2.2 and effect size for DMI, MY, FAT%, and C18:2 and C18:3 fatty acids concentrations were calculated as standardized mean difference (SMD) using a confidence interval of 95%. The model used here was a random effects model and heterogeneity was determined through Q-test and I² statistic.

Results Once papers were selected based on the selection and exclusion criteria, the database for the parameters DMI, MY, FAT%, and C18:2 and C18:3 fatty acids concentrations consisted of 12, 11, 11, 11, and 9 studies, respectively. Table 1 presents the results of the meta-analysis for DMI, MY, FAT%, and C18:2 and C18:3 fatty acids concentrations, using the random effect model. Our findings indicated that supplementing dairy cattle feed with soybean oil reduced DMI while increasing MY, decreasing FAT%, and increasing concentrations of C18:2 and C18:3 fatty acids in milk compared to control group. Heterogeneity results from Q-test indicated existence of heterogeneity between studies on FAT% and C18:2 in milk and the heterogeneity obtained through I² for FAT% and C18:2 in milk were about 65.242% and 81.028%, respectively.

Table 1 Effect size and heterogeneity for soybean oil supplement impact on DMI, MY, FAT%, and C18:2 and C18:3 fatty acids concentrations in dairy cow milk

Outcome	N studies	N comparisons	SMD	95% confidence intervals	P- value	Q	P- value	I ²
DMI	12	16	-0.305	-0.544, -0.06	0.013	9.297	0.861	0
MY	11	15	0.362	0.111, 0.612	0.005	14.393	0.421	2.734
FAT%	11	15	-1.015	-1.483, -0.547	<0.001	40.279	<0.001	65.242
C18:2	11	15	0.854	0.234, 1.475	0.007	73.794	<0.001	81.028
C18:3	9	13	0.147	-0.196, 0.491	0.401	18.887	0.091	36.464

Conclusion Supplementing dairy cow feeds with soybean oil as a rich source of C18:2 fatty acid reduces DMI and FAT% while increasing MY and C18:2 and C18:3 fatty acids concentrations in milk, and given the heterogeneity found for FAT% and C18:2, it can be concluded that several factors, including dosage of soybean oil and basal diet properties, may contribute to the heterogeneity observed.

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The effect of the addition of hydrolysable tannins to lucerne and red clover silages on the performance and milk fatty acid profile in Holstein-Friesian dairy cows

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Application Compared to red clover silage, feeding lucerne silage increased intake, had little effect on performance but improved the fatty acid profile of milk, whereas the addition of hydrolysable tannins had no effect on performance or milk fatty acid profile.

Introduction Increased global demand for soyabean and rapeseed meal, and associated fluctuations in their availability and price has resulted in greater interest in the utilisation of home grown, protein-rich forage sources for ruminants. Lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) are of interest due to their high protein content. However the protein in these forages is highly degraded in the rumen, and does not supply sufficient by-pass protein to meet the requirements of high yielding dairy cows. Tannins can bind to protein and other dietary components in forages to form a pH dependent complex that does not dissociate at rumen pH 4.5 - 7 but will dissociate in the abomasum at pH 2 - 3 (Fraser *et al.*, 2001). Hydrolysable tannins are composed of gallic acid or ellagic acid (McMahon *et al.*, 2000), and can be extracted from temperate plants and added to forages at ensiling or feeding. The study objectives were to determine the effect of the addition of hydrolysable tannins to lucerne and red clover silage at ensiling on the performance and milk FA profile in high yielding dairy cows.

Material and methods Twelve multiparous dairy cows that were 88 ± 26.9 days in milk received one of 4 diets in each of 4 periods of 28 day duration, in a Latin square design with measurements taken in the final 7 days of each period. The proportion of lucerne or red clover to maize silage was 40:60 (DM basis), and all dietary treatments contained 55:45 forage to concentrates (DM basis). There were four dietary treatments; lucerne or red clover silage ensiled without (-Tannin) or with (+Tannin) chestnut tannin added at ensiling at the rate of 25 g/kg DM. The maize silage was harvested at approximately 300 g DM/kg. Diets were formulated to be isonitrogenous. Cows were milked twice daily and yield recorded at each milking, with samples collected for composition and fatty acid content. Live weight was recorded at the beginning and end of each period. Data were analysed as a Latin square design using Genstat (v. 16).

Results The lucerne, lucerne plus tannin, red clover, red clover plus tannin and maize silages contained 456, 452, 215, 217 and 266 g/kg DM, with a crude protein content of 215, 214, 159, 150 and 89 g/kg DM, respectively. Intake was approximately 2.2 kg DM/d higher in cows when fed the lucerne diets, but there was no effect of treatment on milk yield, fat, protein or lactose content, or live weight (Table 1). Milk fatty acids 18:2 n-6 and 18:3 n-3 were highest in cows fed red clover silage. In contrast, there was no effect of tannin inclusion on performance or milk fatty acid profile (Table 1).

Table 1 Milk performance, live weight and milk fatty acid profile of cows fed diets containing lucerne or red clover silage without or with hydrolysable tannins

	Treatment				s.e.d	P value		
	Lucerne		Red Clover			Forage	Tannin	F x T
	-Tannin	+Tannin	-Tannin	+Tannin				
DM Intake, kg/d	21.3	22.9	20.1	19.6	1.15	0.009	0.533	0.206
Milk yield, kg/d	38.2	38.5	38.6	37.2	2.24	0.781	0.739	0.606
Milk fat, g/kg	40.7	42.2	40.9	42.4	1.98	0.900	0.297	0.972
Milk protein, g/kg	34.1	34.5	33.1	33.4	0.96	0.142	0.673	0.899
Milk lactose, g/kg	49.1	48.7	49.0	48.8	0.51	0.965	0.520	0.819
Live weight, kg	644	660	657	650	25.8	0.942	0.799	0.518
Milk fatty acids (g/100g)								
18:2n-6	0.38	0.39	0.42	0.42	0.010	<0.001	0.693	0.285
18:3n-3	0.100	0.095	0.107	0.106	0.002	<0.001	0.128	0.292

Conclusion The inclusion of hydrolysable tannins in lucerne and red clover silage had no effect on DM intake, milk performance or milk fatty acid profile of high yielding dairy cows. Cows fed lucerne silage had a higher DM intake than those fed red clover silage but there was no effect on performance. Forage source had a minor effect on milk FA profile, with 18:2 n-6 and 18:3 n-3 being higher in cows when fed red clover silage.

Acknowledgements The authors gratefully acknowledge AHDB Dairy for funding this project.

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A review of grass silage quality in NI from 1996 to 2015 using data from the Hillsborough Feeding Information System – regression between silage nutritive value and harvesting year

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Application With silage quality improving, it is expected that less concentrate can be used for a similar cattle production.

Introduction Silage ensiling techniques have been improved significantly through scientific research during the past decades. The present study aimed to quantify the variation in silage quality using data of grass silage samples of the last 20 years from commercial farms in Northern Ireland.

Material and methods Several thousands of grass silage samples from commercial farms in Northern Ireland has been analysed every year in the analytical laboratory of Agri-Food and Biosciences Institute using the Hillsborough Feeding Information System (HFIS). The HFIS for grass silages was developed based on data of 136 grass silages of various qualities obtained from a series of systematic studies including scanning with the near-infrared spectroscopy (NIRS), wet chemical analysis, rumen degradability measurements, digestibility trials and feed intake experiments. An important aspect of the HFIS is its capacity of using fresh samples for the NIRS scan to predict nutritive values of grass silages, rather than using dry samples as commonly practised. The HFIS is regarded as a reference technique for calibration of the measurement bias for the UK Forage Analytical Assurance Group. All HFIS grass silage data (first to third cuts) harvested in Northern Ireland commercial farms from 1996 to 2015 were collected in the present study. The majority of these silages was from the first cut grass (60.9%), followed by the second cut (33.6%) and the third (5.5%) cuts only amounted for a small proportion. These data were used to analyse the temporal variation in silage nutritive values using the linear regression technique.

Results The linear relationships of harvesting years (1996 to 2015) against yearly mean data of each cut (1st, 2nd and 3rd) or all data from the 1st to 3rd cuts are presented in Table 1 and Fig. 1. The results indicated that for each harvesting season, dry matter (DM) concentration increased from 1996 to 2015, while crude protein (CP), neutral detergent fibre (NDF) and volatile fatty acids (VFAs) concentrations decreased. Ammonia-N as total N also reduced although the reduction rate was not significant for the 3rd cut silage. There was no improvement in digestible organic matter in total DM (DOMD) or metabolisable energy (ME) concentration, however the intake potential of cattle increased although the increase was not significant for the 2nd or 3rd cut silages. Data for lipid and water soluble carbohydrate (WSC) are only available for harvesting years from 2006 to 2015, which saw a linear decrease in lipid concentration, but a linear increase in WSC concentration in grass silages of the 2nd, 3rd cut or all 3 cuts. There was no significant effect on lactic acid concentration or pH value, although there was a significant increase in pH value with all 3 cut data.

Table 1. The R data in linear relationships between harvesting years (1996 to 2015) and various variables in NI grass silages measured by HFIS[#]

	DM	CP	NDF	Lipid	WSC	DOMD	ME	Intake potential	pH	NH ₃ -N/total N	Lactic acid	VFAs
First cut	0.75***	-0.45*	-0.82***	-0.88***	0.08	0.36	0.36	0.53*	0.32	-0.45*	-0.27	-0.76***
Second cut	0.84***	-0.57*	-0.94***	-0.68***	0.74***	0.12	0.12	0.38	0.36	-0.55*	-0.15	-0.83***
Third cut	0.73***	-0.42*	-0.95***	-0.52*	0.68***	-0.26	-0.26	0.30	0.39	-0.33	-0.13	-0.68***
All three cuts	0.75***	-0.32*	-0.75***	-0.59***	0.51***	0.06	0.06	0.38**	0.34**	-0.45***	-0.17	-0.71***

[#] Unit = g/kg for DM and NH₃-N/total N, g/kg^{0.75} for intake potential, MJ/kg DM for ME and g/kg DM for the remaining variables
 Data with *, **, *** or without any * indicate the relationship was at a level of P < 0.05, P < 0.01, P < 0.001, or P > 0.05

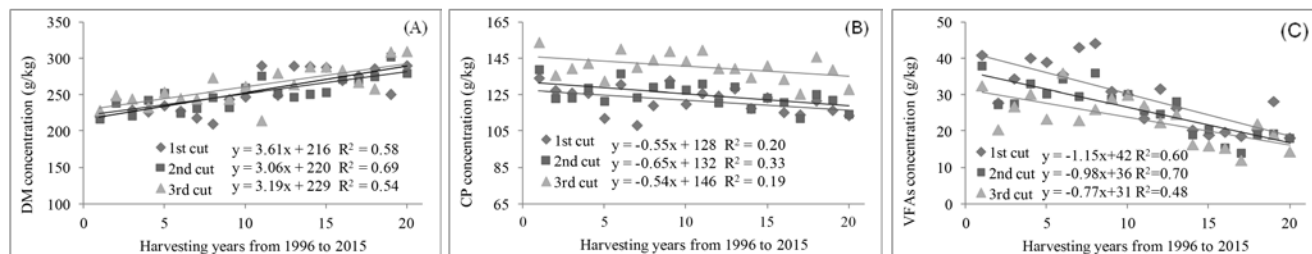


Figure 1 The relationships between harvesting year and DM (A), CP (B) or VFAs (C) concentrations of grass silages

Conclusion The present study found that the silage fermentation quality and intake potential of commercial farm grass silages in Northern Ireland was improved from 1996 to 2015. This may be due to the significant improvement in silage ensiling techniques through scientific research during the past decades.

A review of grass silage quality in NI from 1996 to 2015 using data from the Hillsborough Feeding Information System – effects of harvesting year and harvesting season

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Application This study found that current grass silage has a better quality than before and the harvesting season influences silage quality. These are important factors for developing sustainable feeding strategies for dairy and beef production.

Introduction Harvesting season and ensiling techniques are two key factors influencing the fermentation progress inside the silo and silage nutritive values. The objective was to evaluate effects of harvesting year and harvesting season on silage nutritive variables using data of grass silage samples from commercial farms in NI from 1996 to 2015 harvesting years.

Material and methods Several thousands of grass silage samples from commercial farms in NI have been analysed every year in the analytical laboratory of Agri-Food and Biosciences Institute using the Hillsborough Feeding Information System (HFIS). The HFIS uses scanning data of fresh samples by the near-infrared spectroscopy to predict fermentation variables and nutritive values of grass silages. Silage data from 1996 to 2015 harvesting years were collected and each variable was calculated as an average value based on each harvesting season (1st, 2nd or 3rd cut) on a yearly basis. Lipid and water soluble carbohydrate (WSC) data were only available from 2006 to 2015. The effect of harvesting year was undertaken by grouping the 20 years' data into 4 blocks with 5 years/block. The comparison of variations among harvesting year groups and seasons was performed using the one-way ANOVA.

Results The results are presented in Table 1. In terms of the effects of harvesting years, the fermentation variables were improved from 1996 to 2015, with a lower volatile fatty acids (VFAs) content and ammonia-N/total N in the latter years than in the earlier years, although pH was higher in 2011-2015 than in other 3 groups. The DM content was higher in the 2 late year groups than in the 2 early year groups, while CP and NDF content were lower with the 2 late groups although CP content between 2006-2010 and the 2 early year groups was similar. Lipid content reduced and WSC content increased in 2006-2010 group when compared to 2011-2015 group. Consequently, the silage intake potential for beef cattle was improved with higher intake in the 2 late year groups than with 1996-2000 group. In terms of effects of grass silage harvesting season, the 3rd cut silage had a higher pH, ammonia-N/total N and lactic acid content than in the other 2 cuts, but VFAs content linearly decreased from cuts 1, 2 to 3. The CP content increased linearly from cuts 1, 2 to 3, but NDF content decreased linearly. The 2nd cut silage had lower lipid and ME contents and digestible OM in total DM (DOMD), and consequently a lower intake potential for beef cattle when compared with 2nd and 3rd cut silages.

Table 1. Effects of grass silage harvesting years and seasons on silage nutritive and fermentation variables

	Effect of grass silage harvesting years						Effects of grass silage harvesting season				
	1996-00	2001-05	2006-10	2011-15	s.e.	P value	1st cut	2nd cut	3rd cut	s.e.	P value
DM (g/kg)	236.0 ^a	241.1 ^a	268.3 ^b	280.0 ^b	4.48	<0.001	254.3	252.4	262.3	3.96	0.186
CP (g/kg DM)	131.1 ^b	132.5 ^b	129.8 ^b	123.3 ^a	1.62	<0.001	122.0 ^a	125.1 ^b	140.4 ^c	0.74	<0.001
NDF (g/kg DM)	533.1 ^b	526.1 ^b	488.7 ^a	483.8 ^a	2.55	<0.001	518.7 ^c	513.8 ^b	481.7 ^a	1.68	<0.001
Lipid (g/kg DM)			33.3 ^b	32.2 ^a	0.24	0.002	32.8 ^b	31.9 ^a	33.5 ^c	0.18	<0.001
WSC (g/kg DM)			23.5 ^a	27.3 ^b	1.17	0.031	25.5	24.1	26.6	1.51	0.512
DOMD (g/kg)	665.6	662	659.8	669.7	3.42	0.202	672.1 ^b	654.5 ^a	666.3 ^b	2.67	<0.001
ME (MJ/kg DM)	10.65	10.59	10.56	10.72	0.055	0.202	10.75 ^b	10.47 ^a	10.66 ^b	0.043	<0.001
Dairy intake potential (g/kg ^{0.75})	90.4	89.6	90.6	92.9	0.87	0.069	91.6	89.3	92.1	0.85	0.059
Beef intake potential (g/kg ^{0.75})	74.5 ^a	75.4 ^{ab}	77.1 ^b	77.6 ^b	0.84	0.049	77.2 ^b	74.2 ^a	77.4 ^b	0.77	0.008
pH	4.10 ^a	4.11 ^a	4.13 ^a	4.21 ^b	0.025	0.013	4.13 ^a	4.11 ^a	4.18 ^b	0.010	<0.001
NH ₃ -N/total N (g/kg)	103.8 ^b	117.6 ^c	87.1 ^a	93.3 ^a	2.23	<0.001	98.3 ^a	99.6 ^a	103.5 ^b	1.36	0.029
Lactic acid (g/kg DM)	75.7	72.1	75	72.2	1.44	0.181	70.9 ^a	72.7 ^a	77.6 ^b	0.94	<0.001
VFAs (g/kg DM)	31.5 ^c	32.5 ^c	23.1 ^b	18.3 ^a	1.17	<0.001	29.6 ^c	26.0 ^b	23.4 ^a	0.76	<0.001

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

Conclusion The silage quality in the last 20 years has been improved, with a higher DM content, a lower NDF content and better fermentation variables, which led to a higher intake potential for cattle. On the other hand, these silage data showed little difference in fermentation quality between the 1st and 2nd cut silage, but the 1st cut silage had a higher DOMD and ME content than the 2nd cut silage, thus leading to a higher intake potential for cattle.

The effects of feeding lucerne silage, at two different chop lengths and inclusion rates, as a source of physically effective dietary fibre for lactating dairy cows

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Application Feeding long chop length (CL) lucerne silage at a high inclusion rate (IR; 375 g/kg of diet dry matter (DM)) stimulated rumination and buffered rumen pH relative to a lower IR (125 g/kg diet DM) and a shorter CL.

Introduction To optimise rumen health in lactating dairy cows consuming highly fermentable non-forage carbohydrates, an adequate source of physically effective neutral detergent fibre (peNDF), which stimulates rumination, should be included in the diet. Feeding lucerne promotes rumen health as it contains high concentrations of peNDF however it can reduce milk yield at high IRs (Thomson *et al.* 2016). Forage CL affects peNDF concentration, and there is a need to determine optimum CL to prevent oversupply of long particles which reduce digestibility and encourage diet sorting (Zebeli *et al.*, 2012). The objective of this study was to determine the effect of IR and CL of lucerne silage, replacing maize silage, in a total mixed ration (TMR) with the hypothesis that higher IR and longer CL of lucerne would beneficially affect these variables.

Material and methods Four rumen-cannulated, multiparous, Holstein dairy cows in mid-lactation (161 ± 23 SE days in milk) were used in a 4 x 4 Latin square design study (2 x 2 factorial) with 21d periods. Diets were formulated that contained a 50:50 forage:concentrate ratio (DM basis), and were isonitrogenous (170 g/kg CP). The forage portion of the offered diets comprised maize and lucerne silage in proportions of either 25:75 (high lucerne; HL) or 75:25 (low lucerne; LL). Second cut lucerne was conserved as silage at theoretical CLs of 14 (Long; L) or 19 mm (Short; S). Therefore, treatment diets were either LLS, LLL, HLS or H. Effects on feed intake, milk production, chewing behaviour, and rumen pH and volatile fatty acid (VFA) profile were measured in liquor samples obtained through the rumen cannula. Data were analysed by a Mixed Models procedure of SAS (version 9.4), testing fixed effects of period and treatment and random effect of cow.

Results Both greater IR and CL increased $\text{peNDF}_{>4\text{mm}}$ (Table 1). Dry matter intake was higher (2.7kg/d) when LLS rather than LLL was fed (IRxCL $P < 0.03$), however milk yield was unaffected. Long CL diets reduced total VFA concentration compared to short CL diets ($P = 0.03$) while HL diets increased acetate:propionate ratio relative to LL diets ($P < 0.001$). A number of IRxCL interactions were observed. The number of rumination chews/day was highest for HLL and LLS diets (IRxCL $P < 0.05$) and the HLL diet also tended to produce the highest rumen pH (IRxCL $P < 0.06$). The HLS diet stimulated the least rumination (IRxCL $P < 0.05$) but produced a higher total VFA concentration than other diets fed (IRxCL $P < 0.002$).

Table 1 Effects of lucerne silage chop length (CL), inclusion rate (IR), and their interaction (IRxCL) on DMI, milk yield, rumen pH and VFA concentration (measured over 12h post morning feeding), and rumination activity.

	Diets ¹				SEM	P value		
	LLS	LLL	HLS	HLL		IR	CL	IRxCL
peNDF _{>4mm} , g/kg DM ²	172	199	205	213	3.8	0.003	0.004	0.051
Dry matter intake, kg/d	25.1 ^a	22.4 ^b	23.1 ^{ab}	24.4 ^{ab}	1.08	0.991	0.152	0.027
Milk yield, kg/d	29.3	29.4	29.1	28.4	3.93	0.519	0.704	0.646
Rumen pH	6.25	6.24	6.22	6.38	0.141	0.186	0.103	0.058
Time spent ruminating, min/d	447	453	445	566	41.9	0.106	0.077	0.097
Rumination chews, '000/d	27.9 ^a	26.9 ^b	25.6 ^c	34.9 ^a	2.69	0.192	0.081	0.043
Total rumen VFA, mM	118 ^a	121 ^b	130 ^c	114 ^a	7.1	0.296	0.030	0.002
Acetate:Propionate ratio	3.10	3.05	3.37	3.74	0.257	0.001	0.155	0.079

¹LLS = low lucerne short chop, LLL = low lucerne long chop, HLS = high lucerne short chop, HLL high lucerne long chop

²peNDF = physically effective neutral detergent fibre (particles >4mm length).

Conclusion A longer lucerne CL improved rumen stability when combined with a high IR (HLL) within the TMR, shown by a high acetate:propionate ratio. However, when a long CL was combined with a low lucerne IR, there was no beneficial effect on rumen parameters, likely due to insufficient peNDF concentration. Relatively high mean pH values post feeding (> 6.2) suggest that diets fed in this study did not pose a challenge to the rumen. The true value of a HLL diet would likely be greatest if the TMR had a higher concentration of degradable starch and sugar and thus greater acidosis risk.

Acknowledgements Funding was kindly provided by the AHDB Dairy: Soils, forage and grassland Research Partnership.

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Rate of inclusion of microalgae and its effect on whole tract digestibility and milk fatty acid profile in Holstein-Friesian dairy cows

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Application When added to the diet of dairy cows at 100 g/cow/d, microalgae can increase the milk content of docosapentaenoic acid and decrease the saturated fatty acid content without impacting milk yield or diet digestibility.

Introduction The benefits of long chain fatty acids (FA) on human health have long been recognised, in particular the very long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) such as docosapentaenoic acid (DHA; Allred *et al.*, 2006). The primary producer of LC n-3 PUFA at the bottom of the food chain is microalgae (ALG; Vahmani *et al.*, 2013), which may be added to the diet of livestock to improve the FA profile of milk and meat. The addition of ALG to the diet of dairy cows has been shown to increase the milk content of LC n-3 PUFA (Franklin *et al.*, 1999), although the optimal dose to maximise milk content without impairing whole tract digestibility or performance is unclear. The objective of this study was to determine the effect of rate of inclusion of ALG on milk FA and whole tract digestibility in Holstein-Friesian dairy cows.

Material and methods Twenty Holstein-Friesian dairy cows yielding 40 kg/d at the beginning of the study were randomly allocated to one of four diets in each of four periods in a 4 x 4 Latin square design. Each period was 28 days in duration, with measurements undertaken during the final 7 days. The basal ration contained maize and grass silages and straight feeds, and was supplemented with ALG to provide four treatments; 0 (Control; C); 50 (Low; LA); 100 (Medium; MA) or 150 (High; HA) g ALG/cow/d. The ALG contained 13.5 g/kg DM crude protein, 58 g/kg oil, 0.28 g/100 g FA as C20:5n-3 and 25.7 g/100g FA as C22:6n-3. Cows were milked twice daily with yield recorded at each milking and samples taken on four occasions during the sampling week for subsequent analysis of total fat and fatty acids. Individual intake was recorded daily. Faecal samples were collected twice daily for 5 days during the sampling week from 12 cows. Samples were bulked within days for each cow, oven dried at 65°C and ground prior to subsequent analysis for acid insoluble ash as an internal marker. Data were analysed using Genstat (version 16.1).

Results Supplementation with ALG had no effect on dry matter (DM) intake or milk yield, with mean values of 23.1 kg/d, and 38.5 kg/d respectively, but milk fat content decreased linearly with increasing inclusion rate (Table 1). Whole tract digestibility of DM and organic matter also decreased linearly with increasing dietary inclusion of ALG. Supplementation with ALG reduced the content of saturated FAs from 68.7 g/100g in C to 66.7 g/100g in HA. The milk fat content of polyunsaturated FA (PUFA) and conjugated linoleic acids (CLA) increased linearly ($P < 0.001$) with the addition of ALG in the diet. Milk fat content of DHA was increased by 0.29 g/100g in HA compared with C.

Table 1 Intake, milk yield, diet digestibility and selected fatty acids in cows when fed diets supplemented with different inclusion levels of algae

	Treatment				s.e.d	P value
	C	LA	MA	HA		Lin
DM intake, kg/d	23.7	23.3	23.1	23.3	0.323	0.162
Milk yield, kg/d	38.1	38.8	38.6	38.4	0.305	0.770
Milk fat, g/kg	39.6	38.4	37.1	35.9	1.105	<.001
Digestibility, kg/kg						
Dry matter	0.750	0.710	0.720	0.700	0.018	0.015
Organic matter	0.764	0.726	0.733	0.714	0.017	0.015
Milk fatty acids, g/100g						
C18:0	9.70	9.60	8.58	8.73	0.239	<.0001
C18:2 cis-9 trans-11 CLA	0.61	0.76	0.86	0.90	0.031	<.0001
C18:2 trans-10 cis-12 CLA	0.03	0.03	0.04	0.05	0.005	<.0001
C22:6n-3 (DHA)	0.08	0.15	0.25	0.37	0.017	<0.001
Total SFA	68.7	68.0	67.0	66.7	0.435	<0.001
Total PUFA	4.48	4.79	5.21	5.43	0.084	<0.001

Conclusions Microalgae can be added to the diet of high yielding dairy cows to improve the milk content of DHA although digestibility and milk fat content are reduced when more than 100 g/cow/day is fed.

Acknowledgements The authors gratefully acknowledge Alltech for funding this study.

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Effects of dry period length and concentrate protein content in late lactation on body condition score change and subsequent lactation performance of thin dairy cows

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Application Offering a low protein concentrate to thin cows in late lactation did not improve their body condition score either before or after calving. Adopting an extended dry period improved cow body condition score at calving, but had no longer term performance benefits.

Introduction On many farms, a proportion of cows end lactation with a low body condition score (BCS). Low BCS cows are at increased risk of health and fertility problems following calving, and at increased risk of being culled. There is anecdotal evidence that offering a low protein/high starch concentrate in late lactation may help cows gain body condition. In addition, extending the dry period has been advocated as a strategy by which to improve the BCS of thin cows. The aim of this study was to examine the effects of offering thin cows a low protein/high starch concentrate in late lactation, or of adopting an extended dry period (EDP), on BCS change and performance during late lactation, the dry period, and the subsequent lactation.

Material and methods Sixty-five low BCS cows (mean BCS 2.25 at 14 wks pre-calving) were managed on one of three treatments between 13 and 9 wks pre-partum: NP (grass silage + 5 kg/d of a normal protein concentrate [228 g CP/kg DM]), LP (grass silage + 5 kg/d of a low protein concentrate [153 g CP/kg DM]), or EDP (cows dried off at 13 wks pre-calving and offered a grass silage only diet). Both NP and LP cows were dried off 8 wks pre-partum. All cows were offered a grass silage only diet (plus a dry cow mineral/vitamin mix during the final 4 wks pre-partum) until calving. After calving, all cows were offered a common diet (supplying 11.1 kg concentrate DM/cow per d) for 19 wks.

Results Between 13 and 9 wks pre-partum, LP cows had lower DM intake (DMI), milk yield and body weight than NP cows. However, BCS at wk 9 pre-partum did not differ between treatments. The LP cows continued to have lower DMI and BW than NP and EDP cows between 8 wks pre-partum and calving, but only EDP cows had a higher BCS at calving. Treatment did not affect calving difficulty score or calf birth weight. Although all cows were offered a common diet postpartum, LP cows had lower DMI and milk fat + plus protein yield than cows on any other treatment during the 19-wk period postpartum, but there were no differences in any postpartum indicator of body tissue reserves. The treatments imposed from wk 13 to wk 9 pre-partum did not affect any of the measured fertility or health parameters postpartum.

Table 1 Effects of concentrate protein level in late lactation and of dry period length on the performance of low BCS cows from 13 wks pre-calving until 20 wks post-calving

	Late lactation treatment			s.e.d.	P-value
	Normal protein	Low protein	Extended dry period		
From wk 13 to wk 8 pre-calving					
Silage DMI (kg/day)	10.9 ^b	9.8 ^a	10.8 ^b	0.37	0.004
Total DMI (kg/day)	15.4 ^c	14.3 ^b	10.8 ^a	0.37	<0.001
Milk yield (kg/day)	12.6	11.4	-	0.63	0.050
Milk fat + protein yield (kg/cow per d)	0.97	0.83	-	0.055	0.014
BCS at wk 9 pre-partum	2.33	2.30	2.41	0.054	0.103
From wk 8 pre-calving to calving					
Silage DMI (kg/day)	10.8 ^b	9.5 ^a	10.6 ^b	0.33	<0.001
BCS during the final week pre-partum	2.44 ^a	2.42 ^a	2.60 ^b	0.061	0.006
From calving to wk 20 post-calving					
Total DMI (kg/day)	11.2 ^b	10.8 ^a	11.3 ^b	0.19	0.009
Total DMI (kg/day)	22.9 ^b	21.8 ^a	23.1 ^b	0.47	0.010
Milk yield (kg/day)	37.8 ^{ab}	35.6 ^a	38.4 ^b	1.14	0.040
Milk fat + protein (kg/day)	2.78 ^b	2.55 ^a	2.75 ^b	0.08	0.009
BCS at end of experimental period	2.44	2.45	2.44	0.064	0.984

^{a,b,c} means with different superscripts within rows differ ($P < 0.05$)

Conclusions Extending the dry period for thin cows improved their BCS at calving but did not allow the cows to achieve the target BCS of 2.75 at calving. Also, there were no beneficial effects of extending the dry period on the postpartum performance of these cows. Offering a lower protein diet to thin cows in late lactation did not improve BCS at calving above that of thin cows on a normal protein diet but there were unexplained long term negative effects on cow performance.

Acknowledgements This work was co-funded by DAERA (through the Research Challenge Fund), and by AgriSearch.

Relationship between feed efficiency, milk yield, rumination rate and faecal glucocorticoids in dairy cows

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Application By understanding more about an animal's response to dietary changes, we can better understand influences on feed efficiency within a dairy herd.

Introduction Feed efficiency in dairy cows is likely to be improved by minimising stress; stress can be assessed through faecal glucocorticoid concentration (FGC). Faecal glucocorticoids are metabolites of cortisol, providing a non-invasive alternative to blood cortisol. One potential stressor is a change in diet composition, which may affect rumen function and performance. In a previous study FGC increased in response to mild stress (blood sampling) and FGC increased over 4 successive diet changes (Tennant *et al.*, 2016). The objective of the current study was to further investigate effects of diet change on FGC and rumen function, with diets varying in either forage or concentrate composition.

Material and methods Two trials were conducted with cows fed on partial-mixed rations (PMR) ad libitum, plus concentrate during milking. In Trial 1 (n=39 cows), forage components of the PMR were changed in 4 periods; in the first 3 periods batches of grass silage were changed; in the fourth period wheat straw replaced molasses in the PMR. In Trial 2, (n=43 cows) four sources of protein (soya, rapeseed meal, heat-treated rapeseed and distillers grains) were included in iso-nutrient PMRs during 4 periods of 4 weeks in a 4x4 Latin square design. Cows were housed in a freestall barn, milked (mean 3.2 times daily) in a milking robot (A3, Lely, St Neots), and fed individually through electronic feeders (RIC, Fullwood, Ellsmere). Rumination time, milk yield, dry matter intake (DMI), faecal acid insoluble ash (AIA) and methane emissions were recorded for each individual cow. Feed conversion efficiency (FCE) was calculated as milk yield per kg DMI. An ELISA method was used to measure concentration of the FGC metabolite 11-Oxoetiocholanolon (Mostle *et al.*, 2002). Faecal samples were collected from each cow prior to diet change via natural defecation, and at 12hr post diet change via rectal grab sampling (rectal grab not confounding as no significant increase in FGC output post rectal grab sample (p=0.89)). Data were analysed using the Genstat REML procedure with Period and Diet (Trial 2) as fixed effects, and cow as a random effect.

Results In Trial 1, each silage change increased FGC transiently in the first 3 periods (P<0.010), but there was no period effect on mean FGC (P=0.16) (Table 1). In the fourth period, however, when wheat straw replaced molasses in the TMR, mean FGC increased (P=0.012) after diet change. In Trial 2, protein source and diet change did not affect rumination time, milk yield, DMI, AIA or methane emissions. Protein source did not affect FGC and, in contrast to our previous study in which carbohydrate sources were changed, mean FGC did not increase with successive diet changes across periods (Table 1). In both trials there was a significant correlation between FGC and FCE (Trial 1: P=0.004; Trial 2: P=0.016).

Table 1 Effects of diet change on indicators of rumen function in four successive treatment periods (Trial 1 and Trial 2)

Trial 1	Period				σ	P
	1	2	3	4		
Glucocorticoids (ng/g)	129	154	189	238	41.1	0.16
Rumination (min/d)	339	438	433	436	43.1	0.99
Methane (g/d)	399	328	359	360	15.2	0.38
Milk Yield (kg/d)	43	43	39	38	2.5	0.35
Trial 2	1	2	3	4	σ	P
Glucocorticoids (ng/g)	307	286	529	340	111.2	0.29
Rumination (min/d)	426	421	407	420	8.1	0.71
Methane (g/d)	473	457	463	462	15.4	0.49
Milk yield (kg/d)	38.8	36.1	33.9	29	3.8	0.74

Results of this study confirm that diet changes cause mild stress responses in dairy cows. Increases in FGC induced by diet change were lower than increases induced by procedures such as blood sampling, so are not considered to indicate welfare concerns. Changes in carbohydrate source induce greater increases in FGC than changes in forage or protein source, and effects of varying carbohydrates may be cumulative. Higher FGC following dietary changes were not associated with changes in rumen function. Levels of FGC were consistently correlated with FCE across all trials.

Acknowledgements-This study was funded by AHDB Dairy.

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Evaluation of factors associated with passive immune status, colostrum quality, morbidity and mortality in dairy calves

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Application Passive immune status, colostrum quality and morbidity during calthood (0-4 months) are associated with an increased likelihood of mortality during the same period. Management decisions made during calf rearing are associated with increased morbidity during the first four months of life.

Introduction Dairy calf mortality and morbidity represent a major economic cost to the dairy industry in terms of animal value, loss of genetic potential and the cost of rearing the animal to point of death. Therefore dairy heifer loss is a major area of concern. Decisions made during calf rearing can greatly influence colostrum quality, a calf's passive immune status and morbidity, which in turn can influence mortality (Windeyer *et al* 2014). This study aimed to investigate calf and management factors associated with passive immune status, colostrum quality, morbidity and mortality during the first four months of life on UK grassland based commercial dairy systems.

Material and methods Sixteen commercial dairy farms, geographically spread across Northern Ireland, participated in this study from January 2014 to September 2016. The number of calves born on these farms during 2014 ranged from 76 to 424 (N=1068 calves). Farms that demonstrated excellent record keeping and showed a high level of commitment to an earlier phase of this study were selected to take part in this phase. Farmers were required to collect a colostrum sample from approximately 20 cows as soon as possible after calving. AFBI staff then collected blood samples from the corresponding calves (on average 3.83 days post birth). Colostrum and blood samples were subsequently analysed for their IgG concentration. Data collection forms were filled in by farmers for each animal born. Date of birth, breed and name of sire, and dam freeze brand number were recorded. Farmers were asked to record all deaths, sales and illnesses throughout the study period (birth to first calving). For illness, the type of illness, date of treatment and method of treatment were recorded. All illnesses were self-recorded by the farmer. Date of death was also recorded. Any vaccinations, worm/fluke treatment and dehorning of calves were also recorded. Service dates for all heifers were recorded indicating AI or stock bull, name of sire, breed of sire and date of service. Lastly, first calving information was recorded as and when heifers calved, date of first calving, calving difficulty score and BCS at calving were recorded by the farmer. A questionnaire concerning the management decisions associated with calf rearing was given to each of the 16 farmers to complete. The questionnaire consisted of 55 questions, 25 closed questions and 30 open questions. To allow for the generation of odds ratios during statistical analysis all questionnaire variables were converted to categorical variables. This was simple for closed questions, which were already categorical. For open questions, answers were converted into categories based on the range of answers given by the 15 participating farms. The number of variables generated for each of the 6 sections was as follows: (1) calving pens, 12 variables, (2) calf housing, 7 variables, (3) weaning targets, 6 variables, (4) calf rearing regime, 11 variables, (5) calf feeding equipment, 5 variables and finally (6) concentrate and roughage offered, 4 variables. A total of 45 categorical variables on the management of calf rearing were used for statistical analysis. Statistical analysis was univariate and used a mixture of LMM and GLMM, with farm ID as a random factor in all models.

Results At the calf-level, likelihood of failure of passive immunity transfer (FPT) (IgG <10 mg/ml) decreased by 0.38 (OR) when calves received colostrum of adequate quality (IgG conc \geq 50 mg/ml) ($p=0.02$). A calf with FPT was 2.86 times more likely to die between 0-4 months of age ($p=0.08$). A calf that required treatment for ill-health during the first 4 months of life was 0.55 times less likely to die during the same period ($p=0.01$). If a calf required treatment for pneumonia during the first four months of life it was 0.22 times less likely to die ($p=0.01$). If a calf required treatment for scour during the first four months of life it was 2.15 times more likely to die ($p=0.01$). At the farm-level, using an automatic feeder for longer decreased the likelihood calves were treated for pneumonia by 0.02 times ($p=0.07$). If individual instead of group calf pens were used, calves were 6.43 times more likely to be treated for scour ($p=0.03$). Finally, when calving pens were cleaned out between calving seasons, calves were 0.03 times less likely to be treated for illness ($p=0.05$).

Conclusion These results highlight the importance of further work examining how to reduce FPT and morbidity in the dairy calf. These results also highlight the important calf rearing management decisions that can affect calthood morbidity.

Acknowledgements Wish to thank DAERA for funding this project.

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Immune-related transcriptional response to colostrum feeding in neonatal Holstein dairy calves during the first week of life

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Application This study highlights some of the molecular mechanisms regulating the immune response in neonatal dairy calves. Differentially expressed genes identified are prospective contender genes for the classification of biomarkers for immune-competence development, which may contribute to improved health in dairy calves

Introduction Neonatal dairy calves possess a very immature and naïve immune system compared with adult bovine. The new-born calf lacks immunity and is reliant on the intake of maternal colostrum to protect it from infection and disease (Chase *et al.*, 2008). Therefore, the objective of this study was to examine changes in gene expression and investigate molecular pathways and biological functions involved in the immunocompetence development of neonatal Holstein dairy calves, at two specific time-periods during the first week of life. The hypothesis was that the expression of immune related transcripts would be differentially altered between the two time periods in response to colostrum ingestion

Material and methods Whole blood samples were collected from Holstein dairy calves ($n = 8$) via jugular venepuncture into tempus tubes for subsequent RNA isolation. Blood samples were harvested at 0, 48, 72 and 168 hours post-birth. mRNA was isolated from the whole blood, cDNA libraries prepared from mRNA and subsequently sequenced with single-end reads. Quality analysis of resultant sequence reads was performed using FASTQC. Reads were then aligned to the bovine genome using the aligner software STAR. Mapped reads were subsequently counted using featureCounts and differentially expressed genes (DEGs) identified using EdgeR. DEGs are defined as having a Benjamini Hochberg P value of < 0.05 . Pathway and functional analysis were performed using KEGG and IPA.

Results There were 848 and 903 significantly DEGs identified between 0-48 and 72-168 hour time-periods post-birth, respectively. Immune-related pathways identified ($P < 0.05$) from DEGs at 0-48 hours post-birth included cytokine-cytokine receptor interaction, complement and coagulation cascades, p53 signalling pathway, JAK-STAT signalling pathway, intestinal immune network for IgA production, RIG-I like receptor signalling pathway and primary immunodeficiency. Whereas during 72-168 hours post-birth the following pathways were identified as significantly enriched: cytokine-cytokine receptor interaction, complement and coagulation cascades, p53 signalling pathway, and intestinal immune network for IgA production. The three main enriched pathways at both time-periods were cytokine-cytokine receptor interaction, complement and coagulation cascades, and intestinal immune network for IgA production. Further details of these pathways are described in Table 1.

Table 1 Immune-related pathways significantly enriched across both time-periods examined

Pathway	Over-represented P Value	Number of DEGs	Total number of genes in pathway
<u>0-48 hours post-birth</u>			
Cytokine-cytokine receptor interaction	1.69E-10	35	108
Complement and coagulation cascades	5.31E-09	17	34
Intestinal immune network for IgA production	0.011451506	8	31
<u>72-168 hours post-birth</u>			
Cytokine-cytokine receptor interaction	1.45E-07	31	110
Complement and coagulation cascades	0.000564	11	34
Intestinal immune network for IgA production	0.018076	8	33

Conclusion These data provide an improved understanding of the molecular control of the early development of the neonatal immune system of dairy calves, highlighting some of the molecular mechanisms regulating the immune response in neonatal dairy calves. In particular the cytokine-cytokine receptor interaction pathway, the complement and coagulation cascades as well as intestinal immune network for IgA production were most affected in whole blood of neonatal calves up to the first week of life. DEGs recognized are prospective contender genes for the classification of biomarkers for immune-competence development, which may contribute to improved health in dairy calves.

Acknowledgements The authors gratefully acknowledge Department of Agriculture, Food and the Marine (DAFM) Ireland, research stimulus funding (RSF) (Project 11/S/131).

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The effect of birth delivery method on gene expression in the lungs and jejunum of neonatal beef calves

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Application Delivery by elective caesarean section is postulated to negatively affect neonatal health at birth. This study highlights alteration to expression of key genes involved in overall vitality, successful rapid fluid clearance from lungs, and immunoglobulin absorption in jejunal tissue of elective caesarean compared to trans-vaginally delivered post-natal calves.

Introduction Caesarean section (CS) is one of the most common surgeries performed by veterinarians in cattle, and is considered a routine obstetric technique (Kolkman *et al.*, 2010). However, elective CS before the onset of labour is thought to be associated with loss of ability to rapidly transition at birth, manifested as slower and less effective lung fluid clearance, and a weakened ability to absorb immunoglobulin, critical to the viability of the agammaglobulinemic neonatal calf. There is little evidence to support this however, and the objective here was to investigate the hypothesis that the expression of genes critical to adaptation at birth will be altered in the lungs and jejunum of calves delivered by elective CS

Material and methods Oestrous synchronised Aberdeen Angus heifers (n = 21) were artificially inseminated with frozen thawed semen from a single Aberdeen Angus bull. One week prior to the predicted calving date, heifers were randomly assigned to one of two treatment groups; (i) CS – calves to be delivered via elective caesarean section (n = 10); (ii) TV – calves to be delivered trans-vaginally following normal labour (n = 11). To facilitate the sampling schedule, heifers assigned to the TV group were induced to calve by administration of 2 ml of a prostaglandin F2 α analogue (Estrumate®, Merck Animal Health), 72 hours prior to the expected calving date. Caesarean sections were carried out by an experienced veterinary surgeon using standard protocols. Calves were euthanized within 5mins of birth by jugular vein administration of pentobarbital sodium (Dolethal®, Vetoquinol). Following evisceration, lung and proximal jejunal samples were harvested within 10mins, washed with PBS, and immediately snap-frozen. RNA was extracted using the Qiagen RNeasy plus universal mini kit (Qiagen, UK), and cDNA was prepared using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time quantitative PCR was carried out using the ABI 7500 Fast real-Time PCR System with SYBR green Master Mix (Applied Biosystems, Warrington, UK). Twenty-nine primers were employed to analyse alterations to crucial pathways including immunity, fluid clearance and subsequent absorption. Efficiency of qPCR was calculated for each gene by creating a standard curve from serial dilutions of cDNA. The software package GenEx 5.2.1. 3 (MultiD Analyses AB, Gothenburg, Sweden) was used for efficiency correction of the raw Ct values, normalisation to the 5 selected reference genes, and calculation of quantities relative to the average Ct value for each gene. Data were checked for normality using the UNIVARIATE procedure of Statistical Analysis Software (SAS, version 9.3). Where appropriate, data were transformed by raising co-efficients to the appropriate power of λ using the TransReg procedure. Data were subsequently analysed using mixed model methodology within the MIXED procedure of SAS. The Tukey critical difference test was performed to determine the existence of differences (P < 0.05) between treatment mean values.

Results A total of 5 genes from the lung tissue and 3 genes from the jejunal tissue were identified as differentially expressed (P < 0.05) between TV and CS delivered calves

Tissue	Symbol	Gene annotation	Fold Change ¹	P Value ²
Lung	LAP	Lingual antimicrobial peptide	-2.06	0.0075
	SCN11A	Sodium channel alpha subunit 11	-2.73	0.0002
	SCN11B	Sodium channel beta subunit 11	-1.72	0.0051
	MUC5AC	Mucin 5 subtype AC	2.55	0.0021
	CYP1A1	Cytochrome p450 Family 1, subfamily A, member 1	-3.95	0.0016
Jejunum	IL-6	Interleukin 6	1.23	0.0018
	IL1- β	Interleukin 1 beta	1.92	0.0013
	TNF α	Tumour necrosis factor	1.92	0.001

¹ Fold change represents observed reduction or increase in gene in CS delivered calves

² P values were corrected (bonferroni correction)

Conclusion The lower expression of the sodium channel subunits in the lung in ECS delivered calves provide putative evidence for impaired ability for clearance of lung fluid. Additionally, the increased expression of the pro-inflammatory cytokines in jejunal tissue could lead to altered ability for immunoglobulin absorption in ECS delivered calves.

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Calfhood morbidity affects growth, fertility, first lactation and lifetime performance in dairy heifers

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Application A dairy heifer calf's morbidity during the first 56 days of life was associated with decreased growth, fertility, first lactation yields and decreased lifetime performance.

Introduction Rearing dairy heifers accounts for a considerable proportion of the costs of a dairy system (Bach *et al* 2008). Any delay to a heifer entering the milking herd will increase these costs and increase a heifer's non-productive period (Place *et al* 1998). This study aimed to investigate associations between calfhood morbidity, growth, fertility, first lactation and lifetime performance. The findings of this study will highlight how illness as a calf may have long, even lifelong, impacts on a dairy heifer.

Material and methods This study made use of an existing database of all heifer calves born between spring 2003 and spring 2015 on the AFBI Hillsborough farm (N=1881). Data was available on nutrition, growth, morbidity, fertility, first lactation and lifetime performance. Morbidity variables were examined from 0-56 days old. The morbidity variables examined throughout this study were; 24 hour ZST score, times treated for pneumonia, scour and dullness and the total times treated for ill-health (combination of pneumonia, scour and dullness). This data was used to examine potential associations between (1) calfhood morbidity and growth, (2) calfhood morbidity, fertility and first calving, (3) calfhood morbidity and first lactation and (4) calfhood morbidity and lifetime performance. The above associations were investigated using either LMM or GLMM. The type of analysis used was dependant on the nature of the predictor and response variable and any experimental treatment the heifer calves went through on-farm was included as a random factor in all models. All analysis was univariate.

Results (1) *The effect of calfhood morbidity on growth.* A calf's weaning weight, weaning age, and live weights at 8 weeks, 6 months, 1 year and 1.5 years were all significantly affected by calfhood morbidity. In all cases weaning weight and live weight decreased with increasing morbidity and weaning age increased with increasing morbidity. For example, for every one unit increase in the number of times a calf was treated for ill-health, their live weight at 1 year decreased by 2.79kg. (2) *The effect of calfhood morbidity on fertility and first calving.* Age at first service, age at successful service, BCS at calving and calving weight were all negatively affected by calfhood morbidity. For example, for every one unit increase in the number of times a calf was treated for ill-health, their calving weight decreased by 2.98kg. However, age at first calving was not significantly associated with morbidity. (3) *The effect of calfhood morbidity on first lactation.* First lactation milk, fat and protein yields and 305 day milk, fat and protein yields were all negatively affected by calfhood morbidity. For example, for every one unit increase in the number of times a calf was treated for ill-health, 305 day milk yield decreased by 3.09kg. (4) *The effect of calfhood morbidity on lifetime performance.* Finally, age at death, days in milk and lifetime milk, fat and protein yields were all negatively affected by calfhood morbidity. For example, for every one unit increase in the number of times a calf was treated for ill-health, their total time in milk decreased by 20.03 days.

Conclusion We found that a calf's morbidity during the first 56 days of life was associated with decreased growth, fertility, first lactation yields and decreased lifetime performance. This study therefore highlights the potential lifelong impacts calfhood morbidity can have on the dairy heifer.

Acknowledgements We wish to thank DEARA and AgriSearch for funding this project.

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Quantifying pre-weaning calf growth response to cow milk production in pasture-based beef suckler systems: A meta-analysis

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Application Milk yield (MY) was highly predictive of pre-weaning calf average daily live weight gain (ADG). Calf ADG response to an additional kg of milk was higher for beef crossbred compared to dairy crossbred suckler cows.

Introduction In temperate climates, grazed pasture is generally the principal forage fed to suckler beef cows and calves. In these production systems, calf weaning weight (WW) is a key factor influencing profitability. It is well documented that beef suckler cows with higher milk production produce heavier calves at weaning and that the performance obtained is influenced by cow genotype (McGee *et al.*, 2005; Minchin and McGee, 2011). In the context of beef cattle breeding programs and bio-economic modelling of suckler beef production systems (Crosson and McGee, 2012), it is necessary to predict calf growth response to MY from beef suckler cows with differing genotypes. Therefore, the objective of this study was to conduct a meta-analysis to quantify pre-weaning calf ADG responses to cow MY in pasture-based production systems and to discern how cow genotypes influenced this response.

Material and methods A dataset comprised of 138 treatments from 32 published European pasture-based experiments was collected from the literature. Included studies needed to conduct research on single-suckled grazing beef suckler cows and their calves. The dataset included information on cow MY (kg/d), and birth weight (kg), ADG (g), weaning age (d) and WW (kg) of calves. Cow breed types were classified into four categories: early-maturing beef breeds (EM-Beef), late-maturing beef breeds (LM-Beef), EM dairy crossbred (EM-Dairybeef; EM beef x dairy) and LM dairy crossbred (LM-Dairybeef; LM beef x dairy). A linear mixed model regression analysis that contained fixed effects for parameters of interest and a random effect of study was used to evaluate relationships. The models were derived using lme4 and lmerTest packages (Kuznetsova *et al.*, 2013) of the statistical software R version 3.1.2 (R Core Team, 2014). The general form of the mixed effects model was: $y = X\beta + Z\gamma + \epsilon$, where y represents a vector of observed data, β is an unknown vector of fixed effects parameters with known design matrix X , γ is an unknown vector of random effects parameters with known design matrix Z and ϵ is an unknown random error vector. Models of MY and ADG response of calves were developed and compared across different cow genotypes. Relationships were considered statistically significant when $P < 0.05$ and considered a tendency towards statistical significance when $P < 0.10$.

Results Dairy crossbred cows produced more milk than beef crossbred cows; breed maturity had no effect ($P > 0.05$) on MY within the beef crossbreds, whereas MY of LM-Dairybeef was higher ($P < 0.05$) than EM-Dairybeef (Table 1). Calf ADG from LM-Dairybeef was 113 g higher and LM-Beef (despite similar MY) was 73 g higher than their EM counterparts. Within the LM genotypes, calves from dairy crossbreds had higher ADG than beef crossbreds, whereas within the EM genotypes this difference was not evident ($P > 0.05$), despite a 1.04 kg/d difference in MY. Calf WW followed the same pattern as ADG. The calf ADG response associated with a 1 kg increase in cow MY was highest for the 'low yield' EM-beef (54 g) followed by LM-Beef cows, and lowest for the 'high yield' dairybeef crossbreds, which did not differ ($P > 0.05$).

Table 1 Milk yield, pre-weaning calf ADG and calf WW response to milk yield in pasture-based beef suckler systems

Parameters	Unit	EM-Beef	LM-Beef	EM-Dairybeef	LM-Dairybeef
Milk yield	kg/d	7.31 ^a	7.32 ^a	8.35 ^b	9.43 ^c
Calf ADG	g	968 ^a	1041 ^b	992 ^a	1105 ^c
Calf WW adjusted at 210 d	kg	247 ^a	263 ^b	248 ^a	274 ^c
Calf ADG response/kg MY	g	54 ^a	47 ^b	40 ^c	38 ^c

ADG= Average daily gain; WW=Weaning weight; d = days; EM= Early-maturing; LM=Late-maturing; ^{a, b, c} = means within a row without a common superscript differ significantly ($P < 0.05$).

Conclusion Pre-weaning ADG response to MY was greater for calves from beef crossbred than dairy crossbred cows.

Acknowledgements The authors gratefully acknowledge financial support from the Teagasc Walsh Fellowship Programme.

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Evaluation of feeding elevated levels of milk replacer (750 v 900g) on the performance and health of artificially reared beef calves to 12 weeks

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Application It is standard commercial practice to feed 500-600g/day calf milk replacer (CMR) to artificially reared beef calves. Feeding elevated levels of up to 900g/day will significantly improve performance and reduce feed cost per kg gain.

Introduction In the UK, it is common practice to use dairy-bred and beef cross dairy bred calves for beef production. Around 52% of UK beef originates from the dairy industry. Calf development and growth should be carefully managed early on to ensure that performance is optimised with artificially reared calves. CMR is a common liquid calf feed and the typical feeding levels on commercial beef farms are 500-600g/day to weaning at 6-9 weeks old. There is increasing interest in feeding elevated levels of CMR, especially to dairy heifers with positive benefits on growth and lifetime performance (Khan *et al.*, 2011). The objective of this experiment was to investigate the effect of feeding high (750g/day CMR) and very high (900g/day CMR) levels of CMR on the performance and health of dairy bull calves to twelve weeks.

Material and methods Forty British Blue x Holstein (n=34) and Holstein (n=6) bull calves with a mean age of 15.4 days were artificially reared and assigned in a randomised block designed experiment to one of the following treatments: 750, calves received 750g (air-dry weight)/day CMR ('Wynngold Bloom', Wynnstay Group Plc) in warm (39°C) water (150g CMR/L) fed via buckets at 5 litres per day in two equal feeds; 900, calves fed 900g (air-dry weight)/day CMR in warm (39°C) water (180g CMR/L) at 5 litres per day in two equal feeds. The calves were individually penned and offered ad lib straw, water and concentrates (Start n' Wean, Wynnstay Group Plc) and weaned at 42 days. The calves were moved into group pens at weaning. The CMR and concentrates were analysed to contain 171 and 41g/kg oil and 248 and 183g/kg CP respectively. The data were analysed by ANOVA with calves blocked according to weight and breed.

Results The calves fed 900g/day of CMR recorded significantly higher ($P < 0.01$) DLWGs from start to weaning and gained an extra 5.6kg in weight from start to 12 weeks.

Table 1 Effect of CMR feed rate on liveweight (kg)

	750	900	s.e.d	Sig
Start weight	53.6	51.5	2.35	NS
Weaning weight	84.2	89.7	3.65	NS
12 week weight	135.0	138.5	5.26	NS

Table 2 Effect of CMR feed rate on DLWG (kg)

	750	900	s.e.d	Sig
Start - 3 weeks	0.61	0.78	0.063	*
Start - weaning	0.73	0.91	0.056	**
Weaning - 12 weeks	1.21	1.16	0.092	NS

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

	750	900	s.e.d	Sig
Conc intake (start - weaning)	19.4	17.7	2.09	NS
Milk replacer	28.7	33.5		
FCR start - wean (kg feed: kg gain)	1.57	1.34	0.316	NS

The percentage of calving requiring medical treatments for morbidity was significantly higher for the calves fed 750g/d of CMR (30%) compared to the calves fed 900g/d CMR (10%) ($P = 0.038$). No significant difference was found between treatments for any calf health scores (dehydration, cough, nasal and eye discharge, ear, faecal and coat bloom), however coat bloom and faecal scores improved over time ($P = 0.001$). The results for the cost of feed and morbidity per calf to weaning were £50.74 and £54.10 for the 750 and 900 treatments. The cost per kg liveweight gain were £1.66 and £1.42 respectively based on the feed and veterinary costs prevailing at the time of the study (October 2015).

Conclusion The calves reared on both 750g and 900g/day of CMR exceeded the recognised growth targets for purchased bucket reared calves at 15 weeks of 119-122kg. The calves fed 900g/day of CMR recorded significantly higher DLWGs from start to weaning and by 12 weeks the 900g CMR fed calves had gained an extra 5.6kg in weight. The calves fed 750g/day of CMR having recorded lower DLWGs to weaning did not exhibit compensatory growth.

Acknowledgement Financial support from Wynnstay Group Plc and Trouw Nutrition GB is gratefully acknowledged.

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Comparing greenhouse gas emissions, fibre digestibilities, energy and nitrogen utilisation of pre-weaned calves from forage treatments, reared on an accelerated or conventional milk replacer regimen

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Application Starter concentrate intake of dairy calves is vital during the transition from a liquid based diet to a solid based diet. Little information exists regarding GHG emissions from heifer replacements during early developmental stages.

Introduction A plethora of information exists regarding enteric greenhouse gas (GHG) emissions from dairy cow production; however there are limited research studies which investigate GHG emissions from early growth stages from birth to maturity of dairy heifer replacements. Conflicting conclusions have been drawn regarding the benefits of accelerated milk rearing regimen specifically due to latent rumen development associated with lower solid feed intake. To our knowledge, no study has investigated the effects of milk replacer level, forage type and age at introduction on enteric GHG emissions combined with fibre digestibilities and nutrient utilisation.

Material and methods At birth, (n= 29) dairy heifer calves were allocated to one of 8 dietary treatments balanced for birth weight and breed. Conventional (C) milk replacer (MR) treatment groups were chopped straw introduced at 14d of age (C1), chopped straw introduced at 56d of age (C2), grass silage at 56d of age (C3) or no forage offered until weaning (Control A). Accelerated (A) MR regimen treatment groups were chopped straw introduced at 14d of age (A1), chopped straw introduced at 56d of age (A2), grass silage at 56d of age (A3) or no forage offered until weaning (Control B). Conventional milk feeding regime will be fed 4L/day (150gMR/L) until day 67, then stepped down to 2L/day until weaning at the end of the study. Accelerated milk feeding (9L/day @ 150gMR/L) until 42d; then stepped down in three steps (6L/day, 3L/day and 2/day respectively). Animals from all treatment groups were weaned at 70d old. At 84d post-birth animals were housed in digestibility crates to enable total collection of faeces and urine for a 4d period. 10% of total faeces and urine from total collection were bulked then analysed for nitrogen (N) and gross energy (GE) content. Faeces samples were tested for oven DM and subsequent analysis of ash, GE, ADF, NDF and lipid. Animals were then transferred to chamber facilities for a period of 72h with gaseous exchange of O₂, CO₂ and CH₄ and heat production recorded for the final 48h. All calves received 2L of MR each morning both in digestibility crates and calorimeter chambers. Concentrate, forage and water were offered *ad libitum* with daily intakes recorded. DM of forage, concentrate and MR were taken regularly along with DM of daily feed refusal to determine DM intake. Grass silage was analysed for GE, N, pH, ammonia-N, VFAs, lactic acid and alcohol. Daily oven DM samples were retained for analysis of ash, ADF, NDF, lipid and WSC. Chopped straw was analysed for GE, ash, N, GE, ADF, NDF and lipid. MR was analysed for GE, N, lipid and minerals and concentrate analysed for ash, energy, N, ADF, NDF, starch and lipid. Live weight measurements were taken fortnightly from birth until entering digestibility crates. Further live weight measurements were taken upon entering digestibility crate, entering calorimeter chamber and leaving the study to determine average daily gain (ADG). Data were analysed using REML variance components analysis.

Results No differences were found in digestibilities, gaseous exchange or heat production amongst treatment groups neither did pre-weaning milk replacer level have an effect on DM intakes. However, the provision of any forage type did influence total DMI from concentrate and forage when compared to groups fed no forage.

	CS14	CS56	GS56	Control	SED	P-value
Conc DMI (kg/d)	2.643	2.743	2.617	2.137	0.0188	0.021
Forage DMI (kg/d)	0.081	0.092	0.018	0	0.0374	<.001
Total DMI (kg/d)	3.009	3.127	3.088	2.431	0.0186	0.003
DM digestibility (%)	79.9	81.3	79.6	81.8	1.25	0.255
CH ₄ (g/d)	109.4	98.1	99.9	77.6	19.45	0.451

Conclusion Increased DMI of animals provided with a forage source indicated rumen functioning can be encouraged in animals fed a high level of MR. Consequently, offering forage may provide a solution to maintain ADG throughout the transition from a diet based on a high level of milk to a solid based diet. Amount of forage offered to a calf and method of presentation merit further investigation to understand if limit feeding forage sources can affect gaseous exchange and digestibilities.

Acknowledgements The authors gratefully acknowledge funding from DAERANI

The effect of calf jacket usage on skin surface temperature of pre-wean dairy origin calves

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Application The use of a calf jacket increased average skin surface temperature of pre-wean calves when housed in accommodation with an average ambient temperature below the lower limit of the thermo neutral zone for calves under 4 weeks of age.

Introduction In calves up to 4 weeks of age, the thermo neutral zone, that being the temperature range to which the calf can become acclimatised, is between 10 and 25°C. If ambient temperature falls below this calves must expend energy in order to maintain core body temperature, which could impact on animal health and performance. The aim of the present study was to use data loggers in order to examine if calf jackets produce a significant increase in skin surface temperature when average ambient temperature falls below 10°C.

Material and methods At 3 days of age, eighteen Holstein calves were allocated to either be fitted with a calf jacket for the first 3 weeks of life (C: n=9) or to remain without a calf jacket (NC: n=9). Calves were housed in one of six replicate straw bedded, concrete walled (6 m x 6 m) pens containing no more than 15 calves. Over a 24 hour period for each of the 18 calves, temperature data loggers (Thermochron iButton, model DS1921H-F5#, range 15.0–46.0°C, accuracy: $\pm 1^\circ\text{C}$, Maxim Integrated, CA, USA) were affixed to calves to continuously measure the skin surface temperature. The temperature logger was attached approximately 2cm to the right hand side of the spine at the level of the last rib. In order to attach the data logger, a methodology based on that described by Sutherland *et al* (2013) was employed. A small area of hair ($\sim 2\text{cm}^2$) was clipped to skin level, the data logger was then placed on the area of skin and covered with a 5 cm² square of breathable, elastic adhesive bandage (Elastoplast[®], Beiersdorf UK, Birmingham, UK). The outer edges of the bandage were further secured to the unclipped hair using glue (KAMAR[®], Kamar Products, Inc., Zionsville, IN, USA). Care was taken to avoid using the glue on the clipped area in order to minimise any skin problems when removing the bandage. The data loggers were set to record temperatures every 10 minutes during the 24 h period. A data logger (Thermochron iButton, model DS1921G-F5#, range -30 to +70°C, accuracy $\pm 1^\circ\text{C}$, Maxim Integrated, CA, USA) was affixed to the outer wall of the group pen in order and was set to record temperature every 10 minutes over the same 24 h period in which skin temperature was recorded for each of the 18 calves. Data were analysed using each measurement of skin temperature as a repeated measure in a REML variance components analysis with Sex, Treatment, Time of recording and the interaction of Treatment and Time of recording as fixed effects. Ambient temperature was included as a covariate.

Results When housed in an ambient temperature of 7.7°C (S.D. $\pm 3.82^\circ\text{C}$), calves wearing jackets (C) exhibited a mean skin temperature of 35.2°C compared to 29.1°C in calves not wearing jackets (NC) when measured over a 24h period ($P < 0.001$). There was no effect of sex on average skin surface temperature, nor were there any significant interactions between time of day and skin surface temperature.

Conclusion The usage of a calf jacket significantly increases skin surface temperature in pre wean calves when housed in accommodation with an average ambient temperature below that of the lower limit of the thermo neutral zone (10°C). Further investigation into the use of calf jackets in temperatures outside the thermo neutral zone, such as potential beneficial effects on calf performance and health, are merited.

Acknowledgements The authors gratefully acknowledge the staff from the Sustainable Livestock Systems Unit, AFBI Hillsborough.

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An investigation into the preferred disbudding procedure of beef and dairy farmers in the South West of England, how many farmers follow DEFRA's three codes of recommendations?

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Application The main aim of this study is to investigate how many UK farmers adhere to DEFRA's three codes of recommendations and identify any associations between enterprise and procedure.

Introduction Calf disbudding has become common practice on dairy and beef farms to avoid injury to stockmen and other cattle (Braz *et al*, 2012; Earley *et al*, 2011). Disbudding destroys the horn producing cells (corium) of the horn bud to prevent further growth and can be done using a variety of methods including hot iron (Broom *et al*, 2012) and chemical paste (Passille *et al*, 2014). Dockes *et al* (2015) discovered farmers were unsure of the regulations and recommendations for disbudding, DEFRA has published legislation and recommendations for this procedure. The three recommendations for the procedure are: disbudding should take place when the calf is under 2 months old, chemical cauterisation should be avoided if possible, a hot iron is preferred, and the stockman completing the procedure should have some formal training.

Material and methods A questionnaire, which was sent to farmers within the South West of England, was designed containing 11 closed and 1 open, quantitative questions, no longer than 12 words each (Marshall, 2005). They contained questions relating to farm size, number of calves and number of employees. Previous studies have shown that the size of the herd can influence the decision to disbud (Dockes *et al*, 2015). Three questions related directly to DEFRA's 3 recommendations which included the age of the calves at disbudding, the method used and the training of the stockman. The question regarding training was asked with an open format as no specific formal training was recommended by DEFRA. Two further questions were asked regarding the handling method and post procedure treatment, these were the final two areas of procedure not covered by DEFRA's recommendations. A total of 112 completed questionnaires were received. Data were tested for normality using the Shapiro-Wilk test rather than Smirnov-Kilmogrov test as there were under 1000 participants. Spearman's correlation test was then used to identify associations between variables as the data were non-parametric.

Results results show 89% of farmers use the hot iron method of disbudding, 11% used chemical cauterisation. This agrees with previous literature including Brscic *et al* (2015) who found both beef and dairy enterprises favoured the hot iron method. A correlation ($r=0.78$, $P < 0.001$) was found between age of calves and method of disbudding the hot iron method is used more frequently in older calves. The age of the calves also had an association ($r=0.68$, $P < 0.001$) with enterprise, units having a dairy enterprise, typically disbudded calves at an older age than beef only units. An association ($r=0.64$, $P < 0.001$) between enterprise and method used showed that dairy farms use hot iron disbudding more often than beef units which tend to use chemical cauterisation. There was an association ($r=0.71$, $P < 0.001$) between method used and post procedure treatment of calves, indicating that the use of the hot iron method led to an increase in post procedure treatment.

Conclusion This study provides an estimate of the percentage of UK farmers from the South West of England who follow DEFRA's 3 codes of recommendation. The hot iron method is used by 89% of the population and chemical paste is used by 11% of the population. Beef farmers preferred the chemical paste method, which in turn led to a lower average age at disbudding on these farms, compared to dairy farmers who preferred the hot iron method. Post procedural treatment for pain and infection was used significantly more following hot iron disbudding and by association, more frequently on dairy farms. The post procedure treatments employed included the use of non-steroidal anti-inflammatory drugs (i.e. Metacam[®]) and antibiotic spray, consistent with the perceived more invasive and painful nature of the hot iron compared with chemical paste disbudding procedure

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Evaluation of nitrogen utilisation efficiency in hill ewe lambs with two types of forage

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Application Compared with grass silage grass nuts increased nitrogen (N) intake, faecal and urine N outputs and N retention but reduced the ratio of urine N/faecal N.

Introduction Nitrogen excretion from sheep production systems is an important source of nitrate leaching and nitrous oxide emissions through nitrification and denitrification processes, responsible for groundwater pollution and global warming. The present study aimed to investigate how different forage types and animal genotypes affected N utilisation in hill ewe lambs.

Material and methods Thirty six hill ewe lambs (18 pure Scottish Blackface vs. 18 Texel × Scottish Blackface (50:50)) aged approximately 10 months and weighing 37 ± 5.3 kg were allocated to 2 forage treatments balanced for genotype and live weight. Each genotype was offered 2 forages (grass nuts vs. grass silage) *ad libitum*. Grass nuts were pelleted ryegrass sourced from a commercial supplier (Drygrass South Western Ltd, Burrington, UK). Grass silage was made from the 2nd harvest of perennial ryegrass. The animals were individually housed and offered experimental diets for 14 d before being transferred to individual metabolism crates for further 4 d with N intake, and N outputs in faeces and urine measured. Grass nuts and grass silage contained (g/kg DM): ash 67 and 82; CP 164 and 101; NDF 590 and 597; and GE 19.0 and 19.1 (MJ/kg DM), respectively. Data were analysed in a 2 × 2 factorial arrangement using ANOVA with a probability level of $P = 0.05$ for significance in differences.

Results The effects of forage types and genotypes on N utilisation are presented in Table 1. There were no significant interactions between forage types and genotypes on any variable of N utilisation. Lambs offered grass nuts had greater N intake, faecal N and urine N outputs and N retention ($P < 0.001$) than those given grass silage. However, the ratios of urine N/N intake, urine N/manure N (manure N = faecal N + urine N), urine N/faecal N and manure N/N intake were lower ($P < 0.001$) for lambs offered grass nuts than grass silage, except that forages had no significant effect on faecal N as a proportion of N intake. There were no significant differences between the 2 genotypes of hill ewe lambs on any variable of N utilisation.

Table 1 Effects of forage types and genotypes on N utilisation in hill ewe lambs (n = 36)

	Forage		s.e.	P	Genotype			
	Grass Nuts	Grass Silage			BF	T × BF	s.e.	P
Nitrogen intake (g/d)	26.7	7.2	1.15	<0.001	18.2	15.7	1.46	0.232
Faecal nitrogen (g/d)	12.2	3.2	0.77	<0.001	7.9	7.6	0.97	0.813
Urine nitrogen (g/d)	8.5	4.2	0.40	<0.001	6.1	6.5	0.51	0.555
Manure nitrogen (g/d)	20.7	7.4	1.01	<0.001	14.0	14.1	1.27	0.956
Nitrogen retention (g/d)	6.0	-0.2	0.72	<0.001	4.2	1.6	0.91	0.051
Faecal N/N intake	0.45	0.44	0.024	0.833	0.46	0.43	0.030	0.413
Urine N/N intake	0.33	0.62	0.030	<0.001	0.44	0.51	0.038	0.245
Urine N/Manure N	0.42	0.58	0.020	<0.001	0.47	0.53	0.025	0.081
Urine N/Faecal N	0.78	1.51	0.103	<0.001	0.97	1.32	0.131	0.066
Manure N/N intake	0.78	1.06	0.036	<0.001	0.91	0.94	0.045	0.656

Conclusion Hill ewe lambs offered a grass-nuts diet had higher N intake, N outputs and N retention but a lower ratio of urine N: faecal N when compared to those given a grass silage diet. No significant differences were found in any variable of N utilisation between Scottish Blackface and Texel × Scottish Blackface ewe lambs.

Acknowledgements This work was funded by DEFRA, the Scottish Government, DAERA, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

Effect of nutritional restriction on the response of ewes to vitamin E supplementation during late pregnancy and early lactation

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Application Nutritional restriction, but not vitamin E level influences lamb birthweight. Vitamin E requirements may be higher than current estimates.

Introduction The productivity of a sheep enterprise depends on lamb output. It is estimated that in the UK over 4 million lambs die each year which represents a major cost to the sheep industry. Lamb birth weight and neonatal lamb survival may be enhanced by supplementation of ewes with a high level of vitamin E during late pregnancy. However, these responses are inconsistent. Vitamin E is a fat soluble vitamin that can be stored in adipose tissue, muscle and liver. In addition, vitamin E is assimilated into adipose tissues more avidly, but is depleted at a similar rate to muscle. Consequently, vitamin E availability from tissue stores may be an important factor in explaining the observed responses to additional vitamin E supplementation. The objective of the current experiment is to investigate the effect of nutritional status on the response of ewes to vitamin E.

Materials and methods At day 77 of pregnancy 48 twin-pregnant ewes (Suffolk x Mule) in live weight (LW, 82.89 kg) and condition score (CS, 3.32) were allocated to one of two groups, and fed to achieve a mean CS of either < 2.5 (LC) or > 3.0 (HC) prior to housing. At day 103 (week -6) ewes were individually allocated to one of two concentrates containing either a low vitamin E (L-VE) (50 mg/kg DM) or high vitamin E (H-VE) (500 mg/kg DM) and offered straw ad-libitum. Both concentrates were formulated to supply the same quantities of metabolizable energy (ME), fermentable ME (FME), effective rumen degradable protein (ERDP) and crude protein (CP). Concentrates were fed to meet the nutrient requirements of twin bearing ewes producing 3.0 litres of milk during early lactation (AFRC, 1993). Ewe LW, CS and litter weights at 12 after lambing were recorded. Blood samples were obtained by venepuncture from six representative ewes on each treatment at weeks -6 and -1 pre-partum and weeks +2 post-partum for vitamin E analysis. Ewes were group housed between weeks +4 and +8 post-lambing and the performance was monitored within each treatment. The experiment was analysed by ANOVA as a 2 x 2 factorial design.

Results There was no significant interaction between nutritional restriction and vitamin E supplementation. Unrestricted ewes had a higher CS at week -6 and lost more CS pre-partum. Ewe CS has no effect on post-partum CS loss, but unrestricted ewes had a higher LW loss. They also had a higher litter weight. Level of vitamin E had no effect on ewe or lamb performance, but ewes fed H-VE had higher plasma and colostrum levels (Table 1).

Table 1 Effect of nutritional restriction and vitamin E supplementation on ewe and lamb performance.

	LC		HC		SED	Probability		
	L-VE	H-VE	L-VE	H-VE		CS	Vit. E	Int.
Week-6 CS	2.60	2.54	2.98	2.98	0.065	<0.001	NS	NS
Pre-p. CS change	-0.01	-0.04	-0.21	-0.18	0.101	0.025	NS	NS
Post-p CS change	-0.56	-0.38	-0.32	-0.19	0.219	NS	NS	NS
Pre-p. LW change (kg)	15.84	18.39	15.67	15.13	1.262	0.065	NS	NS
Post-p LW change (kg)	-7.59	-5.93	-9.99	-8.79	1.618	0.029	NS	NS
Vitamin E (µg/ml)								
Colostrum	5.41	16.62	6.46	16.91	1.624	NS	<0.001	NS
Ewes plasma wk. -6	0.94	0.91	1.09	1.23	0.118	0.013	NS	NS
Ewes plasma wk. -1	0.84	2.10	0.86	2.02	0.211	NS	<0.001	NS
Ewes plasma wk.+2	0.68	1.92	0.74	1.52	0.572	NS	<0.001	NS
Litter 12 h weights (kg)	9.78	9.50	11.00	10.30	0.432	0.002	NS	NS

Conclusion Litter weight was reduced by nutritional restriction in mid pregnancy, but there was no benefit from additional vitamin E supplementation. The higher level of vitamin E was required to maintain ewe plasma levels of >2.0 µg/ml recommended by NRC (2007).

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Sensitivity of ewe β -hydroxy-butyrate to blood sample type, by-pass protein nutrition and parasitism

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Application By-pass protein supplementation during late pregnancy and experimental parasite exposure increased ewe blood β -hydroxy-butyrate levels, indicating increased energy requirements. Some effects were more pronounced in serum than in plasma, highlighting that where possible serum samples are preferred, although plasma may be a suitable alternative.

Introduction Ewe metabolizable energy (ME) requirements rapidly rise during late pregnancy (AFRC, 1993). When these ME requirements are not met through dietary intake, ewes mobilise body reserves in an attempt to minimise the resulting negative energy balance. When the rate of such lipid mobilisation is too high, then gluconeogenesis is curtailed in the liver, resulting in hepatic lipidosis and elevated levels of blood β -hydroxy-butyrate (BOHB). Thus, monitoring the latter may assist late pregnant ewe management in terms of energy nutrition. Parasitism may increase ME requirements in response to pathophysiological tissue damage. By-pass protein supplementation (digestible undegradable protein, DUP) may increase ME requirements to make optimally use of the extra amino acids absorbed. We hypothesized therefore that at times of restricted feeding, ewe parasitism and DUP supplementation increase blood BOHB. The latter is traditionally analysed in serum though at times when also plasma collection is required, it would be useful to avoid the need for multiple blood sampling. Here, we additionally hypothesize that serum and plasma do not differ in detecting intervention effects on BOHB levels.

Material and methods A total of 48 twin-bearing Dorset-mated Dorset crosses, with a mean body weight of 59.6 ± 0.8 kg and condition score of 3.14 ± 0.05 at day₋₄₈ (day₀ is parturition), were housed individually and dewormed. Then, half of the ewes were dosed with 10,000 *Teladorsagia circumcincta* larvae every Mon-Wed-Fri (Par) and the others with water (Sham), whilst feeding *ad libitum* hay and 100 g/day commercial concentrates (n=24). From day₋₂₁ until day₁₂ (turn out), ewes were fed at 0.9 times ME requirements (AFRC, 1993) a 30:70 hay:concentrate ration. A 1:1 mixture of xylose-treated rapeseed meal (RaPass[®]) and soya bean meal (SoyPass[®]) was used to increase DUP levels from 25 (LP) to 52 (HP) g/kg (n=24). Thus, each diet-parasitism combination had 12 replicates. Blood samples were taken on day -12 for collection of plasma and serum, and both were analysed for BOHB. Data were analysed using 2×2 split-plot ANOVA, with pen identification as main plot and sample type as sub-plot.

Results Table 1 shows that levels of BOHB were sensitive to sample type, ewe protein nutrition and parasite exposure. BOHB levels were greater in serum samples than in plasma samples (0.62 vs 0.60 U/l; s.e.d. 0.006; P=0.023), were greater in HP ewes than in LP ewes (0.66 vs 0.55 U/l; s.e.d. 0.048; P=0.027) and were greater in Par ewes than in Sham ewes (0.68 vs 0.53 U/l; s.e.d. 0.048; P=0.002). Ewe protein nutrition and parasite exposure did not significantly interact for blood BOHB. However, sample type and ewe parasite exposure did interact (P=0.004); the effect of ewe parasitism was more pronounced in serum samples (0.70 vs 0.53 U/l; s.e.d. 0.059 U/l) than in plasma samples (0.67 vs 0.53 U/l; s.e.d. 0.059 U/l).

Table 1 Effect of sample type, protein nutrition and parasite exposure on 12 days pre-lambing BOHB levels (mmol/l)

Diet (D)	Parasitism (P)	Sample type (S)		Split-plot ANOVA		
		Serum	Plasma	Components		P-values
LP	Sham	0.48	0.48	Main-plot	Diet	0.027
	Par	0.65	0.61		Parasitism	0.002
HP	Sham	0.58	0.58		Split-plot	D×P
	Par	0.76	0.73	Sample		0.023
				S×D		0.738
				S×P		0.004
				S×D×P		0.318
s.e.d. S×D×P		0.069				

Conclusion This data indicate that parasitism and DUP supplementation elevated BOHB levels at times of scarce dietary ME supply, and that BOHB was more sensitive than condition score, which in this work was not affected by both interventions (Houdijk *et al.*, 2016). The basis for elevated BOHB is unclear, as an abundance of glucogenic energy (through metabolism of glucogenic amino acids) was expected from the additional DUP. Although BOHB levels were below the 1.2 mmol/l used to indicate elevated pregnancy toxemia risk, serum samples had greater discrimination power to detect intervention effects. Thus, where possible, serum is the preferred blood sample type for BOHB analysis.

Acknowledgements We thank many SRUC technicians for their help, and SAC-ASD for the BOHB analysis. This research was funded by AHDB Beef and Lamb. SRUC receives financial support from Scottish Government (RESAS).

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Persistence of *Dichelobacter nodosus*, the causal agent of ovine footrot

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Application Ovine footrot is the main cause of lameness in sheep in the UK. Information on sites where the causative agent of footrot *Dichelobacter nodosus* persists may lead to changes in sheep management and disease control.

Introduction Ovine footrot (FR) is an economically important disease that causes lameness and affects sheep flocks worldwide. It is characterized by interdigital skin inflammation (interdigital dermatitis [ID]) with, or without, separation of the hoof horn from the underlying tissue (severe footrot [SFR]). The primary causative agent is the gram-negative anaerobic bacterium *Dichelobacter nodosus*, which is thought to exclusively colonize the hoof and is transmitted indirectly via contaminated surfaces. Periods of apparent zero prevalence of FR in a flock are followed by disease occurrence when climatic conditions become favourable for pathogen transmission. This suggests that there are sites where *D. nodosus* persists. These sites might include the feet, gingival cavity and faeces of sheep and the environment. The aim of this study was to investigate persistence of *D. nodosus*, by investigating possible sites of survival, using a longitudinal study design.

Materials and methods Study 1: From a flock of approximately 150 animals, 5 North Country Mules and 5 lambs (North-country mule x Texel) were selected for the study. All animals and their pasture were examined and sampled (including swabs from feet and the gingival cavity, as well as soil, grass and faecal samples) fortnightly from June to August for a period of 8 weeks. Study 2: A flock of 120 Suffolk x Wiltshire-horn ewe lambs was screened for FR and 40 animals without FR lesions were selected randomly and moved to a study pasture that had been unoccupied for 10 days. The study group and pasture were examined and sampled weekly for five months from January to May. For both studies, sheep were turned and scored for ID and SFR lesions using a 4-point scale (Moore, *et al.*, 2005). Swabs were taken from the interdigital skin of the foot (study 1: n=40, study 2: n=160) and from the gingival cavity (study 1: n=10, study 2: n=40) at every visit. Faecal samples (n=10) were collected from the pasture (study 1 only). For study 2 faecal samples (n=40) were taken directly from the animal. Soil samples (n=22) were collected weekly and grass samples were taken when present at these sites (n=6). Local climate data (ambient temperature [°C] and rainfall [mm]) was collected. All samples from study 1 were analysed resulting in a total of 342 samples. For study 2, DNA was extracted from 7 sheep (5 sheep with footrot lesions and 2 control sheep with no disease), and all samples collected from the pasture; a total of 898 samples. Real-time PCR used to detect and quantify *D. nodosus*. Chi square tests using R software were used for the preliminary statistical analysis.

Results Flock disease prevalence was higher in study 1 than in study 2. The climate during study 1 was warm and initially wet, but became progressively drier and the climate during study 2 was cold and dry. *D. nodosus* was detected in all sample types in both studies but not on all occasions (Table 1). Detection levels differed significantly between the two studies in feet, gingival cavity, soil, and grass samples ($P < 0.01$). The proportion of positive foot swabs was greater than expected by chance ($P < 0.01$) in both studies. The proportion of positive faecal samples (study 1) and grass samples (study 2) was lower than expected by chance ($P < 0.05$).

Table 1 Summary of samples with detectable *D. nodosus* collected from sheep and the farm environment in study 1 and 2

Sample	Study 1		Study 2	
	Number positive	Positive %	Number positive	Positive %
Foot	97/152	63.8	37/172	21.5
Mouth	13/38	32.5	1/43	2.3
Faeces	10/40	25.0	2/43	4.7
Soil	36/88	41.9	19/462	4.1
Grass	10/24	41.7	4/178	2.2

Conclusions For the first time we show that *D. nodosus* can be shed in ovine faeces. The differences in detection between studies are likely to be attributable to differences in disease status of the study groups and climatic conditions. *D. nodosus* was detected in all sample types in both studies but was less prevalent in non-foot sites, especially when the weather was dry, suggesting that feet are the more likely place of persistence. A binomial mixed regression model will be used to further elucidate variables temporally associated with the presence of *D. nodosus* in feet, in the gingival cavity and on the pasture.

Acknowledgements Kat Giebel is funded by a Natural Environment Research Council (NERC) studentship

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Optimising use of labour at lambing time on outdoor sheep farms

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Application Despite lambing being the most labour-intensive part of the sheep year, data for outdoor farms are scarce; estimating labour times and variations within tasks and farms is essential for identifying best practice at lambing.

Introduction On outdoor sheep systems with over 1000 ewes, the biggest expense is labour and the most intense use of it is at lambing. EBLEX figures (2014) show similar trends for similar flocks; labour accounts for around 35% of all costs, with a variation of over 38% of actual cost between the top third and the average producers. At an average labour cost of £30/ewe, and by estimating that a quarter of it spent at lambing time, this represents a significant cost for farmers, especially on larger flocks. It is therefore crucial to obtain a proper estimation of time spent at lambing, and understanding which tasks are involved, to see if there is scope for reducing or at least optimizing labour use. The aim of this study was to investigate the use of labour at lambing time on outdoor sheep farms by recording labour on farms which use best practice techniques, and by identifying the specific tasks at lambing when labour is most in demand.

Material and methods The research was conducted on three outdoor sheep case study farms at lambing time (March-May 2015). Farm 1 had 4050 ewes, farm 2, 855 ewes and farm 3, 1100 ewes. As well as variations in the organisation of lambing time, the three farms also had different periods of lambing (Farm 1 lambed the earliest at the end of March, and Farm 3 lambed the latest (May), representing the variation in outdoor sheep farms activities. For each of the farms, labour has been recorded at two different periods: mid-lambing and end of lambing, to cover the variation in the numbers of lambs being born during the whole lambing period. For each sample period, three different assignments have been considered: i) lambing fields (checking ewes lambing), ii) lambed ewes fields (caring for lambed ewes), iii) other tasks associated with lambing, such as castration. Each of the assignments had been recorded separately, to get a full range of activities and tasks carried out by farmers during lambing. The recordings were carried out using video technology (fitted Go-Pro Hero 3 cameras). The labour tasks from the video recordings were subsequently classified in 15 main tasks (adapted from Kirwan, 2009), each of which with different subtasks. Distance walked/driven and locations were also recorded during the sample days on each farm, using a mobile phone application (www.endomondo.com). Although this application is designed originally for recording physical activity and performance for running or cycling, it was used to gauge the areas and distance covered, in addition to the timed activities. It allowed both spatial and temporal recordings of the lambing activities.

Results Results were compiled by assignment (lambing fields, lambed ewes fields, other tasks), as well as overall. Variations amongst farms for the assignments and activities were also estimated. Overall, for the lambing fields, the main identified tasks were driving (30%) and opening/shutting gates (21%), followed by the tasks related to the ewes and the lambs (18% and 15% respectively). The tasks undertaken with the lambed ewes were however more varied and numerous. A bigger proportion was spent driving (40%) and walking (11%), and less time was spent with the animals (11% with the ewes and 11% with the lambs), compared with the lambing fields. The 'other tasks' (anything not related to the lambing or lambed ewe fields) showed less time was spent driving (29%) and more time was allocated to the lambs (25%) and the ewes (20%). Most of the tasks undertaken with the lambs were to do with feeding the lambs (bottle or tube), tail, castrate, tag and spray mark the animals. There were variations between the three case study farms, due partly to their respective fields distance from the main steading, their flock sizes and their approach to managing ewes and lambs. Distances travelled also reflected this variation over the sampling days, with Farm 1 averaging 25 miles/day, over 5.9 hours/day. Farm 2 averaged 18 miles per day over 9 hours of recording, whilst farm 3 averaged 30 miles/day, over 10 hours.

Conclusion This study showed that driving and opening/closing gates were the most time-demanding tasks at lambing, most often due to the locations of the various fields. Whilst all farms in the study had different settings, they all carried out similar tasks. All three farms used best practices, however, the order and organisation of getting the tasks done also varied. This study was an opportunity for farmers to identify good examples of how to reduce some of the tasks (e.g. mobile pens; timing of recording), and ways to reduce non-animal tasks (e.g. opening gates). Quantifying and visualising lambing tasks via videos and phone app proved useful and should help inform on best practice and advice for farmers by farmers.

Acknowledgements

AHDB (Farm Improvement Grant scheme) and the farmers who took part in this exercise are gratefully acknowledged.

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Response to cobalt supplementation in lambs post weaning

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Application In the context of this study, there was no consistent effect of cobalt supplementation on lamb growth and worm status. Supplementation strategies should be defined at a flock specific level and targeted at deficient animals to maximise their efficiency.

Introduction The ability of lambs to utilize grass efficiently is dependent on their health, nutritional status, and grass feed value. In particular, infection by gastrointestinal parasites is one of the main factors affecting lambs performance and welfare. Trace elements such as selenium and cobalt play an important role within their immune system and are thus likely to influence their ability to tolerate parasite infections. Supplementation with cobalt can increase lamb performance post weaning (Keady *et al.*, 2015). The aim of this study was to assess whether supplementing lambs with cobalt from weaning had an effect on both their growth and worm status.

Material and methods The study was undertaken at 5 commercial lowland sheep farms in Northern Ireland as well as the AFBI research flock. At each farm, 40 twin lambs were selected at weaning (20 female and 20 males or castrates) and included a range of genotypes (Highlander x, Lleyn x, texel x and Belclare x). All lambs were given selenium injection. At each farm, a group of 20 lambs (balanced for gender and live weight) also received a cobalt drench orally (130 mg cobalt sulphate) every four weeks. The cobalt status of the lambs, together with their faecal egg count (FEC) and live weight, were measured at weaning and on at least 2 occasions at monthly intervals thereafter. At each farm, grass samples were obtained from four fields grazed by the study lambs in May, July and September 2014 and analysed for dietary minerals. Data were analysed using mixed models with farm, treatment, breed type, gender as fixed effects and ewe identity as random effect.

Results Cobalt levels in the grass grazed by the lambs were often below recommended levels (0.1 mg/kg DM for Cobalt, see Peers and Philips 2011) with important variability between farms, within farms and throughout the season. Response to supplementation was not consistent across farms, and there were no overall effects on the concentration of blood vitamin B12, daily live weight gain and worm status (Table 1). Several other worm species were monitored, however worm counts were often too low to be able to detect a difference between the two groups of lambs (supplemented vs not supplemented). Concentrations of vitamin B12 in lambs were very variable within and between farms (Figure 1), with 36% of non supplemented lambs with concentrations below 188 pmol/l.

Table 1 Effects of cobalt supplementation on lamb growth and worm status (farms 3 to 6 in Figure 1)

	Treatment		sed	Sig.
	Control	Cobalt		
Live weight gain (g/d)				
Weaning to weaning + 1mth	171	164	12.0	NS
Weaning to weaning + 2mth	144	147	9.6	NS
Worm faecal egg count (strongyle epg)				
At weaning + 1mth	343	348	59.9	NS
At weaning + 2mth	352	275	56.2	NS

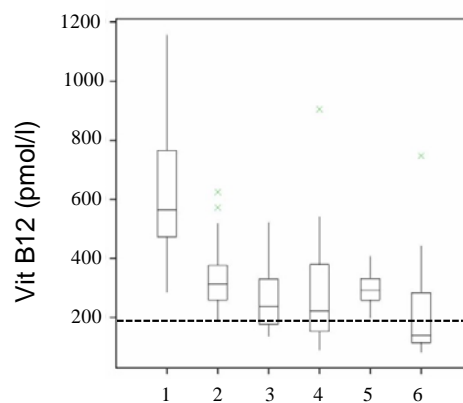


Figure 1 Blood vitamin B12 in all study lambs at weaning+1 month (dotted line represents the minimum recommended concentration)

Conclusion Despite low cobalt levels in grazed grass, no consistent effect of cobalt supplementation was found across the study farms. This was mostly due to low worm burdens and to the important variability in the concentrations of blood vitamin B12 among lambs, indicating that some lambs were not sufficiently deficient in cobalt to respond to supplementation.

Acknowledgements This work was funded by DAERA and AgriSearch.

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Prediction of carcass weight from live weight in cull ewes

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Application There is a linear relationship between cull ewe live weight and carcass weight. Each 1 kg increase in ewe live weight increases carcass weight by 0.53 kg.

Introduction Based on data from the National Farm Survey it is estimated that the mean replacement rate on lowland sheep farms in Ireland is 22%. Thus, approximately 550,000 ewe replacements join the national flock annually. Keady (2014) reported a mean annual mortality of 4.7% for 2 to 6 year old ewes. Consequently about 80% of ewes that exit the flock annually are available for slaughter, equivalent to approximately 450,000 ewes. At the time of culling producers have the option to sell immediately or to retain the ewes on their holdings with the intention of increasing carcass weight, and thus cull value. The purpose of the current study was to evaluate the utility of pre-slaughter live weight and body condition of ewes as predictors of carcass weight.

Material and methods Live weight and carcass weight were available for 473 ewes culled during 3 seasons from the research flocks at Athenry. The ewe genotypes represented purebreds (Belclare, Texel and Suffolk) and crossbreds which were either Scottish Blackface crosses or Suffolk crosses (mainly ≥ 0.75 Suffolk). The ewes were between 2 and 6 years of age at slaughter and had been managed in grass based systems of mid-season lamb production during their productive life, which involved being housed during mid and late pregnancy and turned out to pasture post lambing in March. Ewes were culled from the flocks in September. On the day prior to slaughter, at an EU approved abattoir, ewes were weighed and condition scored (2 seasons only). Carcass weight (cold) was recorded after the carcass had been dressed to meet commercial requirements. Regression analysis, using a model with season and ewe genotype, was used to examine the relationship of carcass weight with pre-slaughter live weight and condition score.

Results Live weights ranged from 41.7 to 118.5 (mean 75.8, s.d. 10.4) kg, condition score ranged from 2.0 to 4.75 (mean 3.5, s.d. 0.49), carcass weight ranged from 14.7 to 57.0 (mean 32.6, s.d. 5.71) kg and kill-out proportion ranged from 323 to 539 (mean 429, s.d. 35.7) g/kg. Regression of carcass weight on live weight (LW) yielded the following linear relationship:

$$\text{Carcass weight (kg)} = -7.53 + 0.53\text{LW} \quad R^2 = 0.84 \text{ ***}$$

(s.e. 0.828) (s.e. 0.011)

The relationship between condition score and carcass weight yielded an $R^2 = 0.52$ (the regression coefficient was 9.03 kg per unit change in BCS); thus live weight was a better predictor of carcass weight than condition score. Adding body condition score to the model with LW had a significant ($P < 0.001$) but minor impact on precision, changing the R^2 from 0.84 to 0.87. Each 1 unit increase in ewe condition score at slaughter was associated with an increase in carcass weight of 2.8 kg at a given live weight.

Ewe genotype was a significant source of variation in the intercept for all models, but not for the regression coefficient. The differences among ewe genotypes largely reflected a significantly greater than average kill-out proportion for Texel and Scottish Blackface-cross animals, and a significantly lower than average kill-out for Suffolk and Suffolk-cross genotypes.

For validation purposes, the dataset was randomly divided, within season, into two sub-groups of 309 and 166 cases. The larger sub-group was used as the calibration data set; the other sub-group was used for validation. The correlation, within genotype, between predicted and actual carcass weight for the validation dataset was 0.92; the overall bias was 0.18 kg and the square root of the mean squared prediction error was 2.55 kg. The bias varied significantly among breeds, from -1.8 (s.e. 0.48) for Texel to 1.69 (s.e. 0.64) kg for Suffolk cross ewes. Prediction based on a model that included BCS and weight yielded a slightly lower correlation between actual and predicted carcass weight (0.89), much greater average bias (-1.85 kg) and a larger prediction error (3.18 kg) than prediction based solely on live weight.

When the prediction equation based on LW was applied to a set of data for purebred Scottish Blackface ewes (average LW 49.1 kg) the correlation between actual and predicted carcass weights was 0.85, the square root of the mean squared prediction error was 2.90 kg while the bias was -2.6 (s.e. 0.16) kg.

Conclusion While the carcass weight of cull ewes, of lowland and hill breed types, can be predicted with satisfactory precision from its relationship with live weight information on breed type is required in order to minimize bias.

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Effect of separation age on weight gain of artificially reared Friesland lambs

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Application Early separation age from the ewe has no implication on weaning weight on artificially reared Friesland lambs. Lamb weights within the first week of life show weight fluctuations which could implicate future productivity.

Introduction In the dairy sheep industry lambs are separated soon after birth following colostrum ingestion. Lambs are then artificially reared to increase the amount of ewe's milk that can be utilized for dairy products and to increase flock value (Sevi, *et al.*, 2003; Hernández-Castellano, *et al.*, 2015). As growth rate directly influences lamb profitability, good management is the least cost method of increasing farm production values (Demiroren, *et al.*, 1995). As different weaning ages impact the lambs so that production is compromised, consideration should be taken when to remove lambs for artificially rearing at a young age (Napolitano, *et al.*, 2008). This study aims to examine effects of removing lambs at two different ages using growth rate as an indication of potential production value.

Material and methods 100 Friesland lambs were allocated a trial group post-partum. 50 lambs were removed from the ewe at 24 hours after birth and assigned group 1. 50 lambs remained with their dam until separation at 72 hours old and assigned group 2. Group allocation ensured that siblings remained together and there was no discrimination in litter size. Artificially reared lambs were socially housed indoors and had access to ad lib. milk replacer, GLW lamb creep, water and hay after two weeks. Trial lambs were weighed at 24 hours, 72 hours, one week, two weeks, four weeks and at weaning at seven weeks old using a weigh crush calibrated to 0.2kg. Data were analysed using a parametric, two-way non-paired t-test on R i386 3.3.1. to evaluate the difference in weight gain between group 1 and group 2.

Results The results show that there is a highly significant weight decrease in group 1 compared to group 2 at 72 hours ($t=-3.32$, $P=0.0013$). However, at seven days, it was observed that there was a significant weight increase by group 1 in comparison to group 2 ($t=2.601$, $P=0.011$). Final weights, at weaning (7 weeks), show that there was no significant weight difference between the two groups ($t=0.79456$, $P=0.4289$).

Conclusion Body weight fluctuations at an early age could implicate future lamb health and longevity; implicating the performance of stock replacements. Despite this, at weaning age, the findings show that the age at which lambs are removed from the ewe has no significant effect on weaning weight. Given this finding, the decision when to remove lambs should instead be influenced by factors such as welfare and individual farm management strategies.

Acknowledgements The author gratefully acknowledges Paul and Laura Mardell at Musson Hall Farm and Dr Anne Carter from Nottingham Trent University.

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The genetic basis of aseasational breeding and pseudopregnancy in dairy goats

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Applications The study has shown that there is a genetic basis to aseasational breeding and pseudopregnancy in dairy goats, the magnitude of which is typical for other documented fertility traits in livestock.

Introduction Goats are highly seasonal breeders, presenting a challenge for producing a year-round supply of fresh milk. Pseudopregnancy (PPG) is relatively common in dairy goats. High levels of PPG within a herd causes delays in becoming pregnant, affecting supply of replacement animals and commencement of subsequent lactations. It may be possible to select against PPG and to select for breeding cycles outside of the typical season. However there are no published studies on the genetic basis of these traits in dairy goats. The aims of this study were to estimate genetic variance of two fertility traits: out of season fertility (OOS) and PPG and to calculate genetic correlations with the production traits 520 day milk yield (MY520), lifetime milk yield (MYLife), and lifetime number of days in milk (DIMLife).

Material and methods Lactation records from 9,546 animals from 2 farms between 1987 and 2015 were used. Pseudopregnancy was diagnosed via the use of ultrasound. Cases (n = 904) and controls (n = 8,642) of PPG were assigned based on whether or not a goat experienced PPG during her first lactation. Peak kidding season was defined as the four weeks of the year where the highest average number of kids were born, and the OOS phenotype was defined as the week an animal first kidded, relative to peak kidding season (0 to 24 weeks). Milk yield up to 520 days of lactation (MY520) was calculated for the first lactation, as per the Test Interval Method (ICAR, 2003). Lifetime production traits analysed included the total milk yield (MYLife) and total number of days in milk (DIMLife) for each animal, across its entire productive life. Production records that fell outside 3 SD of the mean were excluded from subsequent analyses. The pedigree contained 231 sires, and 7,201 dams over 14 generations. ASReml (Gilmour *et al.* 2009) was used to estimate variance components for each trait, using the following model: $y = Xb + Za + e$; where y is the vector of phenotypic observations; b the vector of fixed effects, consisting of herd-year-season (HYS) of birth (all traits); age at first kidding (OOS); HYS of kidding (PPG); number of lactations (MYLife and DIMLife); MYLife (DIMLife); DIMLife (MYLife), a is the vector of random animal effects; e is the vector of random residual effects, and X and Z are incidence matrices relating observations to their respective effects. As PPG was measured as a binary trait, a threshold animal model (Gianola & Foulley, 1983) was applied using a logit link function. A series of bivariate analyses were used to calculate correlations between each trait. Fixed and random effects were fitted for each trait based on the univariate analyses as described above.

Results Heritabilities and correlations between production traits and reproductive traits are presented in Table 1.

Table 1 Heritabilities (h^2) for 520d yield (MY520), lifetime yield (MYLife), total lifetime days in milk (DIMLife), pseudopregnancy (PPG), and out of season kidding (OOS), and their genetic (r_G) and phenotypic (r_P) correlations

Trait	h^2	OOS		PPG	
		r_G	r_P	r_G	r_P
MY520 (kg)	0.35 (0.03)	-0.15 (0.09)	0.01 (0.01)	-0.03 (0.11)	0.09 (0.02)
MYLife (kg)	0.20 (0.02)	-0.17 (0.10)	-0.02 (0.01)	-0.09 (0.13)	-0.08 (0.02)
DIMLife (days)	0.12 (0.02)	0.14 (0.11)	0.01 (0.01)	0.58 (0.11)	0.29 (0.01)
PPG	0.11 (0.02)	0.36 (0.15)	0.06 (0.02)	-	-
OOS	0.11 (0.02)	-	-	-	-

Bold font signifies results that significantly differ from zero. Standard errors are presented in parentheses.

Conclusion This is the only study to have estimated h^2 for pseudopregnancy in any livestock species. No studies have looked at the genetic basis of OOS in dairy goats. Although h^2 for the fertility traits are relatively low, they are well within published ranges for other reproductive traits (Bagnickia *et al.*, 2007). The methods presented here suggest that selection for PPG and OOS may be possible, and without adversely affecting production.

Acknowledgements The authors gratefully acknowledge Innovate UK for funding, and cooperation with the participating goat farms.

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Genetic parameters for longevity traits in UK dairy goats

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Application This study has shown that longevity traits in UK dairy goats are under low genetic control and are suitable for use in selection programs.

Introduction Following an increase in the popularity of goats' milk, much work has been done on UK dairy goat breeding programs, with the main focus on milk yield (Mucha *et al.* 2014). More efficient breeding programs are characterised by incorporating a range of traits that will promote optimum improvement. Goats with long productive lives are desirable as they reduce the number of replacement animals required, allow greater selection intensity, and are a reflection of the health of the herd. Understanding the genetic relationship between longevity and production traits will help in the development of a weighted selection index. The objectives were to estimate the heritability (h^2) of six longevity traits and investigate their genetic and phenotypic correlations with production traits.

Material and methods Data was provided on 16,866 female dairy goats from two farm sites. The goats were a composite of 3 breeds (Alpine, Saanen, and Toggenburg) and genetically linked between the two sites. The pedigree file contained records of 33,327 individuals of which 332 were sires and 13,995 were dams. The dataset consisted of milk yield records, birth year (1989 to 2013), farm (2), maximum lactation number (1 to 11) and age at first kidding (300 to 897 days). The three lifetime longevity traits were age at death (AD) calculated as the number of years from birth to death, age alive (AA) calculated as the number of years from birth to death or to current age if still alive, and predicted age (PA) calculated as the number of years from birth to death or if the animal was alive predicted based on the actual age multiplied by the probability of survival. The three production longevity traits were the number of days in milk over lifetime (LDIM), the average lifetime daily milk yield (ADY), and the total lifetime milk yield (LY). Each longevity trait was fitted in the following univariate mixed animal model in ASReML (Gilmour *et al.* 2009): $Y = X\beta + Za + e$ where Y is a column vector of the trait of interest; β is the column vector of fixed effects which included birth year, farm, maximum lactation number, age at first kidding, birth year by farm and maximum lactation by farm interactions; a is the column vector of random animal effects; X and Z are the design matrices for the fixed effects and random effects, respectively; e is the random residual effect. All of the fixed effects were significant ($P < 0.05$) so were included in the model. A bivariate model was then used to calculate correlations between the lifetime and production longevity traits, with fixed and random effects fitted as described above.

Results The mean (s.d.) in years for AD, AA and PA was 4.55(2.23), 4.32(2.23) and 4.58(2.04), respectively. The mean (s.d.) no. LDIM was 896.9(674.9) days, and for ADY it was 2.85(1.04)kg, and LY was 2970(2117)kg. Heritabilities (s.e) for the three lifetime longevity traits AD, AA and PA were the same 0.08(0.01). For DIM, ADY and LY, heritabilities were 0.08(0.01), 0.36(0.02) and 0.14(0.01), respectively. Phenotypic (r_p) and genetic (r_g) correlations between the lifetime and production longevity traits were all positive with moderate to high correlations (Table 1). LDIM and the three lifetime longevity traits were highly correlated showing animals with longer lifespans had longer lifetime days in milk. Correlations between LY and the three lifetime longevity traits were also high indicating that increased longevity has higher lifetime yields. Positive r_p and r_g correlations among AD, AA and PA, with ADY were moderate and demonstrate that animals with longer lifespans additionally had higher daily milk yields. This suggests that selection for increased milk yields is indirectly selecting for increased animal longevity in these two herds. It also reflects that animals are only kept longer in the herds if they have high milk yields.

Table 1 Phenotypic (left) and genetic (right) correlations between lifetime and production longevity traits with s.e.

	Lifetime days in milk (LDIM)	Average daily yield (ADY)	Total lifetime yield (LY)
Age at death (AD)	0.94 (0.001), 0.99 (0.004)	0.13 (0.011), 0.25 (0.079)	0.74 (0.004), 0.60 (0.057)
Age alive (AA)	0.89 (0.002), 0.98 (0.009)	0.19 (0.008), 0.31 (0.065)	0.76 (0.003), 0.70 (0.041)
Predicted age (PA)	0.79 (0.003), 0.96 (0.019)	0.26 (0.008), 0.33 (0.064)	0.74 (0.004), 0.67 (0.045)

Conclusion Heritability estimates for longevity traits indicate that they would respond to selection. Longevity traits were found to be highly correlated with productive longevity traits at both the phenotypic and genetic level. Positive correlations indicate that increasing longevity in dairy goats' results in an increased number of days in milk during the animals' lifetime, a greater lifetime milk yield and an improved average lifetime daily milk yield. These results will allow genetic improvement in dairy goats by incorporating longevity into a breeding index with production, health and fertility traits.

Acknowledgements The authors gratefully acknowledge funding from Innovate UK, and cooperation with the goat farms.

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Genetic aspects of ewe longevity and fertility traits in Lleyn and Dorset sheep

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Application The genetic parameters estimated indicate that ewe longevity is worthy of inclusion in future breeding programmes. The estimates observed with age at first lambing and lambing interval should also be considered due to their antagonistic relationship with longevity.

Introduction Improving ewe longevity, or productive lifespan in sheep, is of high economic importance due to the potential to reduce culling rates and female replacement costs.

Material and methods Data from commercial Dorset and Lleyn flocks, recording as part of the Signet Sheepbreeder Programme, were used for this study. The three traits studied were: Longevity (LONG), defined as the age (in years) at the last recorded lambing event; Age at first lambing (AFL), defined as the age of the ewe (in years) at her first recorded lambing event; Lambing interval (LI), defined as the number of days between the ewe's first and second lambing event. Ewes born between 1980 and 2000 were used for the LONG analyses, to allow them to achieve their full lifetime in the flock prior to data analysis, whereas data from 1980-2011 were used for AFL and LI. The number of data records per trait, per breed, for LONG, AFL and LI were 14,047, 20,816 and 14,648 for Dorsets (n=60 flocks) and 13,652, 30,597 and 18,391 for Lleyms (n=129 flocks) respectively. The pedigree files used contained sire and dam information for 236,333 and 366,509 Dorset and Lleyn animals respectively.

Genetic parameters were estimated using univariate analyses in ASReml (Gilmour et al. 2009), using the following model:

$$y = Xb + Za + e$$

where y is the vector of phenotypic observations; b the vector of fixed effects, consisting of ewe birth year, flock, ewe age at first lambing, total litter size born and the total number of lambing events (LONG); year of ewe's first lambing, litter size of the ewe when she was born and flock x birth year (AFL); year of ewe's first lambing, the ewe's previous litter size born and flock x birth year (LI); a is the vector of random animal effects; e is the vector of random residual effects, and X and Z are incidence matrices relating observations to their respective effects. Season effects were also fitted for the Dorset analyses due to their ability to lamb all year round.

Results Both breeds had an average (s.d. in parenthesis) LONG value of 3.7 years (1.6). For AFL and LI these were 2.0 years (0.4) and 365 days (100.1) for Dorsets and 2.1 years (0.3) and 390 days (94.5) for Lleyms respectively. Heritability estimates for each trait ranged from 0.05 (Lleyn LONG) to 0.20 (Dorset AFL). The genetic and phenotypic correlations between each trait, for each breed, are shown in Table 1. The highest and lowest genetic correlations, for both breeds, were observed between LONG and AFL and between AFL and LI respectively. Phenotypic correlations ranged from -0.12 to 0.54.

Table 1 Heritabilities (diagonal), genetic and phenotypic correlations (upper and lower diagonal) for each breed

Breed	Trait	LONG	AFL	LI
Dorset	Longevity (LONG)	0.11 (0.01)	0.74 (0.05)	0.55 (0.06)
	Age at first lambing (AFL)	0.54 (0.01)	0.20 (0.02)	0.27 (0.07)
	Lambing Interval (LI)	0.45 (0.01)	-0.19 (0.01)	0.12 (0.02)
Lleyn	Longevity (LONG)	0.05 (0.01)	0.31 (0.08)	0.25 (0.09)
	Age at first lambing (AFL)	0.16 (0.01)	0.14 (0.01)	-0.14 (0.05)
	Lambing Interval (LI)	0.10 (0.01)	-0.12 (0.01)	0.06 (0.01)

Conclusion The traits analysed were low to moderately heritable. The genetic correlations estimated indicate that, for both breeds, age at first lambing can impact on the longevity of the ewe. The older the ewes are at the first lambing event, the older they are when they leave the flock. Additionally, for both breeds, ewes that have longer intervals between lambing events remain in the flock longer and, in the case of the Lleyms, ewes that lamb for the first time at an older age will have a lower lambing interval for her second lambing. Overall, the results suggest that these traits are worthy of inclusion in breeding programmes in order to improve ewe longevity and efficiency.

Acknowledgements The authors gratefully acknowledge AHDB Beef and Lamb for funding and Signet Breeding Services for providing access to the data.

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Novel approach to the reduction of round worm infections in Lleyn sheep

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Application Collecting faecal samples, in order to obtain faecal egg count (FEC) is very time consuming and costly (Bishop & Stear, 2000). Using a 'proxy' such as saliva IgA might be a good way to reduce the number of infected animals in a flock.

Introduction Round worm infections can be very harmful to the animal and may cause serious economic losses to farmers (Stear *et al.*, 2003). A sustainable way to reduce worm burdens in sheep is to breed for reduced parasitic worm burden. FEC has been successfully used in sheep to breed more resistant sheep to internal parasites in the past, but the use of IgA as a phenotype that is indicative of more resistant sheep to parasitic infection, has not yet been examined in the UK. Research indicates that IgA is heritable, with h^2 estimated from 0.39 (0.18) (Shaw *et al.*, 2012) to 0.56 (0.15) (Davies *et al.*, 2005). Hence, developing saliva IgA estimated breeding value (EBV) provides breeders with another tool for monitor and improving the health of the flock, and for use as a breeding goal trait. Based on previous research, it has been shown that IgA is correlated with the amount of round worms infecting sheep (Davies *et al.*, 2005).

Material and methods IgA records from 5,201 lambs in 15 Lleyn flocks, collected over 18 months were used in this study. Mean IgA phenotypes ranged from -0.1411 to 5.0780, with an average of 0.61 and standard deviation of 0.57. As the IgA phenotypes were not distributed normally, a Box-Cox procedure was applied to normalise the data (Osborne, 2010) using $\lambda=0.5$ as it was the best fit. The amount and quality of FEC records were also assessed. Two types of FEC records were available: Nematodes (FecN) and Strongyles (FecS). There were 4,473 animals with all three traits (FecS, FecN and IgA) recorded. Parameters for transformed IgA estimated with ASReml (Gilmour *et al.*, 2009) were fitted into the national genetic evaluation system and a set of EBVs were produced using age of animal when sample was taken, contemporary group, litter size born and embryo transfer flag as fixed effects.

Results After the Box-Cox transformation, IgA ranges from 0.0316 to 2.25 with the average of 0.70. The **Results** from univariate analysis in ASReml indicate that IgA is a heritable trait, with the h^2 estimation of 0.16 (SE 0.04), which is greater the heritability estimated for FecS 0.08 (SE 0.03) and at similar level like for FecN 0.14 (SE 0.04). Moreover, despite no relationship discovered at the phenotypic level, a moderate negative correlation of -0.26 (SE 0.02) is seen at the

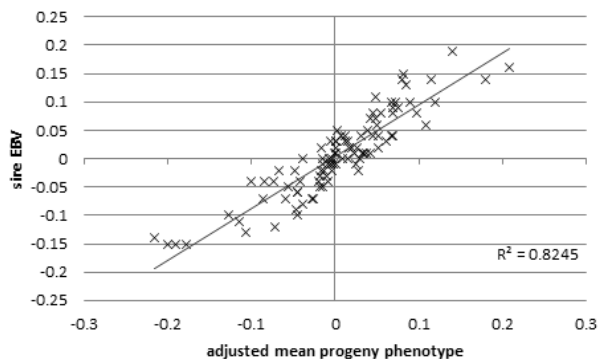


Figure 1 Sire EBV against adjusted offspring phenotype

flocks. The heritability of 16% shows that there is a genetic potential to reduce the number of infected animals using the IgA EBV. Moreover, acquired EBVs as shown on the Figure 1 are predictable and can be used as a selection tool.

genetic level between IgA and FecS, which is in accordance to earlier findings (Bishop & Stear, 2000). The EBVs for transformed IgA ranged from -0.19 to +0.23 with a mean of 0.18 and standard deviation of 0.01. The EBVs for sires with more than 10 offspring with adjusted (for fixed effects) IgA phenotypes are shown in Figure 1, indicating a clear relationship (correlation 0.91) between produced EBV and the transformed IgA phenotype in their offspring.

Conclusion Heritability estimated for the purpose of commercial evaluation is lower than estimated during the research projects. This is due to a different model, which must be able to handle the commercial evaluation including data collected over a long period of time from many different

Acknowledgements The authors gratefully acknowledge AHDB Beef and Lamb for funding, Glasgow University researchers for the introductory research and Lleyn breeders for collecting of the data.

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Phenotyping tools for genomic selection to reduce mastitis in Texel sheep

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Application These results are a first step in the development of genomic breeding values (GEBVs) for hard to measure traits such as mastitis in meat sheep. The relationships observed between Somatic Cell Count (SCC) and the California Mastitis Test (CMT) suggests that this is a useful indicator trait for breeding programmes to reduce mastitis in meat sheep.

Introduction Mastitis is often regarded as one of the most important health problems in dairy ruminants, but it can also have a large impact on non-dairy ruminants, such as those reared for meat production. The disease can prove expensive in terms of the costs associated with treatment methods, poor lamb growth and premature culling of ewes. Conington *et al.* (2008) estimated that a 10% reduction in the risk of contracting mastitis would be worth £8.40 per ewe, or £2.7 million a year for the purebred Texel breed alone.

Material and methods During 2015 and 2016, data were collected from 2,957 Texel ewes from 29 performance-recorded Texel flocks, located throughout the UK. Ewes were recorded twice each year, at mid-lactation and at late-lactation/weaning (at approximately 4 and 11 weeks post-lambing respectively). Traits recorded at each visit included Somatic Cell Counts (SCC) and the California Mastitis Test (CMT), where each half of the udder was awarded a score of 0-4 (according to severity of infection, 0 low, 4 high). The CMT scores recorded were considered as the sum of both scores (SUM) and the maximum score of both scores (MAX). All traits were log-transformed to normalise the data using the formulae:

$\text{Ln}(\text{SCC value})$ and $\text{Ln}(\text{CMT score} + 1)$

Genetic parameters were estimated using univariate analyses in ASReml (Gilmour *et al.* 2009), using the following model: $y = Xb + Za + Zu + e$ where y is the vector of phenotypic observations; b the vector of fixed effects, consisting of ewe parity, scorer (not included for SCC), litter size born x litter size reared interaction and farm x lambing month x score year contemporary group. Lactation stage was also fitted as a covariate; a is the vector of random animal effects; u is the vector of random permanent environmental effects; e is the vector of random residual effects, and X and Z are incidence matrices relating observations to their respective effects. The pedigree file used contained sire and dam information for 31,775 animals.

Results Average cell counts (s.d.) recorded at mid- and late-lactation were 1.7m (3.7m) and 3.0 m (6.4m) cells/ml respectively. Direct heritability estimates for SCC at mid- and late-lactation were 0.11 and 0.08 respectively. The estimates observed for SUM and MAX were 0.09 and 0.08 at mid-lactation and 0.06 and 0.02 at late-lactation respectively (Table 1). Correlations observed between the SCC and the CMT traits were moderate to high at both time points (0.70-0.92) (Table 2).

Table 2 Direct heritability (h^2) and permanent environment (pe) estimates at mid- and late-lactation.

Trait	mid-lactation h^2	mid-lactation pe	late-lactation h^2	late-lactation pe
Average Somatic Cell Count (SCC)	0.11 (0.04)	0.15 (0.05)	0.08 (0.05)	0.16 (0.07)
Sum of CMT Scores (SUM)	0.09 (0.04)	0.15 (0.05)	0.06 (0.04)	0.18 (0.06)
Maximum of CMT Scores (MAX)	0.08 (0.03)	0.14 (0.05)	0.02 (0.04)	0.19 (0.06)

Table 3 Genetic (r_g) and phenotypic (r_p) correlations between Somatic Cell Count (SCC) and California Mastitis Test (CMT) traits recorded at mid- and late-lactation.

Trait	mid-lactation r_g with SCC	mid-lactation r_p with SCC	late-lactation r_g with SCC	late-lactation r_p with SCC
Sum of CMT Scores (SUM)	0.88 (0.10)	0.73 (0.01)	0.70 (0.14)	0.67 (0.01)
Maximum of CMT Scores (MAX)	0.92 (0.10)	0.73 (0.01)	0.70 (0.16)	0.68 (0.01)

Conclusion The **Results** obtained from this study indicate that genetic progress can be made to reduce mastitis in meat sheep. The correlations between SCC and the CMT traits indicate the use of CMT scoring would be worthwhile, as a less expensive and user-friendly indicator trait for selection programmes in the future.

Acknowledgements The authors gratefully acknowledge funding from Innovate UK, The British Texel Society and all participating farmers.

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Effect of breed and sex on production and carcass traits of male lambs following an intensive finishing period

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Application Performance of Texel cross Scottish Blackface (TXSB) lambs was superior in all variables measured compared to pure breed Scottish Blackface (SB) lambs. With effects more pronounced in ram lambs than in wether lambs.

Introduction Scottish Blackface sheep account for approximately 22% of the 2.5 million breeding ewe flock in Ireland. The SB breed was traditionally used to produce light carcasses (10kg-15kg) which were exported to Mediterranean markets. However, due to the economic down turn these markets have declined resulting in reduced demand for light carcasses. In response to the decline of the Mediterranean markets, hill sheep farmers have begun to breed SB ewes to terminal breeds, such as Texel, to produce heavier and better conformed carcasses which will meet higher market specification. The objective of this study was to compare the performance of pure breed SB and TXSB ram and wether lambs fed on an intensive concentrate diet.

Material and methods A total of 200 spring born male lambs were assigned to a 2 × 2 factorial arrangement with two breeds (SB (n=100) and TXSB (n=100)) and two sexes (wether (n=100) and ram (n=100)). Lambs were identified on commercial farms at birth and each alternate male lamb born alive was castrated using a scrotal rubber ring within 48 h of birth (Molony *et al.*, 2002). Lambs were purchased for the experiment at 5 months of age and transported to the research centre. The study was conducted over five slaughter dates between September and April. Within breed and sex the ten heaviest lambs were selected for the finishing period for each of the five slaughter time points. This random complete block design method was favoured over complete random design to ensure that light lambs were not selected for trial too soon thus ensuring approximately equal starting weight across each time point. This method is also reflective of commercial practice. Lambs were individually housed on expanded metal feeding pens (182 cm L × 122 cm W) for the 36 day indoor finishing period. Lambs were allowed a 12 day acclimatization period to adapt to a 95% concentrate diet to minimise digestive upset. For the duration of the finishing period lambs were offered 100 g/day DM of silage and had access to *ad libitum* concentrate. The concentrate used was a 60% cereal-based lamb ration with 15% crude protein and an energy value of 1UFL/kg fresh weight. Cold carcass weights were recorded 24h after slaughter and subsequently used to calculate kill-out percentage (KO). Carcasses were graded for conformation using the EUROP scale which were coded 5, 4, 3, 2, and 1, respectively, for data analysis, and classified for the level of fat cover using a 1 to 5 scale (1=low fat cover, 5=excess fat tissue). Data were analysed using the MIXED procedure (SAS, version 9.4). The model included fixed effects of source farm, breed, sex and slaughter date as well as all appropriate interactions with animal considered as the random effect. Relevant covariates such as initial weight at onset of intensive feeding period and carcass weight were used where applicable.

Results There were no interactions (P>0.10) between breed and sex for any variables, with the exception of carcass fat (P<0.10). Texel cross Scottish Blackface lambs outperformed SB lambs in terms of average daily gain (ADG) (P<0.05) and feed conversion ratio (FCR) (P<0.05) as well as producing heavier carcasses with greater conformation (P<0.05) as shown in Table 1 Ram lambs had greater growth rates (P<0.05) and were more efficient in FCE (P<0.05).

Table 1 Effects of breed (Scottish Blackface (SB) and Texel cross Scottish Blackface (TXSB)) and gender on production and carcass traits.

Variable	Breed				Sex			
	SB	TXSB	SEM	P-Value	Ram	Wether	SEM	P Value
Start weight, kg	36.93	41.24	0.26	<0.05	39.12	39.04	0.25	NS
Slaughter weight, kg	45.61	53.69	0.40	<0.05	50.46	48.85	0.57	<0.01
FCR, kg ¹	6.74	5.17	0.20	<0.05	5.58	6.31	0.20	<0.05
ADG, g/day ²	241.00	349.00	7.00	<0.05	314.00	272.00	7.00	<0.001
Kill out, %	45.46	47.96	0.21	<0.05	45.79	47.63	0.24	<0.001
Carcass fat score, 1-5	3.77	3.21	0.09	<0.05	3.07	3.91	0.07	<0.001
Carcass conformation score, 1-5	2.63	3.38	0.08	<0.05	2.92	3.10	0.06	<0.05
Carcass weight, kg	20.71	25.74	0.20	<0.05	23.14	23.31	0.20	NS

¹FCR = Feed conversion ratio, ² ADG = Average daily gain.

Conclusion Texel cross Scottish Blackface lambs had superior growth rates and feed conversion efficiencies, resulting in higher carcass weights which had a better conformation score and were leaner. This would suggest that TXSB lambs offer an opportunity to improve the sustainability of hill sheep farm systems. The use of ram lambs offered increased growth rates and feed conversion efficiencies while producing a leaner carcass compared to wether lambs.

Acknowledgements This work is funded by RSF grant 11/SF/310.

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Effects of forage type and genotype on methane emissions from hill ewe lambs

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Application Grass nuts increased feed intake, however reduced methane (CH₄) emissions per kg feed intake than grass silage in hill ewe lambs.

Introduction Manipulation of dietary forage type can alter rumen fermentation pattern and consequently influence nutrient degradability and CH₄ emissions from the host animals. Little information is available on CH₄ production of hill ewe lambs. The present study aimed to investigate how different forage types and animal genotypes affected CH₄ emissions in hill ewe lambs.

Material and methods Thirty six hill ewe lambs (18 pure Scottish Blackface (BF) vs. 18 Texel (T) × BF (50:50)) aged about 10 months and weighing 37 ± 5.3 kg were allocated to 2 forage treatments balanced for genotype and live weight. Each genotype was offered 2 forages (grass nuts vs. grass silage) *ad libitum*. Grass nuts were pelleted ryegrass sourced from a commercial supplier (Drygrass South Western Ltd, Burrington, UK). Grass silage was made from the 2nd harvest of perennial ryegrass. The animals were individually housed and offered experimental diets for 14 d before being transferred to individual calorimeter chambers for further 4 d with forage intake, faeces and urine outputs and CH₄ emissions measured. The method of CH₄ emission measurement was as described by Zhao *et al.* (2015). Grass nuts and grass silage contained (g/kg DM): ash 67 and 82; CP 164 and 101; NDF 590 and 597; and GE 19.0 and 19.1 (MJ/kg DM), respectively. Data were analysed in a 2 × 2 factorial arrangement using ANOVA and the differences between treatment means were declared significant if P = 0.05.

Results The effects of forage types and genotypes on enteric CH₄ emissions are presented in Table 1. There were no significant interactions between forage types and genotypes on any variable of CH₄ emissions. Lambs offered grass nuts had greater DM intake, heavier live weight and produced more CH₄ (g/d), however less CH₄ per kg DM intake, digestible DM intake or digestible NDF intake than those given grass silage. Similarly, CH₄ energy (CH₄-E) as a proportion of GE intake, DE intake and ME intake were less for grass nuts than grass silage (P < 0.01). However, CH₄ production per kg live weight was increased by feeding grass nuts (P < 0.001). There were no significant differences between the 2 genotypes of hill ewe lambs in feed intake, live weight or any variable of CH₄ emissions.

Table 1 Effects of forage types (Forage) and genotypes on enteric CH₄ emissions in hill ewe lambs (n = 36)

Enteric CH ₄ emissions	Forage		s.e.	P	Genotype		s.e.	P
	Grass Nuts	Grass Silage			BF	T × BF		
DM intake (kg/d)	1.02	0.45	0.046	<0.001	0.79	0.69	0.058	0.237
Live weight (kg)	40.9	35.5	0.45	<0.001	37.7	38.7	0.57	0.225
CH ₄ (g/d)	15.0	10.5	0.60	<0.001	13.3	12.2	0.76	0.320
CH ₄ /Live weight (g/kg)	0.37	0.30	0.013	<0.001	0.35	0.31	0.017	0.101
CH ₄ /DM intake (g/kg)	15.2	23.9	0.98	<0.001	19.5	19.6	1.24	0.984
CH ₄ /Digestible DM intake (g/kg)	23.4	34.5	2.04	<0.001	30.3	27.6	2.58	0.438
CH ₄ /Digestible NDF intake (g/kg)	40.0	55.0	2.92	0.001	49.5	45.5	3.69	0.449
CH ₄ -E/GE intake (MJ/MJ)	0.044	0.070	0.0028	<0.001	0.058	0.057	0.0036	0.837
CH ₄ -E/DE intake (MJ/MJ)	0.070	0.104	0.0064	<0.001	0.092	0.082	0.0081	0.368
CH ₄ -E/ME intake (MJ/MJ)	0.078	0.125	0.0100	0.002	0.110	0.093	0.0127	0.368

Conclusion Grass nuts could increase feed intake of hill ewe lambs and reduce CH₄ emissions per kg feed intake. However, CH₄ production per kg live weight was greater when feeding grass nuts than grass silage. No significant differences were found in any variable of CH₄ emissions between Scottish Blackface and Texel × Scottish Blackface ewe lambs.

Acknowledgements This work was funded by DEFRA, the Scottish Government, DAERA, and the Welsh Government as part of the UK's Agricultural GHG Research Platform project (www.ghgplatform.org.uk).

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Sheep selected for divergent methane yield on lucerne pellets also express the same trait when fed fresh pasture

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Application The methane (CH₄) yield ranking of low and high CH₄ selection line sheep fed lucerne (*Medicago sativa* L.) pellets was both similar and repeatable over time when fed fresh pasture. This suggests that selection of sheep for low CH₄ yield, currently in progress on lucerne pellets, is relevant to New Zealand pastoral systems.

Introduction Previous work has shown that low and high CH₄ yield selection line sheep differed significantly in both CH₄ yield (g/kg dry matter intake; DMI) and absolute CH₄ emissions (g/d) when fed lucerne pellets at 2 × maintenance metabolisable energy requirements (MEr) in respiration chambers (Pinares-Patiño *et al.* 2013), and when fed pasture *ad libitum* using the SF₆ tracer and portable accumulation chamber methods (Jonker *et al.* 2016). However, there is some doubt about the accuracy of these methods for estimating CH₄ production and DMI from sheep on pasture. The objective of the current study was to determine repeatability of CH₄ emissions measured in respiration chambers from low and high CH₄ yield selection line sheep while fed cut pasture *ad libitum* over three measurement periods.

Material and methods A cohort of 96 low and high CH₄ selection line ewe hoggets born in August/September 2014 (progeny of low and high CH₄ yield selection line sheep) were first measured for CH₄ in respiration chambers (from 3 August to 30 September 2015) while fed lucerne pellets at 2 × MEr with 2 separate 48 h measurements of CH₄ about 14 d apart (Pinares-Patiño *et al.* 2013). After this experiment, three additional periods of CH₄ measurement in respiration chambers were performed (between 7 October 2015 and 12 February 2016) with the same sheep fed cut perennial ryegrass (*Lolium perenne* L.) based pasture *ad libitum*. In each period, good quality pasture was cut daily around 1100 h, with feed bins refilled twice daily around 1530 h and 0830 h the next day. Refusals were collected before the afternoon feeding and an aliquot dried at 65°C for 48 h to determine DMI. Live weight (LW; kg), CH₄ (g/d), DMI (kg/d) and CH₄ yield (g/kg DMI) were analysed separately for each measurement period using a standard least squares model (SAS 2012) with a repeated measures model, fitting animal as a random effect and selection line, sampling group and day as fixed effects, and LW as a covariate. Significance was declared at P < 0.05.

Results Dry matter intake was over 5% higher (P < 0.05) for the low CH₄ selection line sheep compared with the high CH₄ selection line sheep in all periods, but this difference disappeared when LW was included as a covariate (Table 1). Methane yield was 10.4% lower (P < 0.001) for the low vs. high CH₄ selection line sheep fed lucerne pellets, and 8.9, 8.4 and 9.7% lower (P < 0.001) over three consecutive periods for the low vs. high CH₄ selection line sheep fed fresh pasture. Therefore, the difference in CH₄ yield between low and high CH₄ selection line sheep fed lucerne pellets was similar when fed fresh pasture irrespective of measurement periods.

Table 1 Dry matter intake (DMI) and CH₄ yield in low and high CH₄ selection line sheep when fed lucerne pellets and fresh pasture over three periods (P1-P3).

		Lucerne pellets	Pasture P1	Pasture P2	Pasture P3
DMI (kg/day)	Low CH ₄ line sheep	1.54 ± 0.010	1.50 ± 0.025	1.62 ± 0.024	1.93 ± 0.023
	High CH ₄ line sheep	1.48 ± 0.011	1.49 ± 0.026	1.60 ± 0.025	1.88 ± 0.024
	Difference (%)	-3.8	-1.1	-1.7	-3.1
	P-value	0.0003	0.663	0.466	0.093
CH ₄ (g/kg DMI)	Low CH ₄ line sheep	14.0 ± 0.14	21.7 ± 0.28	20.6 ± 0.30	18.9 ± 0.23
	High CH ₄ line sheep	15.5 ± 0.15	23.6 ± 0.29	22.3 ± 0.32	20.7 ± 0.24
	Difference (%)	10.4	8.9	8.4	9.7
	P-value	<0.0001	<0.0001	0.0003	<0.0001

Conclusion Methane yield was 8.4 to 10.4% lower for the low vs. high CH₄ selection line sheep, irrespective of whether they were fed lucerne pellets or fresh pasture, indicating that this trait is stable and repeatable.

Acknowledgements This study was funded by the Pastoral Greenhouse Gas Research Consortium (PGgRc) and New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC). Paul Boma was financially supported by Livestock Emissions & Abatement Research Network award (LEARN).

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Serum cortisol concentrations in horses supplemented with selenium and vitamin E undergoing moderate exercise in an ozone polluted environment

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Application Physical activity affects serum cortisol concentrations; this could be exacerbated in working horses in an ozone polluted environment. In these conditions, oral antioxidants could affect serum cortisol concentrations.

Introduction Physical activity provokes cellular injury, oxidative stress and increases serum cortisol (CORT) concentrations. Human athletes without optimal nutrition present cellular injury, low antioxidant activity and high CORT levels (Díaz *et al.*, 2010). This could be exacerbated in working horses in an ozone polluted environment, since ozone is a powerful oxidant. The objective of this study was to evaluate the relationship between serum cortisol concentrations and supplementation with selenium (Se, selenium methionine) and vitamin E (E, α -tocopheryl acetate) in horses under moderate exercise in an ozone polluted environment.

Material and methods This study was approved by the Institutional Animal Care and Use Committee of the Veterinary Medicine School of the National University of Mexico; it was carried out in the Mounted Police Unit of Mexico City (2240 m above mean sea level) in winter 2014 and lasted 77 days. Data from ozone concentrations were obtained from the Ministry of the Environment (SEDEMA, CDMX). The equipment and the methods used comply with the characteristics demanded by the US Environmental Protection Agency (US EPA). Twenty-four clinically healthy horses, who did not perform physical labour the month prior to this study, were used. They aged 5 to 15 years (450 kg BW), they were randomly assigned in a factorial design experiment (2 Se levels \times 2 E levels) with repeated measures. The level of antioxidant supplementation was the only factor of experimental variation and they met NRC (2007) recommendations (LSe, 0.1; HSe, 0.3 mg Se/kg of DM and LE, 1.6; HE, 2 IU vitamin E/kg of BW). Then, experimental groups with 6 horses each were named using the combination of the supplementation levels, as follows: LSeLE, HSeLE, LSeHE and HSeHE. As diet was poor in experimental antioxidants (Se, undetectable, $<2 \mu\text{g/kg DM}$; vitamin E, 14.4 IU/kg DM), selenium and vitamin E were fully supplied by oral supplements. Day 0 (d0) corresponded to the baseline measurement of CORT before starting the experimental supplementation. The experimental periods were as follows: d0 to d32, adaptation period, not for exercise but for diet; d33 to d56, exercise period (3 consecutive days per horse; daily exercise entailed 5 min warm up, 20 min moderate gallop, 5 min cool down; all horses were exercised in the same polluted conditions); d57 to d77, rest. At d64, supplementation was ended. Once a week, jugular blood samples were taken on the same day (Thursday) and at the same time (11:00–13:00 h) to reduce circadian cortisol variation effect. During the exercise period, blood samples were taken at the end of the last day of exercising. CORT was quantified by ELISA. Data were analysed (SAS PROC MIXED; SAS Inst. Inc., Car. NC.), for the design described above. The fixed effects were Se, E, d and their interactions, while horse nested within treatment was the random effect. Tukey-Kramer's test was used to compare LSM. The relationship between CORT, ozone and time was analysed and visualized using multiple sequence regression to test linear and nonlinear effects (Lenth, 2009) using the RSM package in R. Statistical significant levels were set at $P < 0.05$.

Results Ozone showed a daily pattern during the experimental period, showing the highest concentrations (82.48 ± 24.43 ppb) after 1600 h. CORT was affected by Se level ($P = 0.02$; LSe, 168.9; HSe, 298.4; ± 39.03 ng/mL) and time ($P < 0.001$). Most important differences were observed between initial concentrations (d0–d28, 177.4 ± 18.1 ng/mL) and those from exercise period (d42–d56, 326.3 ± 38.4 ng/mL). As Se level affected CORT, the relationship between CORT, ozone and time was analysed for both experimental group (HSe and LSe). A relationship between studied variables was detected ($P < 0.01$) for LSe (CORT ng/mL = $91.18 + 1.00 \times \text{ozone ppb} + 0.06 \times \text{TIME days}$; $P < 0.05$, $r^2 = 0.10$). According to the response surface obtained with the regression model, a concentration of 185.8 ppb ozone at 70 days would favour the equine to reach 281.18 ng/mL of serum cortisol; which is higher than those observed after 20 min of cantering (Marc *et al.*, 2000).

Conclusion Moderate exercise affected serum cortisol concentrations. The experimental group HSe (0.3 mg Se/kg of DM) had higher serum cortisol concentrations than LSe (0.1 mg Se/kg of DM). However, serum cortisol concentrations from LSe were associated to air ozone. This fact implies that a low supplementation level of selenium could favour high serum cortisol concentrations in horses. These results require further research.

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The effect of home-made semen extenders and commercial semen extenders in the progressive motility of equine spermatozoa

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Application The use of commercially available extenders showed statistically significant positive effects on the maintenance of progressive motility, with homemade products tested in this context performing worst.

Introduction Stallion's fertility is highly dependent on the Progressive Motility (PM) of the inseminated spermatozoa (Loomis and Graham, 2008). Extension of the gel-free fraction of stallion semen aids in the metabolism and preservation of spermatozoa for artificial insemination (Aurich *et al.*, 2007). Home-made or commercial extenders are utilised in the equine industry, therefore the aim of this study was to analyse through Computer Assisted Semen Analysis (CASA) the effects of home-made semen extender, home-made semen extender with Timentin, milk-based commercial semen extender, native phosphocaseinate-containing commercial semen extender, and dual-sugar commercial semen extender in the PM of equine spermatozoa.

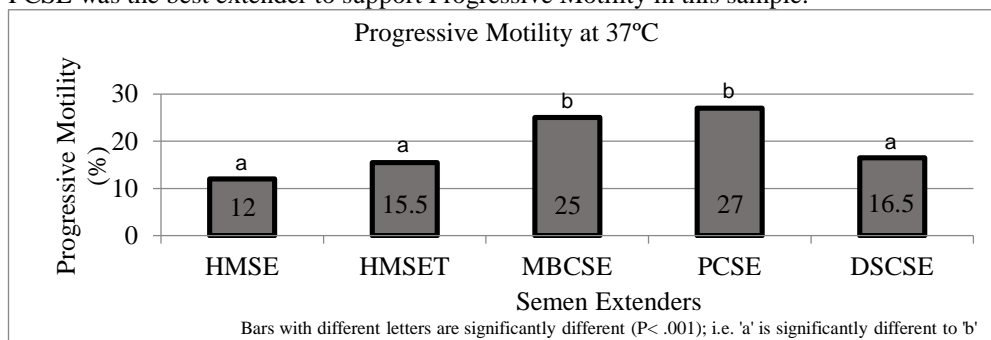
Material and methods Following collection of 10 stallions of varying age and breed, the concentration of 4ml of each sperm-rich semen sample was measured through a light source sperm counter (Quick Check™). The final concentration of each sample was adjusted to contain 35 million spermatozoa per ml. Five samples per stallion were placed in 15ml centrifuge tubes and extended with each of the following pre-warmed extenders:

- home-made semen extender, (HMSE)
- home-made plus Timentin semen extender, (HMSET)
- milk-based commercial semen extender, (MBCSE)
- native phosphocaseinate-containing commercial semen extender, (PCSE)
- dual-sugar commercial semen extender. (DSCSE)

10µl of each sample were pipetted onto a warmed (37°C) microscope slide for microscopic examination using CASA and tested for PM. The samples were maintained chilled in an Equitainer at 5°C for 56 hours after collection, analyses of each sample were performed at 8-hour intervals.

Statistical analyses All statistical analyses were performed using IBM SPSS 23. Assumption of normality was violated; a Friedman Test was used to determine the difference in PM between extenders. Pairwise comparisons, with adjusted P values, were used to compare PM between the extenders.

Results The Progressive Motility of the spermatozoa was significantly different between extenders, $X^2(4) = 106,357$, $P < .001$. Pairwise comparisons, with adjusted P values, were used to follow up this finding. There were significant differences in PM between HMSE and MBCSE (median = 12.00 & 25.00, $P < .001$), HMSE and PCSE (median = 12.00 & 27.00, $P < .001$), HMSET and MBCSE (median = 15.50 & 25.00, $P < .001$), HMSET and PCSE (median = 15.50 & 27.00, $P < .001$), DSCSE and MBCSE (median = 16.50 & 25.00, $P < .001$), and DSCSE and PCSE (median = 16.50 & 27.00, $P < .001$). PCSE was the best extender to support Progressive Motility in this sample.



Conclusion A greater capacity of maintaining a higher progressive motility was shown with two out of three commercial extenders. Thereby, the equine reproduction industry is advised to use commercial extenders for artificial insemination. Further research with different home-made formulas could be performed to demonstrate their full potential.

Acknowledgements The author specially thanks the co-authors Jos Mottershead and Kathy St. Martin of Equine-reproduction.com, LLC for the funding and amazing guidance.

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Current understanding of the role of equine paraprofessionals within therapy and rehabilitation: a study of public perception

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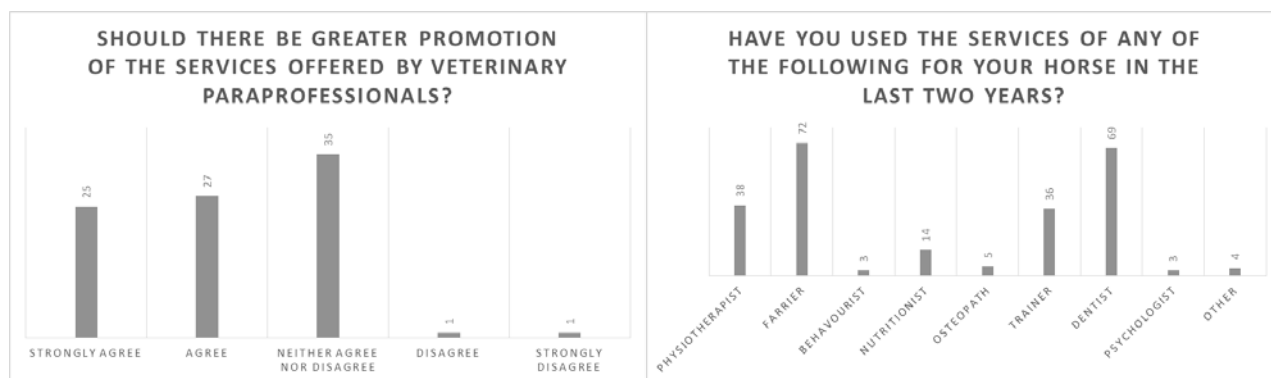
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Application The equestrian public were not aware of the term paraprofessional and did not consider individuals falling under this term as part of a one health approach.

Introduction Despite the title of paraprofessional being used regularly within the veterinary healthcare field, the knowledge and understanding of the title within the general public is limited. The term paraprofessional encompasses many roles within the veterinary healthcare field, including, nutritionists, physiotherapists, dentists and farriers. These roles are an important part of animal healthcare provision, both in the United Kingdom and globally. However there is limited data on public perception, and even awareness, of paraprofessionals in the UK available, with research focussing on roles within the developing world, where paraprofessionals provide many fundamental elements of health care, due to better access to their communities than urban and peri urban based vets.

Material and methods A survey was conducted using the Bristol Online Survey Tool, to gauge current public perceptions of the role of paraprofessionals, within the equine field, with a focus on therapy and rehabilitation. The survey was launched online, using social media to reach equine owners and professionals, all respondents volunteered themselves to complete the survey online, this aimed to remove researcher influence. The survey was available for two months, and question types, such as open, closed and likert scales, were utilised in order to gain a rounded view of respondent opinion and to allow for expansion on points where relevant. Sample size was small and therefore it may be argued that **Results** are not representative of the population as a whole. However McQuarrie and McIntyre (2014) state responses will still reflect the views held by other members of the target population, despite limited response numbers.

Results 100 individuals responded to this self-completion survey, whilst a statistically small survey, 59% of respondents were between the ages of 20 and 30, 88% being female, which is representative of the equine industry as a whole according to the British Equestrian Trade Association (2015). Sixty per cent of individuals were unfamiliar with the term veterinary paraprofessional, although 58% of respondents strongly agreed or agreed that veterinary paraprofessional services should receive greater promotion. Perceptions of the role of the paraprofessional depended on whether the respondent had primary experience of their work, for instance the role of the farrier was seen as essential across the board, whilst physiotherapists were only viewed as vital to those who had previously used their services.



Conclusion Whilst the term veterinary paraprofessional is poorly understood by the wider public, the services of individuals themselves are well received and respected as important in care provision for the horse. As few studies have been published in this area, there is little available data with which to compare findings. However, the findings of this study indicate the general public have a positive perception of the role of veterinary paraprofessionals within the industry.

Acknowledgements

With thanks to Alison Pyatt for her role as supervisor for this dissertation.

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***In vitro* digestibility and nutritional quality of soaked hay and straw for use as forage for laminitis-prone horses**

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Application Soaking hay for laminitis-prone horses reduces the water soluble carbohydrate content and also digestibility to levels equivalent to that of certain straw types. Straw may be another forage option for laminitis-prone horses.

Introduction Horses prone to laminitis require a diet high in fibre (1.5% horses BW/day of forage) and low in sugars in order to reduce recurrence (Geor, 2010). One way to achieve such a diet is to soak conserved forage in water, which decreases water soluble carbohydrate (WSC) concentration. However there are few data on how soaking affects digestibility. In addition, there is little information on whether unsoaked forages low in WSC concentration, such as straw, are as suitable as soaked hay for feeding to laminitis-prone horses. The objectives of this study were to identify whether different soaking treatments affect WSC and neutral detergent fibre (NDF) content and *in vitro* digestibility of hay, and to compare these values with those obtained for different straw types.

Material and methods Two hay (meadow, ryegrass) and three straw (wheat, oat, malting barley) samples were obtained. Hay was soaked as follows; weight:volume ratios of either 1:10, 1:20 or 1:30; environmental temperatures of 4°C or 21°C or time periods of 10, 360 or 960 minutes, to replicate soaking treatments commonly used in practice. Soaked hay, unsoaked hay and each straw were oven dried and milled (<2 mm) prior to analysis. WSC was measured colorimetrically using a segmented flow autoanalyser system. NDF was determined using reference procedures in a fibre analyser (Ankom). True *in vitro* digestibility was measured by incubating samples with incubation medium (Daisy II incubator; Ankom Technology, 2005) and fresh horse faeces obtained from two forage-fed horses. Differences in WSC and NDF content and *in vitro* digestibility between hay soaking methods were analysed using a general linear model ANOVA (Minitab). The analysis was repeated to include the straw samples, including each hay soaking treatment as a separate forage. Results are presented as least square means, considered significant when $P < 0.05$.

Results Dry matter content of hay samples decreased ($P < 0.05$) with increasing weight:volume, increased soaking time and at the higher soaking temperature when compared with unsoaked hay. NDF content of soaked hay was greater ($P < 0.05$) than that of unsoaked hay, due to losses in water soluble components. WSC content was greatest for unsoaked hay (16.8 g/kg DM), decreased upon soaking, and was lowest for the longest the soaking time. This response was also observed for true digestibility of hay samples. When compared with all hay sources, wheat and barley straw contained more NDF than unsoaked hay, but the NDF content of oat straw was comparable to the hay samples. All straw samples had a lower WSC content than all hay samples (0.34 g/kg DM for barley straw compared with 16.9 g/kg DM for unsoaked hay). True digestibility of oat straw was lower than most hay samples, but that of wheat and barley straw was comparable to that of hay soaked for the longest period of time (Figure 1).

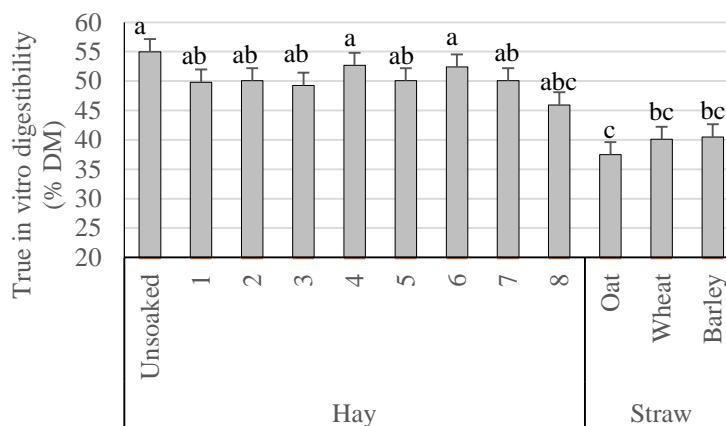


Figure 1 True *in vitro* digestibility of different forage sources

Where 1, 2 and 3 are hay soaked at weight:volume of 1:10, 1:20, 1:30; 4 and 5 are hay soaked at either 4°C or 21°C, and 6, 7 and 8 are hay soaked for 10, 360 or 960 minutes.

Conclusion Soaking hay for longer periods of time decreases WSC content but also *in vitro* digestibility to levels equivalent to that of wheat and barley straw. Results from this study indicate that straw could be included in the diet of laminitis-prone horses who require forage high in NDF but low in WSC concentrations.

Acknowledgements This study was supported by the University of Reading. The authors gratefully acknowledge the assistance of Mr R. Pilgrim for his help with WSC analysis.

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Characterisation of transcriptome profiles of fresh or cultured *ex vivo* equine endometrial explants at different time points

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Application This study determined that an endometrial explant culture (*ex vivo* 24h, 48h and 72h) represents the transcriptome of the live mare mimetic tissue (fresh *ex vivo* 0h), validating the *ex vivo* tissue culture as a model for future studies into equine endometritis.

Introduction An *ex vivo* equine endometrial explant system has previously been proposed to measure uterine inflammation using biomarkers secretion such as Prostaglandin F2 α (Nash *et al*, 2008). However, it has not been determined if the wider transcriptome from explants remain stable in culture. New genomic technologies now make this possible. The aim of this study was to determine whether the transcriptomes of *ex vivo* cultured endometrial explants collected from native pony mares are representative of the transcriptome of a live mare (*ex vivo* uncultured 0h endometrial tissue) in the pre-breeding, non-inflammatory state.

Material and methods Endometrium from native pony mares' uteri (n = 8) at the follicular stage of the oestrous cycle were sampled at 0h (representing the live mare) and tissue explants from the same animals were cultured in triplicate for 24h, 48h and 72h. At the end of each incubation period, tissues were stored in RNA-Later, then total RNA was extracted, quality assessed by agarose gel electrophoresis, followed by Qubit quantification. RNA was subjected to library preparation for RNA sequencing on the Illumina HiSeq 2500 platform. Following the Tuxedo protocol (Trapnell *et al*, 2012) differentially expressed genes (DEG) were analysed using the Cuffdiff package and significance was assumed where $P < 0.05$.

Results From a total of 13,212 genes, none was differentially expressed ($P < 0.05$) when comparing the gene expression in equine endometrium at the four different time points (0h, 24h, 48h and 72h).

Conclusion This preliminary study showed that no significant transcriptomic changes occurred when comparing the endometrial transcriptome representing the live mare (*ex vivo* 0h) with the transcriptome of autologous tissues cultured for up to 72h. Therefore, this tissue culture model has potential application for further interrogation of the mechanisms underlying uterine inflammation.

Acknowledgements The authors gratefully acknowledge Science Without Borders Program for studentship funding and F Drudy & Sons Ltd for enabling sample collection.

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Sodium butyrate modifies fermentation patterns in the gastrointestinal tract of pre-weaned calves, independently of changes in microbial composition

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Application Sodium butyrate (SB) modified fermentation patterns in the gut of pre-weaned calves when added to milk replacer. It had no significant effect on microbial communities.

Introduction The microbiome of the gastrointestinal tract (GIT), represents the interface between nutrition, health, and production in ruminants, and its importance in beef and dairy systems cannot be understated. In addition to their role in fermentation of ingested feedstuffs, these microbial communities contribute to homeostasis in the animal, modulating the host immune system and contributing to host development and physiology. The ban on the use of antibiotics as growth promoters in production animals has fuelled the need for development of novel alternatives, such as dietary butyrate. Butyrate is a natural product of microbial fermentation in the gut, known as a preferred energy source for rumen and colon epithelial cells. Improved growth in the pre-weaning period has been associated with increased milk and meat production in later life, and supplementation with butyrate in early life has been shown to positively impact growth rates, GIT development and health status in calves. However, to our knowledge, there are no data on the effect of dietary supplementation with butyrate on the microbial populations within the rumen and hindgut of the pre-ruminant calf.

Material and methods Holstein Friesian bull calves (n = 16) were blocked according to age and bodyweight, and were randomly assigned to one of two treatment groups; SB (n = 8: Sodium butyrate added to milk replacer at 3g/kg DM/d) or CON (n = 8: Control – no supplement). Calves were offered 6L MR daily (125g/L). Diets were initiated at day 7 of life, and The experimental period extended from day 10 to day 56 when all calves were euthanized. Following evisceration, rumen, caecum and colon digesta were collected and stored at -80°C pending microbial analysis. The fluid fractions of rumen and colon content were also subjected to volatile fatty acid (VFA) analysis using gas chromatography (GC). 16S rRNA libraries targeting bacterial and archaeal populations were constructed following extraction of DNA from rumen, caecum and colon digesta and sequenced on an Illumina MiSeq platform. Taxonomic analysis employed the open reference OTU picking strategy in QIIME using the Greengenes database. Diversity metrics were calculated in QIIME. Non-parametric statistical analyses between treatment groups were carried out in QIIME and R.

Results Pairwise comparison of OTU abundances between treatment groups using a Mann Whitney U-test revealed no significant effect of SB on microbial composition in the GIT regions examined (P > 0.05). This was validated by ANOSIM analysis which showed no effect of SB on microbial diversity in the rumen (p = 0.74), caecum (P = 0.43) or colon (P = 0.37). Metrics of α -diversity (chao1, Shannon) were not significantly changed between treatments. ADG in the SB group tended to be higher (0.59 kg/d vs. 0.69 kg/d; p = 0.08) compared to CON. VFA analysis by GC revealed increased amounts of acetic and propionic acid in the colon, as well as an increase in overall VFAs (P < 0.05). Interestingly, rumen butyric acid was decreased with supplementation (P = 0.04).

Conclusions SB supplementation in milk replacer positively affected VFA profiles and ADG in pre-weaned dairy calves, and these changes occurred independently of any significant changes in GIT microbiota. Propionate and total VFA levels were increased in the colon by SB indicating increased fermentation levels in the colon under SB supplementation. Butyrate is recognised as a major fuel for gastrointestinal epithelial cell growth and proliferation, while propionate is the major precursor for gluconeogenesis in ruminants. Thus, the increased growth rates observed in animals fed dietary butyrate may also be linked to the observed increase in hindgut propionate.

In conclusion, while SB supplementation in milk replacer does not have a statistically significant impact on hindgut or ruminal microbial communities, it significantly influences VFA levels in the lower gut, suggesting that increased growth observed in calves may be due to elevated hindgut fermentation, potentially leading to increased glucose synthesis in the host.

Acknowledgements

All procedures involving animals were approved by University College Dublin, Animal Research ethics committee, under licence from the Irish Department of Health and Children in accordance with the Cruelty to Animals Act (Ireland 1897) and European Community Directive 86/609/EC.

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Weight and piglet vitality at birth are main determinants of piglet performance to weaning

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Application Birth weight and vitality scores can be used as predictors of piglet performance to weaning and hence in identifying piglets requiring intervention in early life.

Introduction The selection for highly prolific sows has led to a rise in the number of low viability piglets (Alonso-Spilsbury *et al.*, 2005). Less viable piglets not only have increased pre-weaning mortality, but they have reduced pre-weaning growth and overall life time performance compared to more viable piglets (Douglas *et al.*, 2013). Factors such as birth weight and birth vitality scores have been identified as traits that affect the viability of piglets in relation to pre-weaning mortality (Baxter *et al.*, 2008); however, their effect on growth and performance to weaning is still unclear. Determining adequate methods of predicting piglet performance is important in order to identify piglets which may be at risk of low growth rates. The aim of this study was to identify the potential risk factors associated with piglet viability and determine their effect on performance to weaning.

Material and methods Seventy-two (Large White × Landrace) sows were moved into the farrowing house on day 112 of gestation and kept in conventional farrowing crates within individual farrowing pens measuring 4.2m². Sows were fed diets throughout gestation and lactation that met all nutrient requirements (NRC, 2012). At birth, piglets were given a vitality score based on their behaviour within the first five minutes after birth. Piglets were then weighed, temperatures taken using a tympanic ear thermometer and tagged for identification. Litter sizes were recorded. Piglets were re-weighed at one week of age and at weaning. All deaths were recorded. Only piglets that survived from birth to weaning were used for analysis. The variables examined were: birth weight, sex, birth order, litter size, body temperature and birth vitality score. Vitality scores were categorised (LOW, MEDIUM, HIGH). A multiple linear regression analysis was used to identify indicators of piglet performance to weaning. Each potential variable was initially analysed as the sole explanatory factor. Significant independent variables were then added to the model and removed in a stepwise manner to determine the combination of variables that best explained variation in average daily gain (ADG) from birth to weaning.

Results Piglet performance from birth to weaning was assessed for 760 piglets. Piglets had an average birth weight of 1.35 ± 0.31 kg and an average weaning weight of 7.06 ± 1.75 kg. As sole explanatory factors, birth weight (P<0.001), litter size (P<0.01) and vitality score (P<0.05) were found to influence ADG to weaning. Sex (P=0.719), birth order (P=0.344) and body temperature (P=0.830) had no effect on piglet performance to weaning. The final model (R²=0.06) found piglet birth weight (P<0.001) and vitality score (P<0.05) to be the best predictors of piglet ADG from birth to weaning (Table 1). As piglet birth weight increased by 100 grams, ADG from birth to weaning increased by 4.4 g/day. As piglet birth vitality score increased by one category, ADG from birth to weaning increased by 6.8 g/day.

Table 1 Final multiple linear regression analysis for indicators of average daily gain from birth to weaning

Variable	Odds ratio	95 % CI	P-value
Birth weight, g	0.044	0.029 - 0.059	<0.001
Birth vitality score	6.785	0.023 - 13.547	0.049

Conclusion Together, piglet birth weight and vitality score were found to be the best indicators of piglet performance from birth to weaning in this dataset. An increase in birth weight or vitality score resulted in an increase in ADG to weaning, indicating that they are good predictors of performance and growth. Low birth weight piglets with low vitality scores may require additional supplementation of colostrum in order to increase their performance to weaning.

Acknowledgements With thanks to ABN, ABNeo and the University of Leeds for funding.

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Acute phase proteins response as biomarkers in the immediate response to vaccination in specific pathogen free layer chicks

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Application Detection of acute phase proteins and stress in terms of innate immune response post vaccination with commercial vaccine in specific pathogen free chicks SPF using commercially available ELISA kits.

Introduction The acute phase proteins (APPs) are an early and non-specific systemic reaction of the innate immune system to local or systemic disturbances, the goal of which is re-establishment of homeostasis and healing (O'Reilly and Eckersall, 2014). APPs are considered to have a reliable diagnostic and prognostic potential because of their response-correlated concentration in sera (Murata *et al.*, 2004). The assessment of the heterophil to lymphocyte (H/L) ratio has been established as means of evaluating stress in chickens (Post *et al.*, 2003). This study aimed to measure AGP, SAA and the H/L ratio in SPF chicks, as a means of measuring their immediate post-vaccination innate immune and stress responses, which would allow subsequent investigations on the pathophysiology of avian APPs and their interaction with the protection of the host provided by vaccination.

Material and methods ELISAs were used to monitor levels of α -acid glycoprotein (AGP) and serum amyloid A (SAA), in 80 7-day old SPF chicks pre-and post-vaccination, i.e. intra-ocular administration of a combined live attenuated ND and IB vaccine (Treatment group). A saline was administered to 80 control SPF chicks. Blood samples were collected from 6 chicks pre-and post treatment at 12, 24 and then every 24 hours for a total of 6 day post treatment. The H/L ratios were estimated each time point as a physiological stress indicator to determine a possible correlation between this ratio and APPs. To check that the vaccination had been successful, blood samples were collected at day 21 post treatment. The antibody response to the vaccine, H/L ratios, and APP (SAA, AGP) of the vaccinated and control groups, were compared at each sampling time point using a Mann-Whitney Test (Minitab 17.1.0). In all analyses, $P < 0.05$ was used to represent statistical significance

Results The antibody titer was significantly higher in the vaccinated group at 21 days post vaccination confirming the efficacy of the vaccine to stimulate the immune system. The H/L ratio was significantly higher in the vaccinated group at 24 and 48 h post vaccination ($P < 0.01$) and at 72 h post vaccination ($P < 0.05$). The concentration of SAA increased by 2.4fold at 24 h post vaccination, from $42.6 \pm 6.89 \mu\text{g/ml}$ (mean \pm SD) in control to $102.5 \pm 20.6 \mu\text{g/ml}$ in vaccinated group ($P < 0.05$). The concentration of AGP increased 2-fold at 48 h post vaccination, from $0.47 \pm 0.17 \text{ g/L}$ in control group to $0.94 \pm 0.35 \text{ g/L}$ in vaccinated group (, with $P < 0.05$). The H/L ratio was not correlated with AGP ($P = 0.6507$, $r = 0.07383$) nor SAA ($P = 0.2363$, $r = 0.1916$).

Conclusion In **Conclusion** vaccination of chicks with N/B vaccine by the intra-ocular route produced a mild acute phase and stress response with the latter shown by the increase in H/L ratio. Indeed, the H/L ratio showed a more consistent change than did the measurement of the APP. It is possible that the vaccine did not elicit more explicit APPs due to the route of administration.

Acknowledgements The authors thank the University of Kufa and the Iraqi Ministry of Higher Education for funding.

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The effect of a high lactose creep feed and a peat product on piglet performance

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Application Feeding a high lactose creep or peat product to young piglets is commercially beneficial as these products produce faster growth compared to piglets only receiving maternal milk in the pre-weaning stage.

Introduction In the UK pig industry, outdoor reared pigs frequently outperform their indoor counterparts. Studies suggest this is the result of multifactorial effects but one factor which appears to be influential is the exposure to natural microorganisms and micronutrients in the soil (Mulder *et al.*, 2009). The use of peat on indoor units is an increasing area of interest to mimic this benefit (Trckova *et al.*, 2005). Peat has a high acidic content, providing bactericidal effects on pathogenic bacteria (Matlova *et al.*, 2012). In addition, indoor reared piglets, are commonly fed a high lactose creep (HLC) to enhance growth, promote gut development and aid adaptation of the gastro-intestinal tract to novel nutrients (Bandara *et al.*, 2013). The aim of this study was to investigate the effects of a specialist HLC and peat product on piglet performance.

Material and methods The trial was conducted on a commercial indoor unit and 4 treatments were allocated to blocks of 6 pens, balanced for parity (6 litters/treatment): Control (no supplementation), commercial HLC (Primary Diets), peat and a 1:1 ratio (w/w) of HLC:peat (combined). Supplemental feeding started at 4 days of age (d4) until d21, when a commercial first stage diet was introduced. Individual weights were recorded at d4, d21, d27 (weaning) and d55. Supplemental feed disappearance was recorded pre-weaning and mortality was recorded throughout the trial. Piglet weight, average daily gain (ADG), mortality and feed disappearance were analysed using a linear mixed model in IBM SPSS v23, with treatment as a fixed factor and litter size and parity as random factors. Statistical significance was declared at $P < 0.05$ and trend at $P < 0.10$.

Results Treatment effects on average piglet weight, ADG and feed disappearance are shown in Table 1. Peat and HLC significantly improved ADG compared to control between d4-21 ($p = 0.041$), and as a trend ($p = 0.065$) between d4-27. Feed disappearance was also significantly different between treatments d4-21 and d4-27, with highest disappearance in HLC and lowest in peat. Mortality through the trial was very low (2/257 piglets) and not significant between treatment.

Table 1 Effect of treatment on Means \pm SE of piglet weight (kg), ADG (kg) and average feed disappearance (kg/piglet)

	Control	HLC	Peat	Combined	P
Weight d4	1.77 \pm 0.189	1.78 \pm 0.196	1.90 \pm 0.189	1.95 \pm 0.199	0.665
Weight d21	5.40 \pm 0.382	5.95 \pm 0.394	6.11 \pm 0.382	5.78 \pm 0.402	0.181
Weight d27	7.31 \pm 0.421	7.98 \pm 0.434	8.11 \pm 0.421	7.86 \pm 0.442	0.149
Weight d55	18.09 \pm 0.411	18.79 \pm 0.401	18.91 \pm 0.395	19.35 \pm 0.417	0.193
ADG d4-d21	0.213 \pm 0.015 ^a	0.246 \pm 0.016 ^b	0.248 \pm 0.015 ^b	0.225 \pm 0.016 ^{a,b}	0.041
ADG d21-d27	0.275 \pm 0.009	0.292 \pm 0.010	0.290 \pm 0.010	0.302 \pm 0.010	0.240
ADG d4-d27	0.241 \pm 0.013	0.270 \pm 0.014	0.270 \pm 0.013	0.257 \pm 0.014	0.065
Feed Disappearance d4-d21	n/a	0.159 \pm 0.011 ^a	0.081 \pm 0.010 ^b	0.118 \pm 0.011 ^c	0.002
Feed Disappearance d21-d27	0.173 \pm 0.013	0.143 \pm 0.015	0.128 \pm 0.013	0.125 \pm 0.015	0.149
Feed Disappearance d4-d27	0.173 \pm 0.020 ^a	0.301 \pm 0.022 ^b	0.209 \pm 0.020 ^{a,c}	0.244 \pm 0.022 ^{b,c}	0.004

Mean values with different superscript indicate significant difference ($p \leq 0.05$)

Conclusion The results indicate positive effects of feeding HLC or peat diets to improve piglet growth pre-weaning. Feed disappearance results indicate that piglets likely consumed both HLC and peat from a young age (d4) with a preference towards HLC. Further area of research would investigate the mechanism of action by which HLC and peat supplementation can improve piglet performance focusing on modulation of gut health and consequent effect on performance.

Acknowledgements The authors gratefully acknowledge Primary Diets for providing the funding for this research.

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High doses of supplementary phytase improve the performance of weaner pigs fed a low iron wheat-soybean meal based diet

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Application Increasing the level of supplementary phytase from 500 to 2,500 FTU/kg may allow for reductions in supplementary Fe concentration with no adverse effects on pig performance during the post weaning period.

Introduction Iron (Fe) deficiency is the most prevalent mineral deficiency in pigs with 35% of pigs on 20 commercial farms tested having low haemoglobin (Hb) status (Perri *et al*, 2015). A key determinant of Fe availability in pig diets is dietary phytate concentration. Phytases are commonly added to pig diets to improve dietary phosphorus (P) availability; however, little information exists as to the effect of phytase on Fe availability. The aim of this study was to determine the effect of a standard and a high phytase dose on the growth performance and Fe status of weaner pigs fed a low-Fe diet.

Material and methods A total of 234 mixed sex weaner pigs (initial BW ~7.6 kg; ~28 d of age) were allocated onto 1 of 6 wheat-soybean meal based diets balancing for weight, sex and litter of origin. Dietary treatments were arranged as a 2 x 3 factorial design with 2 levels of supplemental Fe (analysed total Fe 100 [L-Fe] or 320 mg/kg [H-Fe] as FeSO₄) and 3 levels of supplemental phytase (0, 500 and 2,500 FTU/kg). All diets were formulated to meet or exceed the BSAS (2003) recommendations for all nutrients with the exception of Fe. Treatments were fed *ad libitum* to 8 replicate pens (4 or 5 pigs/pen) for 20 d post-weaning. Pig weights and feed consumption were recorded weekly throughout the experiment for the determination of pen average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). At the end of the experiment, a blood sample was collected from the vena jugularis of one pig per pen for subsequent Hb and haematocrit (Hct) analysis. Data were analysed by two-way ANOVA using the GLM procedure of SPSS (version 22.0) with the pen serving as the experimental unit for the growth analyses, and the individual pig for all blood analyses.

Results Weaner pigs fed the L-Fe diet had a lower ADFI throughout the experiment than those fed the H-Fe diet ($P < 0.01$). Supplementing the diets with 2,500 FTU of phytase/kg increased post weaning feed intake ($P = 0.055$). Pigs receiving the L-Fe diet had a significantly lower ADG than those fed the H-Fe diet. Supplementary phytase at 500 FTU/kg marginally improved the ADG of the L-Fe fed pigs, but had no effect on the H-Fe fed pigs. Supplementary dietary phytase at 2,500 FTU/kg improved pig ADG; however, its effect was greater when added to the L-Fe diet, thus resulting in a significant phytase x Fe treatment interaction ($P < 0.05$). In addition, phytase supplementation at 2,500 FTU/kg improved weaner pig FCR irrespective of dietary Fe concentration ($P < 0.01$). Increasing the dietary Fe concentration from 100 to 320 mg/kg significantly increased Hct ($P < 0.001$) and tended to increase haemoglobin concentration ($P = 0.051$). Phytase had no measurable effect on Hb ($P > 0.1$) but tended to increase Hct concentration ($P < 0.1$).

Table 1 Effect of dietary phytase and iron supplementation on weaner pig growth performance and haematological status.

Dietary Fe FTU/kg	L-Fe			H-Fe			SEM	P-value		
	0	500	2500	0	500	2500		Fe	PHY	Fe x PHY
Growth										
ADFI (g)	288	287	308	312	311	331	7.6	<0.01	0.055	0.213
ADG (g)	189 ^a	219 ^{ab}	253 ^{bc}	247 ^{bc}	246 ^{bc}	264 ^c	9.4	<0.001	<0.001	<0.05
FCR	1.41	1.35	1.26	1.35	1.29	1.19	0.043	0.115	<0.01	0.250
Blood										
Hb (g/dl)	9.7	10.0	10.4	10.7	11.0	11.4	0.55	0.051	0.495	0.879
Hct (%)	25.1	26.5	27.6	29.3	30.6	31.8	0.93	<0.001	0.085	0.777

^{a-c} Means that do not share a common a super-script within a row are significantly different ($P < 0.05$).

Conclusion Weaner pigs responded positively to an increase in dietary Fe concentration from 100 to 320 mg Fe/kg, which suggests that current post-weaning Fe requirement of 80 mg/kg as set by the NRC (2012) may require re-evaluation. Supplementing the low-Fe diet with a high dose of phytase (2,500 FTU/kg) improved piglet performance to a level comparable with those fed a high-Fe diet. The improvements may in part be linked to improved Hct status. The results of this study demonstrate that in the presence of high doses of phytase, dietary reductions in supplemental Fe can be made with no adverse effects on pig performance during the post-weaning period.

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The impact of three contrasting management strategies on hock lesion scores in dairy cows

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Application Confined dairy cows housed within a free-stall cubicle house had a greater risk of hock injury than grazing dairy cows. This highlights the need to develop improved bedding systems for confined cows.

Introduction Hock lesions are described as clinical presentations of hock damage, varying in severity from mild hair loss to progressive ulceration, open wounds and swelling of the hock joint (Laven and Livesey, 2011). The prevalence of hock lesions has been associated with the incidence of lameness, which can have serious economic, production and welfare implications in a dairy herd. The objective of this study was to examine the impact of three contrasting management strategies on the severity of hock lesions in dairy cows.

Material and methods Three management strategies were examined in a 24-week (April – September), continuous design experiment involving 114 Holstein Friesian dairy cows (38 cows/strategy), as follows: i. housed and offered grass silage (Silage), ii. housed and offered fresh grass (Zero-grazing), iii. full-time grazing (Grazing). Concentrate feed levels were common across all three treatments (8.0 and 6.0 kg/day for multiparous and primiparous cows, respectively). Cows within the Silage and Zero-grazing treatments were housed in cubicle accommodation, with cubicles fitted with rubber mats and bedded with sawdust three times each week. These cows were offered either fresh silage or fresh herbage once daily. Cows on treatment Grazing were managed within a rotational paddock grazing system, and given access to fresh herbage daily. Cows were ‘hock scored’ (both right and left hocks, lateral surface) mid way through the study (9th July) and during the final week of the study (26th September), with each hock scored for ‘hair loss’, ‘ulceration’ and ‘swelling’ using a 0 - 3 point scale, as described by Potterton *et al.* (2011). For each of hair loss, ulceration and swelling, the maximum score for each cow within each period was identified, and this data analysed. Due to the low incidence of scores of 2 and 3, these two categories were combined to give a score of ≥ 2 . Data were analysed using ordinal logistic regression, with cow as the random effect, and treatment x period as fixed effects.

Results Management strategy had a significant effect on hock scores for hair loss ($P < 0.001$), ulceration ($P < 0.001$) and swelling ($P = 0.022$). Across all three parameters, grazing cows had better hock health (lower scores) than confined cows offered either grass silage or zero-grazed grass. Hair loss ($P < 0.001$) and ulceration ($P = 0.004$) differed between periods, tending to be poorer during mid season. There were no treatment x period interactions for any parameter ($P > 0.05$).

Table 1 Effect of management strategy on hock scores for hair loss, ulceration and swelling (probability (and s.d. in parenthesis) of obtaining a score of 0, 1 or ≥ 2)

	Treatment	Hock Score			P value
		0	1	≥ 2	
Hair loss	Silage	0.20 (0.051)	0.20 (0.051)	0.60 (0.062)	< 0.001
	Zero-grazing	0.17 (0.047)	0.19 (0.049)	0.64 (0.059)	
	Grazing	0.66 (0.056)	0.17 (0.044)	0.17 (0.043)	
Ulceration	Silage	0.26 (0.056)	0.32 (0.060)	0.42 (0.063)	< 0.001
	Zero-grazing	0.26 (0.054)	0.34 (0.059)	0.40 (0.061)	
	Grazing	0.78 (0.048)	0.15 (0.042)	0.07 (0.029)	
Swelling	Silage	0.38 (0.062)	0.38 (0.062)	0.24 (0.055)	0.022
	Zero-grazing	0.54 (0.062)	0.32 (0.058)	0.14 (0.043)	
	Grazing	0.58 (0.058)	0.30 (0.054)	0.12 (0.039)	

Conclusion Confined dairy cows offered either grass silage or zero-grazed grass had a greater risk of hock injury than grazing dairy cows.

Acknowledgements This study was co-funded by DAERA and AgriSearch.

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Comparison between half and full body weight assessment of Holstein Friesian calves when monitoring pre and post-wean growth

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Application The inclusion of automated half body weigh scales can be used to accurately monitor calf growth.

Introduction The accurate assessment of calf weight to monitor successful growth is paramount to the progressing dairy farm. Half body, or front feet scales as they are commonly known, are a relatively new technology for weighing livestock which enable automated weight calculation on a regular basis. Within commercial practice half body weigh equipment is typically located within a race/stall linked to a water or feed dispensing station. However, little independent research has been undertaken to investigate the use of this equipment and a direct comparison to actual full body weigh scales is needed. The main objective of this study was to examine how half body weigh scales compare to full body weigh scales and to consider the extent to which this technology can accurately be used to assess calf live weight.

Material and methods Assessment of 129 Holstein Friesian calves from the age of 7-70 days old across six half body weigh systems was conducted in comparison to full live weight recorded (n=1216) through pre and post weaning stages. Calves were weighed manually on a calibrated Tru-test weigh crate and directly compared to the half body weight recorded for that time point.

Results A significant positive correlation ($p < 0.001$) was shown between the half body weight to the manually recorded weight ($R^2 = 0.964$) across all time points by regression analysis. Monitoring calves within the different age groups of 7-28 (n=427), 29-56 (n=584) and 57-70 (n=205) days of age demonstrated similar correlation with $R^2 = 0.904, 0.907, 0.909$ respectively. Comparison of the percentage difference in the weight output by half body weigh scales compared to full live weight demonstrated accuracy of <10% difference across 97% of the dataset with min/max % difference +/-18% and Standard Error of 2.53. The percentage difference in output weight was assessed across the different weight ranges collected from 7-70 days of age as shown in the table below.

Weight Range (kg)	n	Average Weight (Full Body) kg	Standard Error	Average % Difference (min to max range)
26-45	168	41.3	1.9	0.4 (-15 to +12)
46-55	315	50.5	2.4	1.3 (-13 to +18)
56-65	274	60.7	2.7	0.8 (-18 to +13)
66-75	273	70.3	2.7	0.1 (-12 to +10)
75+	186	82.3	2.6	-1.0 (-9 to +6)

Conclusion The findings of the current study support the use of half body scales for the robust estimation of calf growth during the first 10 weeks of life across a weight range of 28-108 kg. Automated real-time measurement through half body weigh scales provides an accurate representation of the full live body weight and the opportunity for continual performance assessment in relation to management, nutrition and health of dairy calves with the potential contribution to early warning systems.

Effect of overstocking during the non-lactating (dry) period on behaviour and faecal glucocorticoid metabolites in pregnant dairy cows, and on calf birthweight and growth

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Application Overstocking dairy cows during the dry period resulted in shorter feeding times and more agonistic social interactions at feed-face after feed delivery, but did not have a negative impact on the weights and the growth of offspring.

Introduction The level of agonistic social interactions in dairy cows is known to be affected by space allowance in lying and feeding areas as well as re-grouping frequency. On commercial dairy farms, dry cows (non lactating cows before calving) are usually kept in a dynamic social group, where group size and composition are irregularly changed. If access to resources e.g. feed and/or lying space is restricted, this could increase the number of agonistic social interactions and cows can experience social stress. It has been reported that social stress experienced by pregnant animals can have a negative impact on fetal development, which can have a consequence for the offspring throughout its postnatal life. The aim of this study was to investigate the effects of overstocking and frequent re-grouping on feeding behaviour and the faecal glucocorticoid metabolites of cows during the non-lactating (dry) period. Effects of maternal social stress on calf birthweight and pre-weaning growth were also investigated.

Material and methods Holstein cows (n=48) that were expected to calve between 20th January and 5th July 2015 and their offspring (n=44) participated in this study. Cows were dried off 8-9 weeks before their expected calving date, and kept in cubicle sheds with a yoke feed face system during the far-off period (dry-off to 2-3weeks before calving), and moved to straw yards with a post-rail feed face system during the close-up period (the last 2-3 weeks before calving). Cows were balanced by parity and allocated to either high (H) or low (L) stocking density groups after dry-off. During the far-off period, cows had 0.5 vs 1.0 yokes and 1.0 vs 1.5 cubicles/cow respectively for H and L groups. During the close-up period they had 0.3 m vs 0.6 m feed face and 6 m² vs 12 m² lying space/cow respectively for H and L groups. Five minute-scan sampling was conducted to record the behaviour of cows that were feeding during the 3 hours after feed delivery on three days per week. Agonistic social interactions were also observed two days per week during 0-20min, 40-60min and 80-100 min after feed delivery. Faecal samples were collected at 5 timepoints to measure faecal glucocorticoid metabolites (FGCM). Calves were managed in the same way regardless of maternal treatment. Bodyweight was measured at birth and at weaning (at 49 days old), and daily gain (g/day) from birth to weaning was calculated. Behavioural data and the concentrations of FGCM were analysed by Residual Maximum Likelihood (REML) using GenStat. The model for behavioural analysis included treatment, housing type and observation day as fixed effect and treatment*experimental week as a random effect. The model for FGMC analysis included treatment parity and sampling points as fixed effects and cow as a random effect. Log transformation was performed where necessary. Interactions between treatment and other factors were also investigated. Calf birthweight and daily gain were analysed by General Linear Model using GenStat including maternal treatment, breed, gender and maternal parity in the model. Means and standard errors are presented, and means obtained from the transformed data were back-transformed to the original scale, and confidence intervals are reported.

Results Cows in L group spent a longer time feeding (86.8±4.8 min) than H group cows (72.1±4.4 min) during the 3 hours after feed delivery (W=5.5, p=0.023). There was a significant interaction between treatment and housing type on the number of agonistic social interactions (W=8.3, p=0.005). The number of interactions at the yoke feed face was not different between treatment groups (H: 1.5/group, CI=1.2, 1.92 vs L: 1.4/group, CI=1.0, 1.7, p>0.1), but significantly more frequent social interactions were observed at the post-rail feed face in the H group (5.9/group, CI=4.9, 7.1) than in the L group (3.3/group, CI=2.7, 4.0). The concentration of FGCM was not different between treatments (p>0.1) or between parities (p>0.1). However a significant effect of sampling timing was found (W=85.6, p<0.001), where the concentrations of FGCM during the dry period (week2: 379.3 ng/g, CI=161.1, 893.1, week5: 323.6 ng/g, CI=140.8, 743.7, transition: 368.1 ng/g, CI=160.1, 846.1, pre-calving: 357.3 ng/g, CI=150.0, 851.2) were significantly higher than that at dry-off (184.1 ng/g, CI=80.1, 423.1). No effect of maternal treatment was found in the calf bodyweight at birth (p>0.1), at weaning (p>0.1), or in the growth of calves during the pre-weaning period (p>0.1).

Conclusion Overstocking of dry cows resulted in a shorter feeding time during the 3 hours after feed delivery and more frequent competitions at the feed face, especially in the post-rail feed face system. However overstocking did not affect the concentrations of faecal glucocorticoid metabolites. Maternal competitive experience and altered feeding behaviour during the dry period did not have negative consequences on calf birthweight, weaning weight or daily gain during the pre-weaning period.

Acknowledgements The authors gratefully acknowledge funding from SRUC, as well as support from laboratory and farm technicians and farm staff during the experiment.

Age and diet affect the faecal microbial composition in the domestic cat (*Felis catus*); a 5-year study

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Application Age and diet affect the faecal microbiome of cats fed commercial canned and kibbled diets.

Introduction In humans, ageing is associated with changes in the microbiota with dramatic changes in composition in early life, relative stability in adult life, and then followed by substantial fluctuations in elderly life¹. These changes may explain the increase in many chronic diseases in humans, including type-2 diabetes associated with ageing. Pets have increasing life expectancies and are exhibiting the same types of diseases. While there are a few studies investigating the impacts of diets on gastrointestinal microbiota in young cats², the impacts of ageing in older cats has not been explored. This study examined the impact of diet and ageing on faecal microbiota in a cohort of 5 year old cats maintained on either a kibbled or canned diet since weaning.

Material and methods Kittens were weaned at 8 weeks of age and assigned to either a moderate protein:fat:carbohydrate kibble (35:20:28 % dry matter; n=10) or high-protein:high-fat:low-carbohydrate canned (45:37:2 % dry matter; n=10) diet. DNA was extracted from faecal samples collected at 8 weeks and 5 years of age and microbiota profiles were determined by pyrotag sequencing of the V4 to V6 region of the bacterial 16S rRNA gene³.

Results The relative abundance of Actinobacteria was higher in kittens fed kibble diets, which decreased as the cat aged (Table 1). Bacteroidetes were less prevalent in younger cats fed kibble, as compared to canned diets, although this was not observed as the cats aged. In contrast, Firmicutes were more prevalent in young cats fed kibble diets compared to canned, however these differences were also not observed at 5 years. Fusobacteria were more prevalent in cats fed canned diets both at 8 weeks and 5 years of age, compared to those fed the kibble diet. Proteobacteria abundances were similar in young cats on both diet treatments, but decreased more markedly in the cats fed the canned diets at 5 years of age.

Table 1 Age and diet effects on bacterial abundances (% of sequence reads) in the faeces of cats.

Phylum	Time	Kibble (Mean)	SEM	Canned (Mean)	SEM	P-value (Diet)
Actinobacteria	8 weeks	3.92	0.7	0.33	0.8	0.002
	5 years	0.69	0.1	0.22	0.8	0.030
Bacteroidetes	8 weeks	17.07	0.2	30.44	2.2	0.001
	5 years	41.22	4.5	30.90	4.4	0.122
Firmicutes	8 weeks	72.50	4.4	47.68	5.9	0.005
	5 years	54.16	4.4	59.05	5.1	0.486
Fusobacteria	8 weeks	1.84	1.2	17.08	3.8	0.005
	5 years	0.76	0.2	7.83	2.1	0.011
Proteobacteria	8 weeks	4.57	1.7	4.14	0.5	0.819
	5 years	3.03	0.3	1.80	0.4	0.047

Conclusion The effects of diet on bacterial abundances are consistent with those reported previously in the cat². Increased levels of Actinobacteria and Firmicutes have been reported in geriatric cats⁴, whereas in humans ageing tends to decrease the levels of Firmicutes. Increased levels of Bacteroidetes have been observed in ageing mice⁵ and elderly humans¹, in agreement with our results. In conclusion, age and diet affect the faecal microbiome of cats. The long-term impacts of these factors on metabolic health (e.g., glycaemic responses), remained to be explored.

Acknowledgements We acknowledge funding from the New Zealand Government (MBIE; C10X1501).

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Feed efficiency correlations in beef cattle offered zero-grazed grass and a high-concentrate diet

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Application The low correlations obtained for feed efficiency (FE) measures between the dietary phases may have implications for cattle breeding values derived on energy dense, high concentrate diets.

Introduction Feed efficient cattle are central to the economic and environmental sustainability of beef production. Breeding values for FE in beef cattle are usually generated using energy-dense high-concentrate diets, whereas commercially, cattle are predominantly produced on (grass) forage-based diets. Ranking of beef cattle for FE needs to be consistent over the different dietary and physiological phases of their life. However, there is evidence of FE re-ranking in cattle offered concentrate-based diets differing in energy density (Durunna *et al.* 2011). The objective of this study was to examine the within-animal correlation for intake, growth and FE in Charolais (CH) and Holstein-Friesian (HF) steers offered zero-grazed grass and a high concentrate diet.

Material and methods One hundred and seventy seven steers comprising of 92 CH and 85 HF were used. Following a dietary adaption period, individual dry matter intake (DMI) and growth were measured over two 70-d feeding phases 63 d apart. Throughout the interim period they either grazed pasture or were offered grass silage indoors. For phase 1 steers were offered zero-grazed grass (DM 196 g/kg) *ad libitum* and for phase 2 they were offered concentrate (860 g/kg rolled barley, 60 g/kg soya bean meal, 60 g/kg molasses, and 20 g/kg minerals and vitamins) *ad libitum* plus a restricted (~10% DM of total DMI) allowance of grass silage daily. The grass herbage was harvested twice daily from rotationally zero-grazed *Lolium perenne* dominant swards. The residuals of the regression of DMI on ADG, mid-test metabolic body weight (BW), within each breed, were calculated using the general linear models procedure in SAS, and were used to compute individual residual feed intake (RFI) coefficients for each dietary phase. Within breed and phase, animals were divided into equal terciles and labelled high (i.e. inefficient), medium and low RFI. 'Repeatability' for average daily live weight gain (ADG), DMI, Gain:Feed (G:F) and RFI between the two feeding phases was estimated using Pearson correlation coefficients.

Results In phase 1 high-RFI CH steers consumed 0.03 and 0.07 more, and high-RFI HF steers consumed 0.04 and 0.09 more, than their medium and low counterparts, respectively ($P < 0.001$) (Table 1). Corresponding values for phase 2 were 0.08 and 0.14, and 0.09 and 0.14 ($P < 0.001$). In each dietary phase, mid-test BW and ADG did not differ ($P > 0.05$) between the RFI classifications for CH and HF.

Table 1 Feed intake and growth traits for CH and HF steers ranked high, medium and low RFI in dietary phase 1 and 2

Trait	Breed	Zero-grazing phase					High-concentrate phase				
		High	Medium	Low	s.e.m	P	High	Medium	Low	s.e.m	P
DMI (kg/d)	CH	9.4 ^a	9.1 ^b	8.7 ^c	0.08	***	12.4 ^a	11.4 ^b	10.7 ^c	0.16	***
	HF	10.0 ^a	9.6 ^b	9.1 ^c	0.11	***	13.6 ^a	12.4 ^b	11.7 ^c	0.21	***
Mid-test BW (kg)	CH	563	560	566	7.1	n.s.	727	725	722	9.5	n.s.
	HF	486	500	489	9.8	n.s.	666	656	673	12.4	n.s.
ADG (kg)	CH	1.34	1.35	1.34	0.035	n.s.	1.42	1.32	1.39	0.052	n.s.
	HF	1.26	1.27	1.25	0.045	n.s.	1.28	1.28	1.31	0.071	n.s.

Table 2 Pearson correlation coefficients

Trait	Breed		
	CH	HF	
ADG	-0.01	0.05	In both breeds, moderate correlations were obtained for DMI ($P < 0.01$) and low correlations ($P < 0.10$) were obtained for RFI, between the two dietary phases (Table 2). Contrastingly, no correlations were observed for ADG and G:F.
DMI	0.52***	0.45***	
RFI	0.18†	0.19†	
G:F	-0.07	-0.02	

† $P < 0.10$, ** $P < 0.01$, *** $P < 0.001$

Conclusion Dry matter intake and, to a lesser extent, RFI are somewhat repeatable traits, whereas G:F and ADG are not, when cattle are offered zero-grazed grass and a high-concentrate diet.

Acknowledgements This project was funded by the Irish DAFM, Research Stimulus Fund

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Milk yield, milk composition, and milk fatty acid profile in Holstein dairy cows fed a pomegranate peel extract

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Application Dietary supplementation of pomegranate peel extract (PPE) has increased milk yield and improved the quality of milk and milk fatty acid profile in Holstein dairy cows.

Introduction Public concern about the use of antibiotics in livestock rations to alter the rumen microbiota have led to the use of natural replacements such as plant secondary metabolites (PSM). These were found to have a positive effect on ruminal fermentation parameters, by manipulating bacterial populations involved in ruminal biohydrogenation altering the fatty acid profile of ruminant-derived food products such as milk (Cabbidu *et al.*, 2009; Turner *et al.*, 2005). Pomegranate peel (PP) is a by-product of pomegranate juice extraction and contains PSM such as polyphenolic compounds, primarily punicalagin and ellagitannins, which can improve ruminal fermentation parameters and inhibit ruminal biohydrogenation. Therefore, we hypothesized that the inclusion of PP extract (PPE) into the diet would improve milk yield and fatty acid profile. This experiment was carried out to assess the effect of administering three concentrations of water-extracted PPE on milk yield, milk composition, milk efficiency, and milk fatty acid profile in dairy cows.

Materials and methods Sun-dried PP was extracted at 1g PP/ml of water. The peel was soaked in tap water at 40°C for 72h in a closed tank. The contents were then filtered and the filtrate was stored at 4°C for further use. The experiment was carried out to determine the effects of different levels of PP extract (PPE), which were mixed with the concentrate part of the ration, in the diet on the milk yield and milk fatty acid profile of dairy cows. Four Holstein cows were used in a 4x4 Latin square, simple changeover, design using the MIXED procedure with 28-d periods and 4 treatments which contained 45:55 forage to concentrate ratio and were either PPE0 (no extract), PPE400 (400 ml PPE/cow/d), PPE800 (800 ml PPE/cow/d) or PPE1200 (1200 ml PE/cow/d). Dry matter intake (DMI), milk yield and milk composition were measured (Table 1).

Results Milk yield, milk fat and protein yield (kg/d), and milk efficiency were increased by inclusion of PPE800 in the diet. PPE800 had significantly lower $\omega 6/\omega 3$ ratio and higher content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than milk from cows that received no PPE.

Table 1 Dry matter intake (DMI, kg/w^{0.75}), milk yield (4% FCM, kg/d), milk composition, milk efficiency (kg/kg), and milk fatty acid profile (g/100 g of total fatty acids) of Holstein cows fed PPE supplemented diets

	Diets				SEM	P-value	
	PPE0	PPE400	PPE800	PPE1200		Linear	Quadratic
DMI (kg/w ^{0.75})	4.20	3.84	3.77	4.03	0.135	0.667	0.271
Milk yield	25.2 ^b	28.4 ^{ab}	29.9 ^a	26.6 ^{ab}	1.34	0.360	0.049
Fat yield (kg/d)	1.00	1.13	1.12	1.04	0.030	0.439	0.012
Protein yield (kg/d)	0.96 ^b	1.07 ^a	1.11 ^a	1.02 ^{ab}	0.027	0.119	0.012
Milk efficiency	1.187 ^c	1.372 ^{ab}	1.383 ^a	1.255 ^{bc}	0.058	0.435	0.035
Fatty acid							
C18:0	3.46 ^a	2.41 ^b	2.29 ^b	2.83 ^{ab}	0.219	1.33	0.083
C18:3 c (n-3)	0.11 ^{cb}	0.10 ^b	0.13 ^a	0.11 ^b	0.004	0.046	0.274
C20:3	0.03 ^a	0.007 ^b	0.03 ^a	0.02 ^a	0.003	0.764	0.023
C20:4	0.05 ^a	0.02 ^b	0.04 ^a	0.05 ^a	0.004	0.067	0.011
C22:0	0.01 ^a	0.00 ^b	0.01 ^a	0.01 ^a	0.002	0.012	0.014
DHA (C22:6)	0.006 ^a	0.06 ^a	0.04 ^a	0.01 ^b	0.008	0.575	0.009
EPA (C20:5)	0.001 ^b	0.05 ^a	0.03 ^a	0.01 ^b	0.007	0.707	0.012
$\omega 6/\omega 3$	7.00 ^a	2.98 ^b	4.20 ^b	6.88 ^a	0.559	0.872	0.001

EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid.

Conclusion

The results suggest that PPE supplementation at the PPE800 level leads to improved milk yield, milk composition, milk efficiency, and an improvement in the milk fatty acid profile in dairy cows.

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The effect of dog safety education on the recognition of dog visual behavioural cues in “at risk” scenarios in primary school children in the UK

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Application Only 43% of primary school-aged children recognised scenarios associated with potential dog aggression, but education projects may be effective in increasing understanding.

Introduction Dog attacks on children have become an increasing concern on an international scale, with research identifying a false sense of security and a significant lack of awareness regarding warning signals and safe dog interaction in children (May and McBride, 2014). Recent high-profile cases in the UK have raised wide societal discussion, and a number of awareness projects are currently underway. However, with the majority of previous research being performed outside the UK, the aim of the current study was to identify and compare children’s understanding of dog behavioural cues in at-risk scenarios within UK primary schools.

Material and methods Primary school children (n=563) across years 2-6 (ages 6 – 11) in three primary schools in the Borough of Chelmsford, Essex, were asked to complete a questionnaire in class. One of the three schools (school A) used a school dog and a Dogs Trust workshop during year 3 to educate pupils on dog safety. This workshop took place before the questionnaire was administered. School tutors distributed and collected the questionnaire, but did not coach the pupils. Respondents were asked to identify dogs’ visual behaviours from photos displaying scenarios in which children are commonly bitten (Lakestani *et al.* 2014) by matching them to images from a modified Wong-Baker FACES® scale (Keck *et al.* 1996) displaying sad to happy faces. Additionally, children were asked to judge statements describing their reaction to dog visual cues in various scenarios by selecting either a “thumbs up” or “thumbs down” answer. The percentage of correct answers between all three schools was analysed using a one-way ANOVA. To assess the effects of the dog workshop the results of school B and C were combined and Fishers Exact tests to identify associations between attendance at a dog workshop and recognition of at risk scenarios were performed. This study was approved by the Writtle University College Ethics and Animal Welfare Committee on 9 November 2015. Consent from parents/carers and school head teachers was obtained before the questionnaire was distributed.

Results Overall, school A children gave more correct answers ($59.0\% \pm 1.20$) than schools B and C ($43.9\% \pm 1.43$ and $42.9\% \pm 1.47$ respectively, $P < 0.001$), indicating that pupils who had received a dog education workshop answered more questions correctly. Signs of aggression were most often identified correctly, with no effect of dog safety education (Fisher’s Exact, $P = 0.9$). However, Fishers exact analysis showed scenarios relating to resource guarding ($P < 0.001$), food protection ($P < 0.001$), lactating ($P < 0.001$), sleeping ($P < 0.001$) and identifying the meaning of a yellow ribbon (an international sign recognising “a dog needs space”) tied to the dog’s collar ($P < 0.01$) were recognised correctly more often by pupils who received dog safety education compared to pupils who had not received dog safety education. From year 4 onwards pupils scored significantly better (Figure 1).

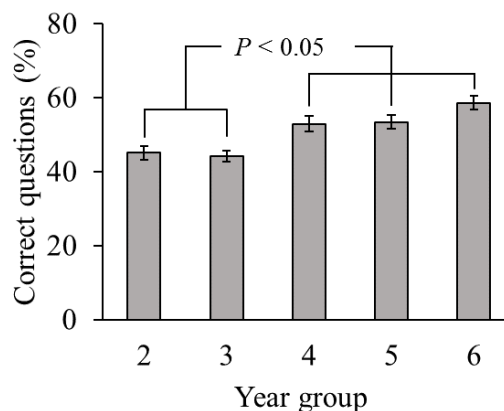


Figure 1 Correct questions (%) by year group. Data represent mean + SEM. Unequal superscripts indicate significant difference (One-way ANOVA, $P < 0.01$).

Conclusion Most pupils (65 %) in this study showed a concerning lack of understanding of visual behavioural cues in dogs in “at risk” situations, especially in years 2 and 3. However, pupils who received dog safety demonstrations scored significantly better. This indicates that dog safety sessions in the classroom could be an effective tool to raise childrens’ awareness of safety around dogs.

Acknowledgements The authors are grateful to the staff and pupils who gave up their time to take part in this study and to Dr Jonathan Amory for useful feedback on earlier versions of this paper.

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Digestibility and nitrogen balance in beef cattle offered autumn grass herbage differing in fertiliser nitrogen application rate

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Application Reducing fertiliser nitrogen (N) application rate is a means of reducing N excretion in beef cattle consuming autumn grass.

Introduction Cattle contribute significantly to emissions of N to the environment as their efficiency of dietary N utilisation is typically low (Dijkstra *et al.*, 2013). Nitrogen intake has been identified as the primary driver of N excretion in cattle. Compared to dairy cows, less is understood about the relationships between nutrition and N excretion for beef cattle, and even less so regarding cattle consuming fresh grass, which is often the cornerstone of beef production systems in temperate climates. Therefore, the objectives of this study were to examine the effects of fertiliser N application rate on autumn grass chemical composition, and the intake, digestion and N-balance of beef cattle consuming this grass.

Material and methods Treatments comprised of rotationally zero-grazed *Lolium perenne* dominant swards harvested ca. 21 d post application of one of two inorganic fertiliser N application rates - 15 (LN) and 80 (HN) kg N/ha/rotation – during August/September. Herbage was harvested daily at 08.00 h to a stubble height of 4 cm using a zero-grazer. Four steers (611±8.2 kg) were used in a 2 (treatments) × 2 (periods) cross-over design experiment. Each period consisted of 24 d with 16 d of dietary adaptation and then 8 d of sampling in purpose-built metabolism stalls. Herbage was offered *ad libitum* in two equal meals daily during the adaptation phase and subsequently at 0.9 of the pre-determined dry matter intake (DMI). Total faeces and urine (in 9 M sulphuric acid) were collected on a 48 and 24 hour basis, respectively. Data were statistically analysed using the GLM procedure of SAS. The statistical model had fixed effects for treatment, animal and period.

Results Herbage DM concentration was 26 g/kg lower, and CP concentration was 35 g/kg DM higher for HN compared to LN (Table 1). Observed treatment differences in herbage DMI, *in vivo* DM digestibility and N intake were non-significant ($P>0.05$). Digestible N intake was higher ($P<0.05$) for HN compared to LN. Mean total N and urine N losses were, respectively, 40 g/d and 44 g/d greater ($P<0.05$) for HN compared to LN, but faecal N loss was not significantly different ($P>0.05$) between treatments. Nitrogen retention and therefore, N-use efficiency did not differ ($P>0.05$) between LN and HN (25 vs. 22 %).

Table 1 Herbage composition (s.d), and intake, digestibility and nitrogen balance (s.e.m) in beef cattle consuming autumn grass differing in fertiliser nitrogen application rate

	Low Nitrogen	High Nitrogen	s.e.m	P-value
Herbage composition				
Dry matter (g/kg)	172 (8.3)	146 (11.8)	-	-
Crude protein (g/kg DM)	127 (4.1)	162 (31.7)	-	-
Dry matter intake (kg/day)	8.9	8.6	0.51	0.74
<i>In vivo</i> dry matter digestibility (g/kg)	773	782	9.9	0.59
Nitrogen balance				
Nitrogen intake (g/day)	182	223	10.7	0.11
Digestible nitrogen (g/kg)	690	760	13.0	0.05
Nitrogen loss (g/day)	136	176	4.1	0.02
Urine nitrogen loss (g/day)	80	124	6.1	0.03
Faecal nitrogen loss (g/day)	55	52	2.7	0.45
Retained nitrogen				
g/day	47	46	9.0	0.96
g/kg Nitrogen intake	246	216	32.5	0.58
g/kg absorbed	347	287	49.1	0.84
mg/kg bodyweight	75	77	14.9	0.92

Conclusion Reducing fertiliser N application resulted in lower herbage N concentration and lower (urinary) N excretion but had no statistically significant effect on DMI, apparent dry matter digestibility and N-use efficiency.

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Effect of Docosahexaenoic acid (DHA) supplementation on equine (*Equus caballus*) reactivity and physiological (stress) response

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Application This study was carried out to determine whether the presence of Docosahexaenoic acid within the diet of *Equus caballus* could reduce physiological response and therefore act as a calmer to potentially improve welfare.

Introduction There are a variety of supplements available claiming to reduce the equine stress response available for horse owners to purchase (Nicol, 2005). This study aimed to determine if the physiological responses of horses could be altered subsequent to consumption of DHA with Flavonoids, which is an antioxidant (QLC) that has been proven to reduce stress and increase cognitive ability in humans Meyer, 2016).

Material and methods Prior to DHA supplementation a random sample of horses ($n = 15$) from Sparsholt College Equine Centre underwent a novel object umbrella test (Malmkvist, 2007) in order to establish their reaction test score, two observers rated the horses' reactivity on a scale of 1-5, and the mean result was assigned to each horse. Resting heart rate using a Polar Equine heart rate monitor, and mean eyeball temperature at rest were recorded, using a FLIR E4 thermal imaging camera. Continuous focal sampling was used to record behaviours when performing a maze test then a second eyeball temperature reading taken on completion of the maze (Malmkvist, 2007). A barrier constructed to limit access to a feed source was used within an indoor arena, horses were required to navigate around it to reach the food source. The feeding ingredients are all sourced from Dodson & Horrell Ltd, for the supplement were as follows DHA / Flavonoid treatment: 11% starch binder, 17% lucerne, 5% fructose, 40% of DHA gold, 27% QLC, DHA treatment: 11% starch binder, 44% Lucerne, 5% fructose, 40% of DHA gold, Control treatment: 11% starch binder, 84% lucerne, 5% fructose Horses were allocated to three treatment groups ($n = 5$) balanced by reactivity scores. Each group continued their existing concentrate diet, the control group (1) with no additions, Group 2 with 150g DHA daily and Group 3 150g of DHA+QLC over a six week continuous period. At the end of the treatment period horses were retested for reactivity and physiological responses during the maze test, resting heart rate was also measured post treatment.

Statistical analysis: All heart rate and eye temperature data were normally distributed, and differences tested using a one-way analysis of variance (ANOVA). Behavioural indicators of stress were grouped together to provide a sum of behaviours for each group of horses and tested using a Chi Squared test.

Results Reactivity scoring reduced significantly after DHA + QLC supplementation when compared to both the control group and the DHA only supplementation group ($F=6.68, P=0.011$). Change in eye ball temperature was not significantly different in horses on any supplement ($F=0.96, P=0.410$) nor was the change in resting heartrate ($F=0.67, P=0.529$). Behaviours observed during the maze test showed no significant differences between groups ($X^2=0.36, DF=2, P=0.83$).

Conclusions Results confirmed that there were significant reductions in reactivity scores of horses consuming DHA+ QLC compared to DHA and control, therefore feeding DHA+QLC could be utilised to reduce reactivity within horses. This could be of benefit for owners with highly reactive horses and may improve trainability or performance within this population. However DHA only and DHA+QLC supplementation appears to not significantly reduce commonly recorded indicators of stress, meaning that future research of supplementation effect using only physiological parameters could furnish misleading results. Future studies should include pre and post treatment reactivity scoring to provide greater insight into supplementation impact.

Acknowledgements Dodson & Horrell Ltd for providing the supplement.

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The use of cholecalciferol supplementation in beef heifers, to increase beef vitamin D content and improve beef tenderness and sensory evaluation

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Application The biofortification of beef is a potential food based strategy for increasing human vitamin D dietary intake, with proceeding benefits of improved beef tenderness.

Introduction Vitamin D deficiency is now regarded as a major issue in northern Europe. There is now growing evidence that low vitamin D status can now be linked to non-skeletal health problems such as various cancers, muscular weakness, multiple sclerosis, type 1 diabetes and depression. Increasing vitamin D intake by dietary means has become extremely important, however only few rich natural sources of vitamin D exist. Beef is among the few dietary staple foods that contain naturally occurring vitamin D. Recently, there has been a growing interest in the bio-addition approach as a means for increasing vitamin D intake through biofortification of livestock feeds. Although, there is limited research investigating this biofortification of beef animal diets using allowable EU vitamin D levels. The use of supplemental vitamin D₃ prior to slaughter in beef animals may also have additional benefits of improving meat tenderness. The objective of the present study was to examine the effect of Cholecalciferol (vitamin D₃) inclusion rates allowable in the EU in be heifers on beef vitamin D content and its subsequent effects of beef tenderness and sensory quality.

Material and methods Thirty continental (Charolais + Limousin cross) heifers were blocked (on the basis of live weight and age) and randomly allocated to 3 dietary treatments (T1) basal + 0IU D₃, (T2) basal + 2000IU D₃ and (T3) basal + 4000IU D₃. Experimental design was a randomised complete block design. Dietary treatments were offered for the final 28 days of an 80 day intensive finishing period. The basal diet consisted of a standard ad-libitum finishing regime of concentrates and forage (straw) offered at a ratio of 90:10. Animal growth was recorded weekly and individual dry matter intakes were recorded using Calan Broadbent controlled feeding system. *Longissimus dorsi* (LD) muscles were excised 10 days post slaughter for vitamin D₃ analysis carried out by analytical High Performance Liquid Chromatography. *Longissimus dorsi* warner bratzler shear force (WBSF) analysis was carried out according to Wheeler *et al.* (1996) with minor modifications. Sensory analysis of cooked LD steaks was performed in duplicate by a total of 40 naïve assessors over two analysis days as described by O'Sullivan *et al.* (2003). Data was analysed using the MIXED procedure of SAS (SAS, version 9.4). The experimental model included fixed effects of treatment and pen, with block included as a random effect.

Results *Longissimus dorsi* total vitamin D content linearly increased ($P < 0.001$) as dietary cholecalciferol levels increased as illustrated in figure 1. Heifers fed 4000 IU of vitamin D₃, had higher LD cholecalciferol and 25-hydroxyvitamin D₃ metabolite activity, compared with those fed 0 IU of vitamin D₃ and 2000 IU of vitamin D. Heifers offered 4000 IU of vitamin D₃ diet had a decreased LD WBSF value ($P < 0.05$), after 10 days chilling post slaughter, compared to other dietary treatments. Mean sensory scores parameters including appearance, odour, texture, flavour, overall accept and off flavour were not affected ($P > 0.05$) by dietary treatments offered. Similarly, dietary treatment had no effect ($P > 0.10$) on animal performance parameters or any carcass related variables.

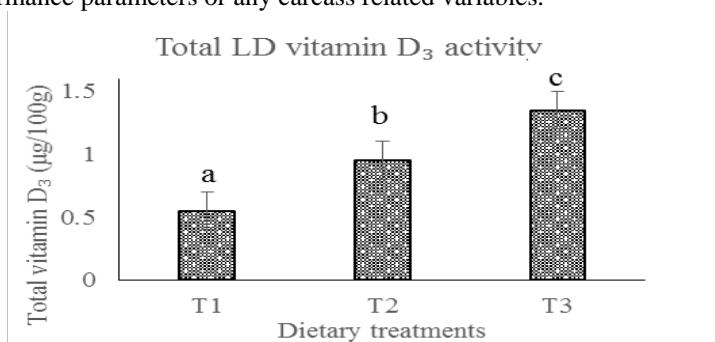


Figure 1 Effect of dietary treatment on total LD vitamin D₃ activity.

Conclusion In conclusion results indicate that vitamin D₃ content of beef can be successfully enhanced through short term dietary supplementation. The improvement of beef tenderness through vitamin D supplementation, may also be beneficial to the beef industry to improve beef quality.

Acknowledgements The authors gratefully acknowledge funding from Irish Department of Agriculture, Food and Marine under its Food Institutional Research Measures.

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Productive traits and blood insulin alterations by two Saudi local sheep breeds supplemented by Quebracho tannins in their diets

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Application Tannins represent a significant constituent of forages that might alter the metabolic as well as productive performance of grazing animals. The association of the tannins in feedstuffs with animal metabolism and productivity still ambiguous.

Introduction Tannins were found to have a positive effect on reducing crude protein (CP) degradation in the rumen (Al-Dobaib 2009) and increasing the CP reaching the small intestine which animal utilizes for milk and meat production. Therefore, this study aimed at investigating the consequences of supplementing various levels of tannins to growing sheep lambs of two local breeds [i.e. Najdi (NJ) and Noemi (NO)] on their circulating concentrations of insulin relative to their productive traits. Recently, Attia *et al.* (2016) showed changes in insulin due to supplementing dairy cows with quebracho tannins.

Material and methods Forty eight growing male lambs (n=24/breed) were randomly allocated into four treatment groups (6 animals/group/breed). Quebracho tannin is a commercial name of condensed tannins derived from Quebracho trees raised in south America. It has the ability to precipitate the dietary protein to avoid its ruminal degradation. Lambs were fed alfalfa hay and barely grains with Quebracho tannin (QT) at levels of 0 (control), 1, 2 and 3% of DM in the diets of the four experimental groups (QT0, QT1, QT2 and QT3, respectively) for a period of 120 days. Jugular blood was collected from 3 animals per treatment within a breed once a week for serum determinations of insulin. Daily feed intake, average daily gain and feed conversion ratio were estimated. Significant differences were considered at ≤ 0.05 . Data were statistically analysed using GLM procedure of SAS using the following model:

$$Y_{ijk} = \mu + T_i + B_j + TB_{ij} + e_{ijk}$$

Where Y_{ijk} = parameter measured on ijk^{th} animal, μ = overall mean, T_i = effect of i^{th} QT level, B_j = effect of j^{th} breed, TB_{ij} = effect of two order interaction of T_i and B_j , and e_{ijk} = random error. Hormone data were analysed by the least square analysis for repeated measures on the same animal.

Results Circulating insulin levels were not different ($P > 0.05$) among tannin levels, whereas insulin concentrations were significantly ($P < 0.05$) higher in Noemi (71.22 ± 3.56 $\mu\text{IU/ml}$) than Najdi (52.2 ± 3.13 $\mu\text{IU/ml}$) lambs. **Results** showed that total dry matter intake (TDMI/120 days) was higher ($P < 0.05$) for NJ than NO, while relative gain (RG) and feed conversion ratio (FCR) were better ($P < 0.05$) for NO than NJ. Total gain (TG) and average daily gain (ADG) were not affected by sheep breed. Regardless of breed, lambs supplemented with QT 2% showed the highest ($P < 0.05$) TG and ADG and the best FCR, however TDMI and RG were not affected by QT level.

Table 1 Effect of breed and tannin level on some productive traits and insulin level

Breed	Tannin level	Total intake (kg)	Total gain (kg)	ADG* (g/d)	RG **	FCR***	Insulin (μIU)
Najdi	TQ 0	181.9 \pm 5.53	23.8 \pm 1.01	198 \pm 8.00	0.90 \pm 0.06	7.73 \pm 0.44	46.12 \pm 6.10
	TQ 1	200.6 \pm 5.03	27.1 \pm 1.06	226 \pm 9.00	1.00 \pm 0.07	7.47 \pm 0.38	43.35 \pm 5.77
	TQ 2	186.3 \pm 6.55	28.9 \pm 0.90	241 \pm 7.00	0.94 \pm 0.03	6.49 \pm 0.32	59.70 \pm 7.11
	TQ 3	200.7 \pm 8.55	26.9 \pm 1.30	224 \pm 11.00	0.94 \pm 0.07	7.53 \pm 0.39	60.10 \pm 8.35
Noemi	TQ 0	162.9 \pm 9.43	24.5 \pm 2.30	204 \pm 19.00	1.12 \pm 0.12	6.80 \pm 0.39	75.79 \pm 8.21
	TQ 1	167.9 \pm 8.80	25.9 \pm 1.60	215 \pm 13.00	1.19 \pm 0.10	6.53 \pm 0.24	72.15 \pm 6.91
	TQ 2	151.8 \pm 10.6	27.6 \pm 1.75	230 \pm 15.00	1.12 \pm 0.11	5.51 \pm 0.24	70.88 \pm 7.38
	TQ 3	148.8 \pm 6.60	24.8 \pm 1.56	206 \pm 13.00	1.18 \pm 0.09	6.10 \pm 0.383	66.15 \pm 8.12
Significance level	B	<.001	0.37	0.36	<0.001	<0.001	<0.01
	QT	0.27	0.07	0.05	0.78	0.006	0.55
	B \times QT	0.24	0.82	0.82	0.98	0.86	0.76

ADG: *Average daily gain; ** RG: Relative gain= Total gain (kg for 120 d)/ Initial body weight (kg); FCR: Feed conversion ratio = Kg intake/ kg gain.

Conclusion Supplementing sheep diet with Quebracho tannins at 2% could be a good approach to improve metabolic and productive efficiency.

Acknowledgements The author extends his gratitude to Prof; Suliman N. Al-Dobaib and Prof; A. Babji for guiding the study.

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Differences in conformation of Holstein heifers in herds adopting different grazing systems

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Application Heifers in dairy herds operating different grazing systems have significantly different conformation scores. This work demonstrates that type classification of heifers can be used to breed cows adapted to specific farming systems.

Introduction Type Classification is a functional tool used for the improvement of individual and whole herd conformation whilst promoting increased production and animal longevity (The CDI, 2016). The classification system provides a scaled image of the animal through the assessment of trained Classifiers (IHFA, not dated) of 22 linear type traits (conformation traits), and 4 subjectively assessed composite scores (Holstein UK, 2016). Research has established associations of type traits with numerous health traits but little research has investigated type classification scores in cows farmed in different systems.

This project compared the linear type scores of heifers in commercial herds operating different grazing systems.

Material and methods The grazing system data were collected as part of a structured interview conducted by the CDI classifiers during classification visits to 261 commercial Holstein herds over a 4 month period between December and April 2016. 22 herds operated extended grazing (EG), 76 permanently housed (PH) the herd and 163 grazed cows in the spring-summer season only (SG). The conformation data of all heifers classified in those herds since 2012 (4 539) were used to compare the mean herd score at each classification visit for 3 composite conformation traits (mammary, feet & legs and body conformation), overall type score and 21 individual linear type traits. The data was analysed using one-way ANOVA and Tukey's test.

Results The feet and leg composite scores of heifers in grazing herds (EG and SG) were significantly higher ($P<0.001$) compared to heifers in PH herds, but there were no significant differences in scores for overall type, mammary or body conformation.

Heifers in EG and SG herds had significantly lower ($P<0.01$) scores for rear udder height and width than those in PH herds, and heifers in EG herds had significantly lower ($P<0.01$) lower (wider) scores for teat placement (rear, front and position) than those in SG and PH herds.

Heifers in EG and SG herds had significantly lower foot angles, and rear leg set than heifers in PH herds. Heifers in EG herds had significantly lower stature ($P<0.01$), angularity and rump angle scores ($P<0.05$) than heifers in SG and PH herds.

Table 1 Mean (\pm se) feet and leg type scores of heifers in herds with different grazing systems

Herd type	Rear Leg Set	Foot Angle	Bone Quality	Rear Leg Rear View	Locomotion
Extensively Grazed (EG)	5.07 \pm 0.108 b	4.85 \pm 0.103 b	6.11 \pm 0.099	5.61 \pm 0.113	5.27 \pm 0.096 b
Spring-Summer Grazed (SG)	4.94 \pm 0.040 b	5.02 \pm 0.038 b	6.08 \pm 0.036	5.45 \pm 0.041	5.24 \pm 0.035 b
Permanently Housed (PH)	4.72 \pm 0.053 a	5.28 \pm 0.051 a	6.07 \pm 0.049	5.47 \pm 0.056	5.01 \pm 0.048 a
Significance	$P<0.001$	$P<0.001$	n.s.	n.s.	$P<0.001$

Conclusion Small, but statistically significant, differences in conformation were found between heifers classified in herds with different grazing systems. Heifers in grazing systems tended to be smaller and less angular, with lower foot angles and wider teat placement than heifers in herds that permanently housed cows.

Acknowledgements

The authors gratefully acknowledge Holstein UK and the Centre for Dairy Information Classification team for data collection.

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Modelling the food-feed competition for agricultural land under heterogeneous soil fertility

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Application The proposed framework allows us to investigate the consequences of the food-feed competition for land under a realistic setting, and thus helps derive a clear pathway for the UK livestock industry to achieve sustainable intensification.

Introduction Pasture based ruminant production systems can utilise land unsuitable for food production and therefore have the potential to contribute to global food security. This concept is of particular importance in the UK context, as only 33% of the country's agricultural land is estimated to be croppable (Defra, 2016). At the same time, ill-considered expansion of grasslands could also lead to reduction of human-edible cereals and unnecessary global warming, and therefore the role of livestock production in society has to be defined in a clear manner (Eisler *et al.*, 2014). To date, investigations into the issue of the food-feed competition for land have primarily been conducted by the means of economy-wide land allocation modelling (Van Kernebeek *et al.*, 2016). However, the vast majority of these models implicitly impose the assumption that the government can freely designate agricultural land use ('dictatorship assumption') and fail to show the policy mechanism with which to achieve the resource allocation shown to be optimal. The objective of the present study was to design a general equilibrium model (a class of economic models in which all input and output markets within the economy clear simultaneously), whereby the aforementioned resource competition between livestock and humans is internalised.

Material and methods The model was set up with three players, namely a representative producer, a representative consumer and the government. The representative producer tries to maximise their profit by allocating the total land available to them between arable cereal production and grasslands, the latter of which is used for pasture-based livestock production. The marginal productivity of cereal production drastically decreases as they expand the arable land, while the marginal productivity of livestock production remains relatively constant regardless of the area of grassland. The representative consumer tries to maximise their utility by allocating the total budget available to them between cereal consumption and meat consumption. For both the commodities, the marginal utility decreases as the level of consumption increases. Being the only 'citizen' within the economy, the representative consumer also owns the representative producer, and the latter's profit is returned to the former in the form of dividends. The government tries to maximise the social welfare, hereby defined as the weighted sum of the representative consumer's utility and the (negative value of) environmental footprint, by intervening with the market by means of imposing production tax against meat production (or equivalently against landholdings for pasture-based livestock production) at a constant rate, as suggested by a recent study Springmann *et al.* (2016). Because this tax has an effect to decrease the effective meat price for producers, meat production is expected to decrease post-taxation. The tax revenue raised through this intervention is immediately given back to the representative consumer, so as to ensure that the market becomes distorted from the free-market equilibrium without draining any resource from the economy. The model was found to have a mathematically explicit solutions and thus could be solved either analytically or computationally.

Results Irrespective of parameters set to define the distribution of land productivity, consumer preference and the overall social welfare, the meat production tax was shown to have a monotonically (but not linearly) negative effect on livestock production. However, excessive taxation on livestock production was consistently found to be associated with lower social welfare, as a higher tax rate would deter livestock production, drive up the meat price and consequently reduce the representative consumer's purchasing power. The economy-wide production (real GDP) also shrank as a result of expansion of arable production into less fertile land, as resource use efficiency decreased because of the low marginal productivity.

Conclusion The above findings challenge the real-world applicability of dictatorship models and reiterate the importance of considering price effects in policy analysis. While the model was originally designed for the UK market, it is presently being calibrated for other member countries of Global Farm Platform (<http://www.globalfarmplatform.org>), the largest consortium of livestock research farms in the world. The resultant analysis is expected to contribute to evidence-based debates on the role of livestock production systems in the context of global food security.

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Case management of traumatic injury in an alpaca and of an outbreak of haemonchosis in an alpaca herd

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Application Description of the case management of traumatic injury to an alpaca and a herd outbreak of Haemonchosis suggest that both cases were manageable by a competent practitioner of limited camelid expertise..

Introduction Camelids may present to the veterinary surgeon with problems which resemble those affecting other species and demand a similar or identical approach. Therefore, the practising veterinary surgeon should not be immediately intimidated by their unfamiliarity with the species. Granted there may be species specific issues which require some knowledge of camelid physiology and anatomy, which may pertain to the presenting problem or may be relevant during periods of aftercare.

Material and methods This paper describes cases of camelids requiring veterinary assistance in which triage was completed by a referring veterinary surgeon and subsequent care continued by the University of Liverpool Farm Animal Practice referral services. It should be evident as the cases are described that much was accomplished by the primary veterinary surgeons and that given sufficient time, stabling and nursing staff, the care could have been continued. The herd problem described began as an emergency referral of an individual but then followed the lines of a parasitology investigation, similar to that which a veterinary surgeon would carry out for a cattle herd or flock of sheep.

Results We describe the case of an alpaca unfortunately attacked by a dog and sustain severe soft tissue injuries. It required general anaesthesia which is described, debridement of soft tissue damage to the axilla and ventral abdomen and replacement of the penis which had been displaced entirely from the prepuce and required novel methods to replace it ; this was followed by a period of nursing and soft tissue wound management with eventual creation of a urethrostomy.

The Haemonchosis investigation began as referral of an anaemic alpaca and concurrent deaths in the herd. Post mortem examination revealed a heavy burden of *Haemonchus contortus*. The treated individual required blood transfusion and subsequent colloid and crystalloid fluid therapy whilst the diagnosis also prompted assessment of risk factors to the herd. An anthelmintic protocol is described which includes 5 day courses of fenbendazole and a grazing strategy.

Conclusion The cases described illustrate how case management was not unique to South American Camelids and was successful.

Acknowledgements K.M. Mueller MVSc DCHP DipECBHM MRCVS; AHVLA surveillance centre, Leahurst, University of Liverpool.

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A reverse-transcriptase PCR assay to assess the survival time of 16s ribosomal RNA of *Mycoplasma hyopneumoniae*: A potential diagnostic methodology tool

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Application Development of a diagnostic tool using reverse-transcriptase PCR for the rapid detection of viable *Mycoplasma hyopneumoniae* organisms.

Introduction *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is the causative agent of enzootic pneumonia in pigs. *M. hyopneumoniae* affects pigs worldwide causing economic losses due to reduced growth rate, lowered market carcass value and increased veterinary costs (Maes *et al*, 2008). Control of this important disease requires specific and sensitive diagnostic tools. Culture is considered the 'gold standard' diagnostic, but due to the fastidious nature of *M. hyopneumoniae* culture techniques are often halted by contamination with other bacteria. Therefore other diagnostic tools are needed which are sensitive, specific and able to detect viable organisms. Reverse-transcriptase PCR (RT-PCR) is a rapid and highly sensitive diagnostic method recently used for the detection of other *Mycoplasma* species and has been shown to provide greater sensitivity than conventional culturing techniques, whilst indicating if the organism is still viable (Marois *et al*, 2002).

Material and methods *M. hyopneumoniae* laboratory strain 232 (confirmed by PCR) was cultured in Friis broth and was incubated at 37°C until an acid colour change (red to orange) was observed indicating growth. Once a colour change was observed, growth was quantified using serial dilution measuring the colour changing units (CCU). Once the CCU was performed, one ml of *M. hyopneumoniae* was centrifuged at 7200 x g and immediately killed by heat lysis (15 min at 95°C). Total RNA was extracted using PureLink[®] RNA Mini Kit (Lifetechnologies, UK) immediately after heat lysis (0), 1, 6 and 24 hours. RT-PCR was performed using QIAGEN[®] OneStep RT-PCR kit following the manufactures' instructions. *M. hyopneumoniae* 16S rRNA was reverse transcribed into cDNA with the RT Mixture. *M. hyopneumoniae* primers were based on Madsen *et al.*, (2006) and were evaluated for their suitability using the Pubmed Blast (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), Primer3 web (<http://primer3.ut.ee>) and IDT (<https://www.idtdna.com/calc/analyzer>) programmes. Products of the RT-PCR were analysed by gel electrophoresis in 1.5% agarose gel with 0.5 µg of ethidium bromide per ml. Doubled distilled water replaced the template RNA to act as a negative control and *M. hyopneumoniae* 232 without heat lysis was used as a positive control. The cellular RNA present in RT-PCR was degraded prior to gel electrophoresis by addition of 1 µL of RNase and incubated for 15 minutes at 37°C (Peredeltchouk *et al*, 2010). A culture comparison was used alongside the RT-PCR in order to validate the test.

Results *M. hyopneumoniae* RNA was detected by RT-PCR immediately (0) and one hour after heat lysis. No visible bands were seen at six or 24 hours on the gel. The culture comparison showed that no viable organisms were detected by CCU after heat lysis.

Conclusion Accurate and sensitive diagnosis of *M. hyopneumoniae* is crucial to understanding the pathogenesis and developing control programmes. The RT-PCR of 16S rRNA of *M. hyopneumoniae* was found to be viable up to one hour after death after heat lysis, suggesting that RNA is degraded in *M. hyopneumoniae* between one and six hours after heat lysis. These results show that RT-PCR can be used as a diagnostic tool to detect viable *M. hyopneumoniae* in pigs and the environment. Future work is now required to test the use of RT-PCR in the field.

Acknowledgements Professor Andrew Rycroft (The Royal Veterinary College) for the *M. hyopneumoniae* strain and Friis broth.

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