

Detection of dairy cow behaviour changes associated with claw horn lesions using biosensors

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Application Differences in dairy cow feeding behaviour detected by sensors may offer a method of predicting lameness on farms.

Introduction Recent technological developments in biosensors offer the potential for automatic real-time monitoring of animals. Early detection and therefore prompt treatment of lameness case reduces severity and duration of cases (Leach *et al.*, 2012). Observational studies have shown that changes in behaviour often precede clinical signs of disease and may therefore be useful predictors of disease (e.g. Gonzalez *et al.*, 2008). This study investigates the potential for a sensor combining location, activity and orientation to detect changes in time budget associated with lameness.

Material and methods All cows from a group of 120 high yielding dairy cows were fitted with neck collars mounted with Omnisense 500 ® sensors between July and December 2014. A support vector machine was used to classify cows as standing, lying or feeding similar to the method described by Vazquez Diosdado *et al.* (2015). Cows were mobility scored every 2 weeks using the DairyCo. mobility score (score 2 or 3 were classified as clinically lame). ZB observed all trimming sessions by the foot trimmer and all lesions treated by the farm staff were recorded by the researcher in a weekly interview. The 7 days prior to lesion treatment were used in the analyses for cows clinically lame with sole ulcers (SU) or white line disease (WL). Cows with no lesions and no lame scores during the study period were classified as non-lame. Standing, lying and feeding behaviour variables were compared for SU, WL and Non-lame cows using a one-way ANOVA (Genstat v17).

Results The duration of feeding bouts were significantly shorter for cows lame with SU compared with non-lame cows without lesions. There was also a non-significant trend for increased numbers of feeding bouts for cows with SU.

Table 1 Behaviour of cows lame with sole ulcer, white line disease or never lame with no lesions recorded

	SU	WL	Non-lame	SED [*]	p-value
Number of cows	9	6	8		
Number of Feeding Bouts	14.5	11.1	11.2	1.67	0.08
Mean Feeding Bout Duration (mins)	20.0 ^a	25.4 ^{ab}	32.5 ^b	5.12	0.05
Total Feeding Duration (hrs)	5.3	5.4	5.5	0.38	ns
Number of Lying Bouts	20.2	21.2	19.5	2.28	ns
Mean Lying Bout Duration (mins)	40.4	40.5	41.2	4.55	ns
Total Lying Duration (hrs)	12.5	13.0	12.7	0.65	ns
Number of Standing Bouts	22.6	20.8	18.1	2.50	ns
Mean Standing Bout Duration (mins)	8.4	7.9	7.2	1.05	ns
Total Standing Duration (hrs)	3.3	2.8	2.2	0.56	ns

^{*}SED; Standard Error of Difference. ^{a,b} Different letters in the same row indicate Tukey *post hoc* test difference P<0.05

Conclusion The pain associated with claw horn lesions whilst standing to feed may explain the larger number of shorter feeding bouts for the SU cows compared with the non-lame cows. WL may also cause a similar effect, but a larger sample size is needed to confirm this. Alternatively recent evidence suggests that cows may already be thin cows before becoming lame and so these alterations in feeding behaviour may begin before the onset of lesions (Lim *et al* 2015). Detection of behaviour changes could make either the early detection of lameness or the prediction of cows at risk of lameness possible.

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A new system for automatic weighing of beef cattle

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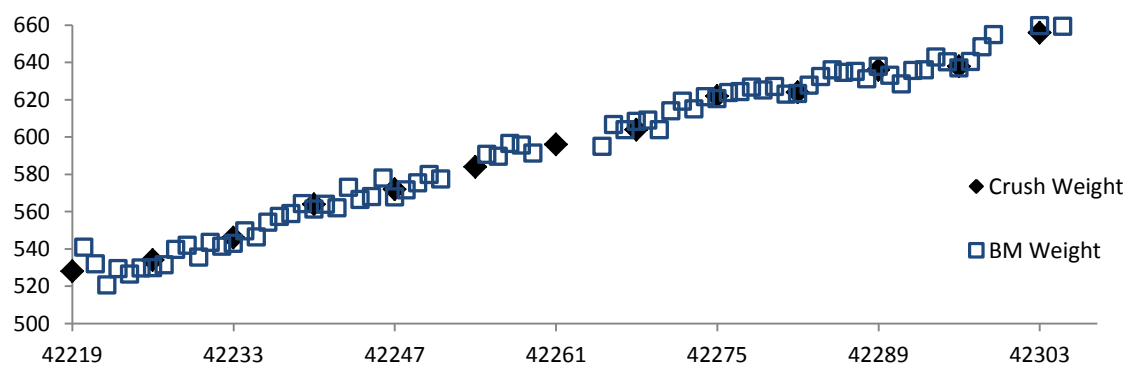
Application A configuration that allows automatic weighing of livestock at a water trough. Integrated with electronic identification this could provide a valuable management tool for monitoring growing and finishing cattle, and provide a platform for complimentary technologies.

Introduction Growth rate is an important measure in assessing individual beef animal finishing performance, and is a key variable in assessing feed efficiency, an economically important trait of beef cattle (Nkrumah *et al.*, 2007). Average daily gain (ADG) systems are available that integrate at feed points (e.g. Growsafe Beef Feedlot), or as walkover systems (e.g. Fullwood WS). This embodiment validates the watering point position, in combination with individual electronic identification (EID). The objectives were to develop and test a passive recording system (in the form of an open ended-crate construct) designed for use by multiple animals, at a common watering point.

Material and methods 78 steers of various breeds were split into two adjoining pens (n=39) and offered *ad libitum* one of two contrasting finishing diets: (i) forage-based diet (Mixed, 500 g concentrate / kg DM), or a high concentrate diet (Conc, 920 g concentrate / kg DM). Within each pen a BeefMonitor (BM) weight recording station was positioned at a water trough to automatically weigh each animal at every drinking event, using EID tags to identify the animal. Once per week the steers were also removed from their pens prior to receiving fresh feed and weighed using a calibrated weighing platform (Tepari Cattlemaster “Titan”). The steers remained on the trial for a minimum of 5 weeks, after which time they were sent for slaughter in 4 groups over a period of 8 weeks. Daily BM weights were calculated by averaging the individual animal BM weights collected over a 24 hour period. ADG (kg/day) for both the BM and crush weights were calculated by regressing weight against days on trial. ADGs for both weigh stations were analysed using general linear models with fixed effects of diet and weigh station.

Results ADG did not differ between weigh stations ($P>0.05$; BM: 1.3 ± 0.04 kg/day, Crush: 1.3 ± 0.04 kg/day). Steers receiving the Conc diet grew at a faster rate than those steers receiving the Mixed diet ($P<0.0001$; Conc: 1.5 ± 0.04 kg/day, Mixed: 1.1 ± 0.03 kg/day).

Diet	Mixed		Conc		Significance		
Weigh Station	BM	Crush	BM	Crush	Weigh Station	Diet	Weigh Station * Diet
ADG (kg/day)	1.1	1.1	1.5	1.4	NS	***	NS



Conclusion There is no difference in ADG gain values calculated from weights gathered automatic using a weighing station positioned at a water trough and from weekly weights measured in a weighing system integrated into a handling system. All animals adapted to the physical BM system arrangement.

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Effect of oestrous synchronisation programme and season on pregnancy rate to timed artificial insemination in beef cows

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Application Artificial insemination (AI) is the most used and valuable reproductive biotechnology for genetic improvement in beef cattle. Timed AI (TAI) based programmes are required to reduce labour requirements and the necessity for heat detection.

Introduction The use of AI in the Irish beef industry is limited with typically only ~20% of calves born to beef cows sired by an AI bull. One of the principal reasons for the poor penetration of the technology is the challenge of detecting animals in oestrus. In particular, small herd size with resultant lower incidence of oestrus-related activity, land fragmentation and the part-time nature of the enterprise mitigate the use of AI, where heat detection is required. Oestrous synchronisation protocols which facilitate the use of timed AI (TAI) provide the opportunity to dramatically increase the use of AI on beef cows (Bó and Baruselli, 2014), but must be both labour efficient and effective. The aim of this study was to evaluate the use of three TAI protocols on pregnancy rate of beef cows under field conditions.

Material and methods A total of 1410 suckled cows located on 61 farms throughout the island of Ireland were enrolled on the study, which was replicated in spring (S; n=703 cows) and autumn (A; n=707 cows) of 2014. A voluntary waiting period of 35 days *post partum* was employed. Body condition score (BCS) was assessed and ovarian ultrasonography was conducted to determine the presence of a corpus luteum (CL). Cows were randomly assigned to receive a 7 day progesterone-releasing intravaginal device (PRID) without (Group 1) or with (Group 2) administration of gonadotropin releasing hormone (GnRH) analogue at PRID insertion and a luteolytic dose of prostaglandin F_{2α} was given at PRID removal. A third group of cows (Group 3) received 400 IU equine chorionic gonadotropin (eCG) at PRID removal. GnRH was administered at TAI 72 h after PRID removal. Pregnancy diagnosis by transrectal ultrasonography was conducted 35–40 days after FTAI. Data were analyzed using the GENMOD procedure of SAS, appropriate to the 2 (seasons) x 3 (treatments) factorial design employed.

Results Mean BCS was 2.75 and 2.92 for S and A cows respectively and was not different between treatments. Overall BCS was positively associated with pregnancy rate (P=0.003). Presence of a CL at treatment initiation increased pregnancy rate independent of either treatment or season (S 50.58%; A 69.97%; P=0.03). There was a treatment by season interaction for pregnancy rate (P=0.0002; see figure 1). Mean pregnancy rate was 59.1% (416/703) for spring calving cows and was affected by treatment (49.6 v 59.3 v 68.5%, for groups 1, 2 and 3, respectively P<0.05). In contrast, in autumn, overall pregnancy rate (52.6%, 364/707) was unaffected by treatment (53.7 v 52.0 v 48.7%, for groups 1, 2 and 3 respectively).

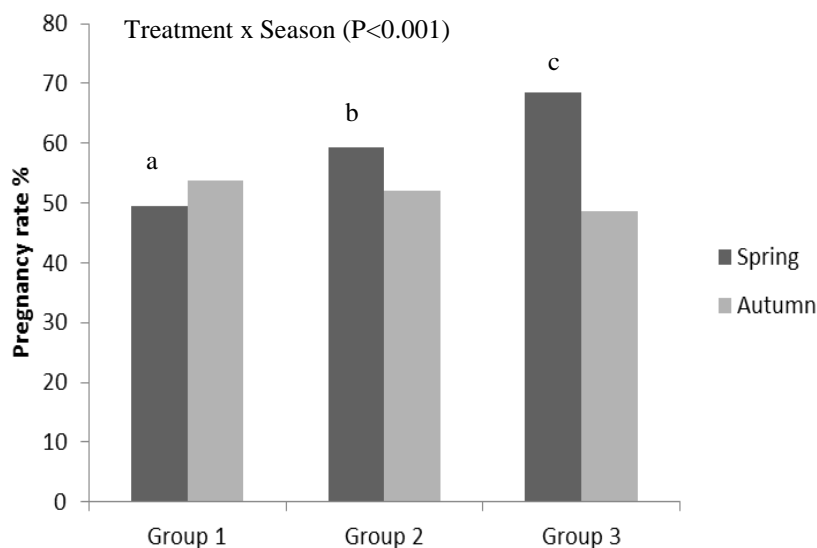


Figure 1 Effect of treatment and season on pregnancy rate to TAI in beef cows.

Within season superscripts are different (P < 0.05).

Conclusion The administration of GnRH at the initiation of a 7 day Co-Synch+PRID protocol as well as supplementation of 400 IU of eCG at PRID removal, both increased pregnancy rate in spring-calving beef cows. However, these effects were not evident in autumn calving cows. Seasonal differences in outcome may reflect different management practices (grazing v confinement) or duration of interval from calving to treatment and remain to be elucidated.

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Comparative live weight, body condition score at breeding and reproductive performance of high and low replacement index beef cows

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Application Replacement index is used to identify animals suitable for breeding or selecting replacements on the basis of their genetic merit

Introduction Producing a live calf every spring in a defined calving period is critical to achieving a profitable suckler system in Ireland (Crosson *et al.*, 2009). However, current industry figures show that cows are producing 0.82 calves per year with a calving interval of 407 days (ICBF, 2015). With the inception of a replacement index by ICBF in 2012, availability of maternal genetic evaluations should enable breeders to improve current industry performance and ultimately improve maternal efficiency of the beef sector. Therefore, the objective of this study was to compare the live weight, body condition score (BCS) at breeding and reproductive performance of high and low replacement index cows.

Material and methods Data were available from 179 cows over two consecutive calving seasons; 101 and 78 cows of high and low index, respectively. Animals were selected from high reliability (>70%) Aberdeen Angus and Limousin sires. The replacement index was €119 and €50 for high and low index cows, respectively. Cows were targeted to calve for the first time at 24 months of age. Live weight and BCS (0 to 5) were recorded every three weeks. Breeding commenced in late April in 2014 and 2015 and lasted 13 weeks. Tail paint and vasectomised bulls were used as aids for heat detection. Cows were artificially inseminated for six weeks in 2014, nine weeks in 2015 and stock bulls thereafter. Reproductive variables included: calving to service interval, 24 day submission rate, pregnancy rate to first service, six week in-calf rate, final in-calf rate, calving to conception interval and the number of services per cow. All pregnancy traits were defined based on trans-rectal ultrasound imaging (Aloka 210D * II, 7.5 MHz) 70 days after the end of the breeding season. The effects of cow genotype on cow live-weight, BCS and reproductive performance was analysed using mixed models in PROC HPMIXED (SAS Inst. Inc., Cary, NC). Sire of the cow was included as the random effect. Analysis of binary fertility data was undertaken using PROC GENMOD assuming a logit link function. Fixed effects included in all models were genotype of cow, year, parity and heterosis and recombination loss of the cow.

Results Live weight, age at first calving, actual calving interval, overall pregnancy rate and all aforementioned reproductive variables investigated were similar across genotypes (Table 1). However, low genetic merit cows had 0.08 of a greater BCS ($P < 0.05$) than high merit cows.

Table 1 Effect of genetic merit on live weight, body condition score and reproductive performance.

	High Genetic Merit	Low Genetic Merit	s.e. ¹	P-value
Live weight (kg)	556	572	9.7	0.1453
Age at first calving (d)	756	758	5.9	0.7481
BCS at breeding ²	2.70	2.78	0.024	<0.05
Calving to service interval (d)	61	58	2.7	0.3737
Submission rate in first 24 days (%)	0.67	0.55		0.0918
Pregnancy to first service (%)	0.50	0.48		0.8478
6 week in-calf rate (%)	0.56	0.57		0.9209
Pregnancy rate (%)	0.89	0.86		0.722
Calving to conception interval (d)	77	74	2.8	0.3742
No. of services per cow	1.81	1.65	0.094	0.2294
Actual calving interval (d)	360	354	4.8	0.3344

¹Weighted standard error of the mean, ²BCS = body condition score: 0 = emaciated, 5 = extremely fat

Conclusion Results from the current study suggest that high merit animals had a lower BCS at breeding than low merit animals. However, genotype had no effect on live weight or any other reproductive traits investigated.

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Effect of altering plane of nutrition during the first and second six months of life on age at puberty onset in Holstein Friesian bulls

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Application Earlier onset of puberty in young genomically-selected high genetic merit dairy bulls will advance the availability of semen, shorten the generation interval and accelerate the rate of genetic improvement. Our data demonstrate that increasing the plane of nutrition during calf-hood advances the age at which puberty occurs.

Introduction With the advent of genomic selection, dairy bulls can now be identified as sires for use in artificial insemination much earlier than was previously possible, creating demand for semen from young sires as early as possible. Data from beef (Brito *et al.*, 2007) and dairy (Dance *et al.*, 2015) bulls show that offering bulls a high plane of nutrition in early life can hasten puberty onset. However, whether suboptimal performance in early life can be mitigated through improving plane of nutrition during the pre-pubertal period is unclear. The aim of this study was to examine the effects of plane of nutrition during the first and second 6 months of life on bull performance, scrotal circumference and age at puberty.

Material and methods Autumn-born Holstein-Friesian bull calves (n=83) with a mean (\pm S.D.) age and bodyweight of 17 (\pm 4.4) days and 52 (\pm 6.2) kg, respectively, were blocked on age, bodyweight and sire and assigned to a high [H] or low [L] plane of nutrition for the first 6 months of life. Calves were individually offered milk replacer and concentrate using an electronic feeder. After five days acclimatisation H (n=37) and L (n=46) calves received 1200 g or 450 g of milk replacer, respectively. H calves were offered concentrate *ad libitum* while L animals received a maximum of 1 kg concentrates daily. Calves were weaned when consuming a minimum of 1 kg concentrates for 3 consecutive days, at a mean age (\pm S.D.) of 78 (4.4) days in H and 79 (5.8) days in L calves. Following weaning, H calves were offered *ad libitum* concentrates while L calves received 1 kg of concentrate daily. All calves had *ad libitum* access to hay. At 24 weeks of age, calves were re-assigned, within treatment, to either remain on their existing plane of nutrition or to change to the opposite diet, until puberty. This resulted in four groups: HH; HL; LL and LH (n=19, 18, 22 and 24, respectively). Animals were turned out to pasture at 26 weeks of age where HH and LH calves received grass and concentrate *ad libitum* while LL and HL calves received grass to appetite plus 0.5 kg concentrate daily. Animals were weighed weekly pre-weaning and fortnightly post-weaning. Scrotal circumference (SC) was measured every two weeks beginning at 15 weeks of age and electro-ejaculation commenced when a SC of 24 cm was reached. Puberty was defined as an ejaculate containing 50 million sperm cells with >10% motility. Data were analysed using mixed models ANOVA and orthogonal contrasts (SAS, version 9.3).

Results H bulls grew faster than L bulls during the first 6 months of life ($P<0.001$; Table 1). There was a pre x post 6 months diet interaction for average daily gain; growth slowed in bulls moved from H to L and accelerated in those moved from L to H. Diet affected weight at puberty during the second 6 months of life with HH and LH being heavier than either LL or HL ($P<0.001$). Bulls offered HH had a larger SC than LL at puberty ($P<0.05$). There was a tendency towards an interaction of diets pre and post 6 months, as bulls offered a H plane of nutrition during the first 6 months of life reached puberty 30 days earlier than L bulls ($P<0.001$) irrespective of diet post 6 months. There was no effect of plane of nutrition during the second 6 months of life on age at puberty ($P=0.35$).

Table 1 Effect of plane of nutrition (PON) on growth and age at puberty in Holstein-Friesian bulls

PON pre 6 months	High		Low		Significance ¹		
PON post 6 months	High	Low	Low	High	Pre	Post	Pre*Post
ADG Pre 6 months ²	0.96 (0.03) ^a	0.96 (0.04) ^a	0.59 (0.03) ^b	0.60 (0.05) ^b	***	NS	NS
ADG Post 6 months ²	1.60 (0.03) ^a	0.70 (0.05) ^b	0.96 (0.03) ^c	1.60 (0.03) ^a	***	***	***
ADG Overall ²	1.24 (0.02) ^a	0.84 (0.02) ^b	0.57 (0.03) ^c	0.96 (0.03) ^b	***	***	NS
Weight at puberty (kg)	404 (11.8) ^a	287 (6.52) ^b	269 (5.52) ^b	374 (15.0) ^a	NS	***	NS
SC at puberty (cm)	31 (0.52) ^a	29 (0.34) ^{ab}	28 (0.39) ^b	29 (0.74) ^{ab}	*	0.06	NS
Age at puberty (days)	298 (6.3) ^a	283 (5.6) ^a	319 (3.9) ^b	323 (6.5) ^b	***	NS	0.09

¹*= $P<0.05$, ***= $P<0.001$. ²kg/day ^{a, b, c}= values within row with different superscripts differ significantly. ADG = average daily gain; SC = scrotal circumference

Conclusion Feeding an increased plane of nutrition pre 6 months of age hastens puberty onset in dairy bulls. Bulls offered a high plane of nutrition post 6 months did not reach puberty earlier than bulls restricted throughout life, despite having an improved growth rate and reaching live weights similar to those of unrestricted bulls.

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The effect of early life plane of nutrition on testicular development of Holstein Friesian bull calves

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Application It is desirable for potential A.I bulls to reach puberty as early as possible and to have the capability of producing high volumes of good quality semen early in life, which is dependent on early testicular development. This study demonstrates that early calthood nutrition can advance testicular development in Holstein Friesian bull calves.

Introduction Developing sperm cells in the testes are supported by Sertoli cells; which are laid down in utero and from birth to puberty. Sertoli cell numbers are directly correlated with adult testicular size and the rate of daily sperm production post puberty (Curtis and Amann, 1981). A high plane of nutrition during early calthood has been shown to advance puberty in both dairy (Dance *et al.*, 2015) and beef (Brito *et al.*, 2007) bulls. The aim of this study was to examine the effect of plane of nutrition during the first 18 weeks of life on testicular development in Holstein Friesian bull calves.

Materials and Methods Holstein-Friesian (HF) bull calves with a mean (\pm S.D.) age and bodyweight of 19 (\pm 8.2) days and 47.5 (\pm 5.3) kg, respectively, were sourced from commercial dairy farms blocked on breed, age, sire and liveweight and subsequently assigned to either a high (H; n=10) or low (L; n=10) plane of nutrition. Calves were individually fed milk replacer and concentrates using an electronic feeding system. After five days acclimatisation, H calves received 1200 g of milk replacer daily, with concentrate *ad libitum*. L calves were allocated 450 g of milk replacer plus a maximum of 1 kg of concentrates daily. All bulls were weaned when consuming a minimum of 1 kg of concentrate for 3 consecutive days, at a mean age (\pm S.D.) of 82 (\pm 3.9) days. After weaning, H calves were offered *ad libitum* concentrates, while L calves received 1 kg of concentrates daily. All bulls had daily access to approximately 0.5 kg of hay and *ad libitum* access to fresh water. At 126 days of age, the bulls were euthanized using an intravenous overdose of sodium pentobarbitone and testes were excised. The epididymides were removed and testes were weighed. Two sections of the parenchyma were dissected from each testis and fixed in formalin. Sections (5- μ m thick) were stained using periodic acid-Schiff and assessed for outer seminiferous tubule (ST) diameter and stage of spermatogenesis. The outer ST diameters were quantified using an eyepiece micrometer at x400 magnification. Measurements were made on 20 different round tubules, selected at random from each testis. The sections were analysed for the most advanced stage of spermatogenesis at x1000 magnification. In the cross-sections of 20 ST per testis, the most mature sperm type was established using the methods of Curtis and Amann (1981).

Results H bulls grew faster and had a greater seminiferous tubule diameter than L bulls (Table 1). H bulls had a higher percentage of spermatogonia denoted as the most mature cell type in spermatogenesis than L bulls. L bulls had a higher percentage of gonocytes and prespermatogonia.

Table 1 Effect of plane of nutrition on calf performance and testes development

	Low	High	S.E.M.	Significance ¹
Pre weaning ADG (kg)	0.43	0.73	0.04	***
Post weaning ADG (kg)	0.57	1.46	0.11	***
Slaughter weight (kg)	107.1	160.9	6.54	***
Paired testes weight (g)	31.4	55.4	3.41	***
Seminiferous tubule diameter (μ m)	72.5	85.4	2.72	**
Gonocyte and prespermatogonia (%) ²	57	31.5		***
Spermatogonia (%) ²	43	68.5		***

ADG: Average Daily Gain ¹** P<0.01; ***P<0.001; S.E.M: standard error of the mean. ²% seminiferous tubules with the most advanced stage

Conclusion HF bull calves fed a H plane of nutrition from 2 to 18 weeks of age had greater testicular seminiferous tubule diameter and a greater percentage of seminiferous tubules with spermatogonia compared to calves fed a L plane of nutrition.

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Sero prevalence of reproductively important pathogens in beef cows on the island of Ireland

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Application Sero prevalence of reproductively important pathogens such as leptospirosis, bovine viral-diarrhoea, infectious bovine rhinotracheitis and neosporosis were determined for the first time in beef cows on the island of Ireland.

Introduction Numerous bacterial, viral and protozoan pathogens have been associated with poor fertility and abortion in cattle. Leptospirosis (hardjo-bovis and -prajitno genotypes), bovine viral-diarrhoea (caused by bovine viral-diarrhoea virus; BVDV), infectious bovine rhinotracheitis (IBR) (caused by bovine herpesvirus-1) and neosporosis (caused by *Neospora caninum*) 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 (Tiwari *et al.*, 2007) and reproductive (Van Leeuwen *et al.*, 2010) efficiency. Therefore the aim of the study was to quantify the sero prevalence of the above listed pathogens in both vaccinated and non-vaccinated herds across the island of Ireland.

Material and methods In the months of May through August of 2014 and 2015, a total of 5554 cows from 155 spring calving suckler cow herds across the island 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 with serum and plasma stored until subsequent laboratory analyses. All serum samples were tested for antibodies against the above listed pathogens using commercially available diagnostic tests at the Department of Agriculture, Food and Marine in the Republic of Ireland. Additionally a short questionnaire was carried with the herd owners to determine if cows were routinely, or had been previously vaccinated for leptospirosis, BVDV or IBR. There are currently no vaccines available for neosporosis in Ireland or Northern Ireland.

Results Percentage sero prevalence of leptospirosis, BVDV and IBR in both vaccinated and non-vaccinated suckler cow herds sampled across the island of Ireland are shown in Table 1. Sero prevalence of leptospirosis, BVDV and IBR was 88%, 92% and 33% respectively in vaccinated herds. Sero prevalence of leptospirosis, BVDV and IBR was 71%, 78% and 44% respectively in non-vaccinated herds. Sero prevalence of neosporosis was 5% (289/5554) with a within-herd prevalence range of between 0 – 29%. Mean BCS \pm s.e.m was 2.57 ± 0.01 for all cows sampled.

Conclusion The results from this study indicate, for the first time, the sero prevalence to leptospirosis, BVDV, IBR, and neosporosis in both vaccinated and non-vaccinated beef cow herds on the island of Ireland. Further investigation will be required to determine the effect of these pathogens on reproductive and performance measures in these herds.

Table 1 Percentage sero prevalence of leptospirosis, Bovine viral-diarrhoea (BVDV) and infectious bovine rhinotracheitis (IBR) in both vaccinated and non-vaccinated herds

Pathogen	Sero-prevalence %	No. of herds Vaccinated	Within - herd Range %	Sero-prevalence %	No. of herds Non-vaccinated	Within-herd Range %
Leptospirosis	88% (2685/3041)	76	0-100%	71% (1779/2513)	79	0-100%
BVDV	92% (2134/2314)	54	43-100%	78% (2518/3240)	101	0-100%
IBR	33% (454/1380)	27	0-84%	44% (1847/4174)	128	0-100%

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Bovine viral diarrhoea virus infection alters uterine prostaglandin profiles and inhibits interferon tau initiated pregnancy recognition in cows

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Application A high rate of early embryonic mortality leads to poor fertility in lactating dairy cows, which is, at least in part, due to failed pregnancy recognition (PR). The situation is even worse if the cows are infected with the bovine viral diarrhoea virus (BVDV), which is currently highly prevalent in the UK dairy population. Understanding the mechanisms how BVDV interrupts PR will help prevent PR failure.

Introduction Poor reproductive performance, of which early embryonic mortality is a major component, causes significant economic loss to the dairy industry. Embryonic mortality rates in cattle can be as high as 40%, with 70-80% of losses occurring before day 16 of gestation (Diskin *et al.* 2011). Evidence has shown that early embryonic mortality in cows can be caused by failed PR and BVDV infection (Grooms 2014). Prostaglandins (PG) play important roles in many reproductive processes, including luteolysis, pregnancy recognition and implantation. The aims of the present study were to investigate the effect of BVDV infection on uterine PG production and PR using an *in vitro* endometrial PR model.

Material and methods Fresh and apparently healthy uteri from 10 BVDV free cows in the early luteal phase of the oestrous cycle were collected at the local abattoir. Uterine endometrial cells (a mixture of primary epithelial and stromal cells) were isolated and cultured and half the cultures were infected with non-cytopathic BVDV (ncpBVDV) for 4 days following the methods described previously (Oguejiofor *et al.* 2015). Four days after infection the cells were treated with 0 or 100 ng/ml interferon-tau (IFNT) for 24 h. PGF_{2α} and PGE₂ concentrations in the spent medium were measured using radioimmunoassays. Data were expressed as mean ± SE. Statistical analysis was carried out using analysis of variance with repeated measure via a linear mixed effect model built in SPSS version 23.

Results The cultured uterine endometrial cells produced a significant amount of PGE₂ and PGF_{2α} (Table 1). Compared with the control, IFNT challenge significantly stimulated PGE₂ production (P<0.05). However, in the cells infected with the ncpBVDV, this stimulatory effect was neutralised (P>0.05) and the PGF_{2α} concentration was lower than in the control cells (P<0.05). ncpBVDV infection alone significantly increased PGE₂ and decreased PGF_{2α} production (P<0.05), leading to an increased PGE₂:PGF_{2α} ratio (P<0.05). Both IFNT and IFNT+ncpBVDV treatments appeared to increase the ratio of PGE₂:PGF_{2α} and IFNT appeared to decrease PGF_{2α} production, but the differences were not statistically significant due to large variations between animals (P>0.05).

Table 1 Effect of ncpBVDV infection ± IFNT treatment on PG production by cultured uterine endometrial cells#

Treatment	PGE ₂ (ng/ml)	PGF _{2α} (ng/ml)	PGE ₂ :PGF _{2α}
CONT	4.8 ± 0.55 ^b	13.3 ± 2.27 ^a	2.5 ± 0.82 ^b
IFNT	9.3 ± 1.78 ^a	11.2 ± 1.75 ^{ab}	5.0 ± 2.15 ^{ab}
ncpBVDV	10.8 ± 2.35 ^a	10.2 ± 1.47 ^{bc}	6.1 ± 1.83 ^a
IFNT+ncpBVDV	3.9 ± 0.40 ^b	9.0 ± 1.59 ^c	4.3 ± 1.42 ^{ab}

The complete experiment was replicated using cells from 10 individual cows. Within columns a>b>c, P<0.05-0.01.

Conclusion Our study suggests that BVDV infection may impair PR by 1) inhibiting the effect of IFNT on uterine PG production and 2) inducing an endocrine switch of PG production from PGF_{2α} to PGE₂. This in turn is predicted to decrease uterine immunity, so potentially predisposing the animals to uterine disease.

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Interaction of preimplantation factor with the bovine global endometrial transcriptome

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Application This is the first study to show that preimplantation factor (PIF) interacts with the bovine endometrial transcriptome, paving the way for future research to investigate the importance of PIF in bovine pregnancy.

Introduction Preimplantation factor (PIF) is a novel peptide secreted from viable embryos as early as the 2 cell stage. Synthetic PIF (sPIF) has been shown to interact with human endometrial stromal cells in culture through three distinct pathways relating to the implantation and acceptance of the embryo by the maternal immune system (Paidas *et al.*, 2010). There is limited knowledge related to bovine PIF, although it has been determined that the peptide is secreted by viable embryos and detectable in maternal serum (Ramu *et al.*, 2013; Stamatkin *et al.*, 2011). The present preliminary study aimed to improve the current knowledge on the interaction of sPIF with the bovine endometrium through RNA-sequencing.

Material and methods Endometrium from bovine heifer uteri (n=4), in the follicular stage of the oestrous cycle, were sampled and intercaruncular tissue explants from each replicate were cultured with or without sPIF (100 nM) for 24 hours. Medium was replaced with fresh medium containing sPIF (100 nM) for a further 6 hours. At the end of the incubation, tissue was stored in RNA later and total RNA subsequently extracted and quality assessed. Extracted RNA that was of a suitable quality was subjected to library preparation for RNA sequencing on the Illumina HiSeq 2500 platform. Following sequencing, a previously described data analysis workflow was adapted for the sample set to determine differentially expressed genes (DEG) and biological pathways modulated by sPIF treatment (McCabe *et al.*, 2012). A p-adjusted value of less than 0.1 was used to determine statistically significant differential expression in the transcript data set.

Results A total of 60 DEG were identified, with 16 down-regulated and 44 up-regulated following treatment with sPIF; however, none showed greater than 2 fold change in expression. There was a strong influence of animal replicates on the data variance, with the native gene expression differing between animals. sPIF treatment up-regulated several genes shown to be important in the endometrium, such as OXTR (oxytocin receptor); PTGER2 (prostaglandin E receptor 2) and IL6R (IL-6 receptor). Furthermore, LEF1 (lymphoid enhancer-binding factor 1) and ITGA2 (integrin alpha-2), shown to be up-regulated in human endometrium following sPIF treatment were also upregulated in the bovine samples. Six biological pathways were over represented in the set of DEG following sPIF treatment (P<0.01) such as the 'Immunoregulatory interactions between a lymphoid and a non-lymphoid cell'. The KEGG pathway 'Biosynthesis of unsaturated fatty acids' was also over represented following sPIF treatment, through upregulation of genes coding for the enzymes fatty acid desaturase 1 and 2.

Conclusion This preliminary study showed that sPIF interacts with the bovine endometrial transcriptome, although the response appears to be relatively weak compared to that identified in humans. Bovine homologs of genes altered by sPIF in the human endometrium were upregulated, however only 2 genes of the 60 DEGs were in that set. The tissue used in the present study was non decidualized endometrial tissue, sPIF may have a greater effect on the pregnancy primed bovine endometrium and future work will reveal if it may show a more similar response to that observed in human studies.

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The effect of dietary omega-6 and omega-3 fatty acid supplementation on semen volume and quality in young post-pubertal dairy bulls

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Application Supplementation with dietary polyunsaturated fatty acids does not lead to an improvement in gross *in vitro* semen quality parameters.

Introduction Sperm utilise polyunsaturated fatty acids (PUFA), in particular n-3 and n-6 PUFA, to maintain normal cell membrane function (Wathes *et al.*, 2007). As ruminants lack the desaturase enzymes which allow *de novo* synthesis of such fatty acids, they require dietary supplementation (Mattos *et al.*, 2000). Recent work has shown some positive effects of dietary supplementation of n-3 PUFA on semen quality in rams (Fair *et al.*, 2014) and bulls (Gurler *et al.*, 2015); however, published data on bulls are limited. The aim of this study was to examine the effects of dietary supplementation with both n-3 and n-6 PUFA on semen volume and aspects of semen quality in young post-pubertal dairy bulls.

Material and methods Holstein-Friesian (n=43) and Jersey (n=7) bulls with a mean \pm s.e.m. age and bodyweight of 420.1 \pm 5.86 days and 382 \pm 8.94 kg, respectively, were blocked on breed, weight, age and semen quality (motility and concentration per ml; based on two pre-trial ejaculates) and randomly assigned to one of three diets, namely (i) a non-supplemented control (CTL, n=15), (ii) rumen protected safflower (n-6 PUFA, n=15), or (iii) rumen-protected n-3 PUFA-enriched fish oil (n-3 PUFA, n=20). Both fats were added at 2% of dietary dry matter (DM) and all three diets were isonitrogenous (160g/kg crude protein). Diets included: 25% rolled barley, 20% maize, 15% soyabean, 17% beet pulp, 12% soy hulls, 4% oil, 2% minerals, 5% molasses. Animals were housed in a slatted-floored shed and fed individually, for the initial 6 weeks, using an electronic feeding system (Calan Inc., Northwood, New Hampshire). Animals were allowed two weeks acclimatisation to the facility followed by ten days acclimatisation to the diets, and were then offered diets *ad libitum* for 12 weeks. All animals were offered 5 kg (fresh weight) of grass silage daily. Semen was collected via electro-ejaculation (Pulsator, Lanes, CO, USA) at weeks -2, -1, 0, 10, 11 and 12 relative to the beginning of the trial period (week 0). On collection, semen volume and progressive linear motility (PLM), assessed using a phase contrast microscope with a heated stage, were recorded. Sperm concentration was measured using a photometer (Minitub, Germany) calibrated for bovine semen. Semen was then diluted to 80x10⁶ sperm per ml in Bioxcell (IMV, L'Aigle, France) and loaded into 0.25 ml straws (IMV, L'Aigle, France). Straws were cooled gradually from room temperature to 4°C over a period of 90 min and allowed to equilibrate at 4°C for 3 h. They were then frozen to -140°C over 9 min (-15.5°C/min) in a programmable freezer (Planar, Birmingham, UK) followed by immersion and storage in liquid nitrogen until use. Post-thaw PLM (n=1 straw per collection for each bull) was assessed as described earlier, after thawing by immersion into a beaker of water at 37°C. Data were analysed by repeated measures ANOVA (PROC MIXED, SAS, version 9.3). Fixed terms for diet and week along with their interactions, where appropriate, were included in the model. Week was included in the analysis as a repeated term.

Results All values represent mean \pm s.e.m. Concentrate intake across the three diets did not differ with bulls on the CTL, n-6 PUFA and n-3 PUFA diets consuming; 9.54 \pm 0.37 kg, 9.54 \pm 0.31 kg, 9.34 \pm 0.35 kg DM respectively. The average daily gain between diets was also similar, with CTL, n-6 PUFA, n-3 PUFA bulls gaining 1.4 \pm 0.19, 1.4 \pm 0.17 and 1.6 \pm 0.29 kg per day, respectively. No effect of week was detected after 70 days feeding; therefore, these data (three successive collections beginning on week 10), were pooled. There was no effect of diet (P>0.05) on semen concentration, PLM or drop in post-thaw PLM (Table 1.). Throughout the trial period, the average volume of semen collected at each ejaculation did not differ between diets (P>0.05).

Table 1 Effect of dietary treatment on semen volume and quality after 10 weeks feeding (average of three collections)

	Treatment			SEM	P-value
	CTL	n-6 PUFA	n-3 PUFA		
Volume (ml)	42438	42434	42435	0.15	0.64
Concentration (x10 ⁶)	1069.3	1139.3	1132.7	40.65	0.83
PLM (%)	28703	29983	28550	1.32	0.39
Drop in PLM post- thawing (%)	20.0	42513	42417	1.85	0.50

Conclusion Dietary supplementation with either n-3 or n-6 PUFA to young post-pubertal bulls had no effect on semen volume or gross measurements of semen quality. Further investigation into post-thaw *in-vitro* quality parameters of sperm from these treatment groups is on-going.

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The development of a UK national terminal combined breed genetic evaluation

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Application The development of a UK terminal combined breed genetic evaluation (CBGE) will enable, for the first time, accurate genetic evaluations for cross bred animals. In addition, it will provide an improved service to the UK sheep industry allowing more frequent genetic evaluations. Both these aspects will help to engage farmers, resulting in increased use of Estimated Breeding values (EBVs) and ultimately improving the genetic progress of the UK sheep industry.

Introduction UK genetic evaluations of sheep have traditionally been undertaken within breeds. However, the UK sheep industry is a mostly stratified system with approximately 56% of ewes in the national flock being crossbred. This represents a 6% increase in the past decade (Pollott *et al* (2012); Pollott and Stone (2003)). This shift has also been noticed in the rams that are used in the national flock. The recent sheep census found that 5% of rams used in the national flock were crossbred. Increasingly, breeders are incorporating crossbred sheep into genetic improvement systems, and it is then not surprising that requests for combined breed EBVs from these cross-breeding flocks have also increased. CBGE allows multiple breeds of sheep to be evaluated simultaneously and animals from different breeds to be compared directly via the resulting EBVs. The benefits of CBGE are that they can accelerate genetic improvement as they increase the accuracy of EBVs (more data is included in the analysis) and allow more intense selection (as the gene-pool to select the next generation can be substantially widened as a result of CBGE and the increased EBV accuracy). Rates of genetic improvement can also be increased indirectly by the ability to provide a more efficient service. At the moment, genetic evaluations are run 3-4 times a year for individual breeds. In a combined-breed scenario only 1 genetic evaluation is required to service many breeds. Therefore, it will be possible to provide up-to-date genetic evaluations more frequently, meaning that breeders always have up-to-date information when they are making their selection decisions. This will help to engage more farmers in performance recording and result in farmers making better use of EBVs, ultimately improving the genetic progress of the national sheep industry.

Material and methods To enable CBGE accurate knowledge of the breed make up is required and full details of this are reported in Kaseja *et al* (2016). The biggest changes to the genetic evaluation system were genetic groups, genetic parameters and the inclusion of heterosis and recombination coefficients to account for hybrid vigour. Previously genetic groups were defined based on gender and year of birth, and varied depending on the breed. In the new system a breed element was incorporated to allow animals of different breeds to be separated. Initially, the genetic group definitions have remained the same as those used in the within breed evaluations. Previously each within breed evaluation had their own set of genetic parameters, however in the CBGE we can only use one set of parameters and a 'best-fit' set of parameters was determined after considering the individual parameters from the within breed evaluations and phenotypes scaled based on the different phenotypic variances. As heterosis/recombination coefficients between all breeds are impractical for computational reasons, two approaches are being considered. The first is to group individual breeds into 3 breed types; European terminal, British terminal and non-terminal, and estimate coefficients for the different breed type combinations. The second is to determine the coefficients on an individual breed basis, but assume the heterosis/recombination effects are the same regardless of breed, and sum the coefficients into a single term.

Results Twelve common terminal breeds were combined to make up the terminal CBGE; Texel, Suffolk, Charollais, Meatline, Hampshire Down, Beltex, Dorset, Shropshire, Blue Texel, Blue du Maine, Vendeen and Southdown. The terminal CBGE runs in approximately 22 hours from extraction, to analysis and then post processing ready for publication. Given it would take approximately 48 hours to run all 12 breeds individually, this represents a saving of 104 hours in a year, assuming all breeds are run 4 times a year. This can result in an extra 4-5 CBGE in the year without using any more resources. Initial quality assurance of the results produced from the terminal CBGE has shown between 0.7-0.95% correlation in EBVs when comparing purebred animals, although the most benefit will come from having EBVs for cross bred animals which were previously unavailable.

Conclusion The system for CBGE has been developed and it is planned to run the within and combined breed systems in parallel in 2016 before fully implementing the CBGE for terminal breeds in 2017.

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Gene pool fishing by out-crossing and back-crossing cycles to accelerate genetic improvement in carcass traits of sheep

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Application The enormous potential of outcrossing and composite breeding is currently mostly unused in the UK sheep industry, largely due to the shackles of breeding society rules. However, its exploitation could have a large impact on the economy of the UK sheep industry, as it brings the best of breeds together, increases genetic variance and heterozygosity and thereby accelerates genetic progress. This project is a blueprint and a cost-efficient demonstration for employing genetic progress made in other breeds to further enhance the performance of a developed terminal sire breed on a reasonable budget.

Introduction There are ~90 recognised breeds of sheep in the UK (Pollott, 2014), which represent a vast gene pool. The wide genetic variation between breeds is largely under-exploited. Improvements in the productivity of sheep are needed, and out-crossing using composite breeding provides solutions with a large impact, bringing the best of breeds together, increasing genetic variance and heterozygosity and accelerating genetic progress. The UK's many sheep breeds and their variation allows the introduction of valuable alleles from one breed into another (target breed) where these alleles do not segregate or do so at very low frequencies. This can be done using molecular genetic methods, e.g. marker assisted selection. However, this is usually expensive and requires knowledge of the trait relevant genes/QTL. Also, there are too few known genes and these explain only small proportions of the genetic variance, a situation that will not change in the next few years by the use of SNP chips or sequencing. A cost effective approach to improve a target breed using unknown genes is using a system of out-crossing followed by backcrossing (to re-establish the typical breed characteristics), whilst keeping useful alleles from the 'donor' breeds. The main focus in terminal sire (TS) breeds is on muscle growth and -shape.

Material and methods An open composite breeding system with outcrossing to Charollais (CH) and Texels (TX) followed by repeated backcrossing has been used to further develop a terminal sire breed (MeatLinc, ML) to become superior to other TS Breeds. This genetic approach, paired with the exploitation of computer tomography (CT), will retain the newly captured muscling alleles. Additional CT traits (not presently available as selection traits via SIGNET) for TS, e.g. muscling, spine traits, killing out % and lean meat % have also been measured on all male selection candidates, with the potential to include these traits in future selection. The initial outcross (F1), was followed by a cross of the two crossbred types (F2, MLxTX and MLxCH), an inter-se mating between these (F3) and a backcross to ML ewes (F4, lambs born 2015). All available ram lambs (n=43; expected gene contribution 75% ML, 12.5% TX, 12.5% CH) were scanned ultrasonically and using CT at about 132d and 155d, respectively. To shorten the generation interval in the *newML* line, ewe lambs and ram lambs were used for breeding. In 2015, in both the *new* and *oldML* lines, mature ML ewes served as mothers, allowing a initial comparison, which was somewhat restricted, since ram lambs from the *oldML* were strongly pre-selected to go forward to CT scanning, whereas all available ram lambs from the *newML* were used. ProcGLM from SAS was used for the statistical analysis. Data were adjusted for live weight.

Results The LS-means and their standard errors (SE) for CT traits in both lines are shown in Table 1.

Table 1 Initial comparison New ML vs. Old ML

Variable	Unit	NewML		OldML		Dif	P*
		Mean	SE	Mean	SE		
CT_Fat	kg	3.50	0.09	3.64	0.10	0.15	0.287
CT_Muscle	kg	13.03	0.11	12.59	0.12	-0.44	0.011
CT_carcass	kg	20.13	0.12	19.77	0.13	-0.36	0.060
CT_KO_P	%	45.41	0.27	44.52	0.31	-0.88	0.045
LM_P	%	29.54	0.24	28.45	0.28	-1.09	0.006

* significant values are shown in bold

- CT_ refers to CT measured tissue weights
- KO_P- killing out percentage,
- LM_P - Lean meat percentage (CT_Muscle * 100/live weight)

NewML have slightly less fat, P=0.15) but significantly more muscle. They have a significantly (P = 0.045) KO_P, they are slightly leaner and have a higher muscle percentage (results not shown). There is a significant improvement of the LM_P by 1%, which is an indicator of economic return and carcass waste. *NewML* would be more superior if a similar pre-selection would have been applied as among the *oldML*. The results of such simulations will also be presented to prove that the applied out-and backcrossing with a special selection focus on muscle growth and -shape has been very effective.

Conclusion A system of outcrossing and repeated backcrossing with an accurate and stringent phenotyping screen (here CT) is a cost-effective way to improve the performance of a TS breed using genetic variants in other developed breeds.

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Determining accurate proportion of breed make up of UK sheep to enable combined breed genetic evaluations

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Implications Creating the Proportion of Breed (PEB) value for each sheep allows to calculate accurate EBVs (Estimated Breeding Values) from a terminal combined breed genetic evaluation, which will satisfy the increasing appetite for combined breed evaluations as a result of the increasing levels of cross breeding in the UK sheep industry.

Introduction The UK sheep industry has a stratified structure with specific breed types and crosses; i.e. hill breeds in the uplands and terminal breeds in the lowlands. Pollott (2012) reported that the amount of cross breeding is increasing indicating that about 56% of all ewes in the national UK flock were crossbred. This along with increased pressure from breeders who performance record has meant that development of a combined breed analysis is required for the national genetic evaluations. In order to provide a national combined breed evaluations accurate knowledge of the breed makeup is required to enable heterosis and recombination coefficients to be calculated and fitted in the model, resulting accurate EBVs of cross bred animals.

Material and methods The UK National genetic evaluation system for sheep has traditionally been undertaken on a within breed basis. A fundamental step to produce EBVs for the combined breed genetic evaluation is correct knowledge of the animals breed make up. Currently, in the BASCO (Beef and Sheep Company) database, an animals breed is recorded as ‘breed 16ths’ and can include up to 4 different breeds. The composition of all 4 breeds must sum up to 16. This record is not suitable for the combined breed analysis as it does not allow precise recording of the breed make up or allow additional breeds as animals become increasingly crossbred. Consequently, EGENES created a system to calculate a PEB value for every animal. The PEB values sum to 100% and currently contains between 1 (for pure breed animals) and up to 12 (although more is possible) different breeds for each animal. The procedure of obtaining the PEB values is fully automated, with the values calculated each day as a part of a snapshot taken from the BASCO database. PEB are obtained by a cycle of iterations starting with the oldest animals iterating to the youngest, where at the end all animals have a PEB value. For all animals the PEB is calculated as half the PEB of the sire + half the PEB of the genetic dam. However, where both or one parents are not known some assumptions are applied. If both parents are unknown, then providing a valid breed 16th is supplied then the PEB will be based on the breed 16ths, alternatively the animal will be assumed to be 100% of the breed code associated with that animal. In the case on one parent known and the other unknown, then again the breed 16th value of the animal, if valid, will be adjusted to subtract the PEB of the known parent and then used to determine the breed of the unknown parent. For example, if the breed 16th is 8 Suffolk and 8 Texel, the animal is a Suffolk*Texel cross, if the known parent has a PEB of 100% Texel then we can deduce that the unknown parent must have been 100% Suffolk. Alternatively if the breed 16th is not available, then the unknown parent is assumed to be 100% of the breed code associated with the animal.

Results Considering performance recorded animals born 2010-2014, the largest purebred (PEB>87.5% of breed) terminal breeds were Texel, Suffolk, Charollais and Hampshire Down with 49%, 20%, 17% and 4% of purebred animals recorded in BASCO coming from these breeds, respectively. The remaining 10% of purebred terminal animals came from 13 other breeds. Animals were assumed to be a terminal first cross if the PEB were approximately 50:50%. The most numerous first crosses are shown in Table 1. As can be seen, majority of 1st cross animals are Texel*Charollais and Texel*Suffolk, namely the crosses between the biggest recorded breeds. A further 21% of first cross terminal animals are included in a further 58 first cross combinations indicating the large diversity of cross breeds in the UK sheep industry.

Table 1 Percentage of 1st cross animals

BreedA	BreedB	% of total
Texel	Charollais	27
Texel	Suffolk	21
Suffolk	Charollais	8
Texel	Shropshire	5
Suffolk	Dorset Down	5
Oxford Down	Oldenburg	4
Meatline	Ile De France	3

Conclusion The breed make up in the current national sheep population support the need for combined breed genetic evaluations. This new method of determining PEB will provide more accurate EBVs and enable better selection decisions to be made when choosing crosses and the animals mated for crossing. Moreover, the frequency of evaluations could increase from 3-4 per breed per season to monthly for all recorded animals, which undoubtedly will be the benefit to all breeders.

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A comparison of the reproductive performance of Lleyn and Scottish Blackface ewes mated to same-breed rams and managed together post-mating in a Scottish hill farm

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Application The aim of this work is to investigate breed substitution *versus* breed improvement in a hill sheep system. Lleyn ewes producing pure-bred Lleyn lambs could potentially improve output and profitability of hill sheep enterprises.

Introduction One of the major constraints of hill sheep breeds such as the Scottish Blackface (BF), is low productivity, and most hill sheep farming systems are not financially viable without support. Lleyn sheep, a more prolific lowland/upland breed, might improve output and profitability of hill sheep enterprises. However, harsh hill environments and poor nutritional supply in such conditions could compromise their reproductive performance, leading to high barren rates and low lamb birth weights, the latter a key factor for lamb survival (Dwyer, 2008). The aim of the current study was to investigate the effects of a harsh hill environment on the reproductive performance of Lleyn ewes, comparing lambing outcomes with those of BF sheep (2 lines) in the same environment.

Material and methods This 2014-2015 study was part of an ongoing project at SRUC's Hill & Mountain Research Centre (56°N, 4°W; elevation: 200 to 1025 m; mean annual rainfall: 2528 mm). The flock comprised approximately 150 control line BF ewes, 150 selection line BF ewes (Conington *et al.*, 2006) and 150 Lleyn ewes. One management system was applied to all ewes, with winter feeding levels tailored to individual weight changes. Lleyn x Lleyn & within-line BF x BF mating was from mid-November for two oestrous cycles; ewes were ultrasound-scanned in mid-February and ewe live weights (LWT) and body condition scores (BCS) recorded pre-mating and at scanning. Two supplementary feeding levels (low/high) were applied from January 8th to lambing, initially on the basis of LWT change (*versus* a target LWT change adjusted for pre-mating LWT and BCS) and, after scanning, number of foetuses carried. Lambing data recorded included litter size and weight, lamb weight and gender. Data analysis used a Generalized Linear Model (GenStat 16; VSNi). Terms in the model for analysis of ewe litter weight data were genotype, ewe scanning LWT, feeding level and ewe age.

Results Barren rates among control BF, selection BF and Lleyn ewes were proportionately 0.17, 0.12 and 0.06, respectively. Among ewes lambing, control BF had the highest incidence of singletons (0.60) while selection BF had the highest incidence of twins (0.54). Litter weights, as recorded, are in Table 1. In the analysis, litter weight was influenced by ewe genotype ($P < 0.001$), ewe age ($P = 0.016$) and post-scanning feed level ($P < 0.001$). Litter weight was positively associated with LWT at scanning ($P < 0.001$). Of lambs born to control BF, selection BF and Lleyn ewes, proportionately 0.75, 0.76 and 0.87, respectively, weighed more than 3 kg at birth. Incidence among twins is illustrated in Figure 1. Among singles and twins, incidence of birth weight above 5 kg was 0.03, 0.03 and 0.13 for control BF, selection BF and Lleyn, respectively. Perinatal mortality incidence did not differ among genotypes (control BF: 0.12; selection BF: 0.10; Lleyn: 0.10).

Table 1 Overall mean \pm sem litter weights (kg) at birth for singleton, twin, triplet and quadruplet offspring of control BF, selection BF and Lleyn ewes

Genotype	Control BF	Selection BF	Lleyn
Singleton (n)	4.2 \pm 0.10 (65)	4.1 \pm 0.10 (55)	4.6 \pm 0.10 (80)
Twin (n)	6.8 \pm 0.19 (42)	7.1 \pm 0.15 (66)	7.3 \pm 0.16 (66)
Triplet (n)	9.6 \pm 0.35 (2)	7.4 \pm 1.26 (3)	9.2 \pm 0.80 (4)
Quadruplet (n)	- (0)	- (0)	13.2 (1)

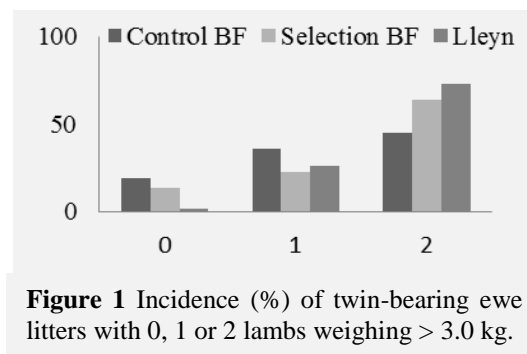


Figure 1 Incidence (%) of twin-bearing ewe litters with 0, 1 or 2 lambs weighing > 3.0 kg.

Conclusion This preliminary study indicates that, on the basis of litter weight, the Lleyn genotype can produce lambs of sufficient birth weight (above 3 kg) to emulate BF counterparts in a harsh hill environment. While the data also show that selection BF ewes achieved higher twin-bearing and lower barren rates compared to control BF counterparts, a notable feature of the investigation was the productivity of Lleyn ewes. Of course, these results are from one season, so future investigations will further assess the comparative resilience and reproductive competence of Lleyn ewes and their offspring.

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Investigating the measurement of feed efficiency in sheep

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Application Recording feed intake in sheep could enable feed efficiency to be included as a goal in UK breeding programmes. This could lower production costs, or allow more sheep meat to be produced from a given area of land.

Introduction Sheep produce meat from grazing land that is often marginal and unsuitable for other agricultural production. High quality grazing is limited in most systems, at least seasonally, and supplementary feed is provided at key times. Feed and forage are the most significant costs across different UK sheep production systems. Producing sheep that use feed more efficiently for growth and reproduction could lower feed costs, or allow more sheep to graze per hectare of land. Livestock feed efficiency (FE) impacts on land use and methane emissions, as well as productivity and profitability. Genetic improvements in FE are expected to help meet these challenges, but many knowledge gaps exist in the best methodology to enable this. To allow genetic selection, genetic parameters must be estimated in a relevant breed. However, there is no current method to measure individual feed intake at pasture with sufficient accuracy for use in a breeding programme. Technological advances in electronic identification of animals and computer-controlled feeding equipment present an opportunity to measure individual feed intake during performance trials in growing lambs. However, the best methodology to use such equipment to allow full expression of feeding behaviour in sheep should be investigated in a relevant system and breed. Relationships with feed intake on pasture and feed intake in mature ewes should also be examined. The objective of this study was to investigate the best way to record feed efficiency in sheep for use in future genetic selection.

Material and methods A desk-based review was undertaken, to assess available technical solutions and methodological approaches to measure and select for FE in sheep. A literature review on FE in sheep (and beef cattle) was produced, concentrating on the use of automated feeders to record feed intake. Other information sources included reports from visits by academic and industry representatives to relevant companies in the USA and Netherlands, and e-mail communications with a research group in New Zealand that is designing a similar experiment in a maternal sheep breed. The resulting review summarised current knowledge and published research, considering installation of recording equipment, recording of feed intake and related traits, and incorporation of FE into a sheep breeding programme.

Results Evidence exists in the literature of variation in FE traits in sheep (Table 1). Moderate heritabilities have been estimated for FE traits in growing sheep (Pulina *et al.*, 2013), as in cattle. Methodological recommendations for using automated feeders to record lamb feed intake include: 9-11 lambs/feeder; 1-2 week adaptation period; 6-8 weeks on test; a forage-based diet. This information could provide a relevant starting point for UK trials. To incorporate FE into a breeding programme, individual feed intake records would be required from 1000-2000 animals at the same stage of development, for robust genetic parameter estimation. Selection for (residual) feed intake should only be considered as part of a multi-trait selection index, since evidence suggests that selection for FE could reduce fatness and may delay maturity. Findings from cattle studies suggest that selection for FE in young growing animals (using automated feeders and a pelleted diet) affects growth and FE on pasture and FE of mature females (Herd *et al.*, 1998).

Table 1 On-test differences between groups of growing lambs identified as high or low for residual feed intake (RFI)

Study	Feed intake	Live weight gain
Redden <i>et al.</i> 2013	17%	NS
Cockram <i>et al.</i> 2013	30%	NS
Johnson <i>et al.</i> 2015	20%	NS

NS = not significantly different (P>0.05)

Conclusion Potential exists to record feed efficiency in individual sheep. Metrological research is required to refine feed intake measurement methods, to allow FE to be assessed in sufficiently powerful samples of relevant UK sheep breeds from grass-based systems. Genetic relationships with other important traits should be investigated to allow recommendations for the incorporation of FE in UK sheep breeding programmes. This is likely to require industry-wide collaboration.

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Ewe body condition scoring (BCS) as a key performance indicator on commercial sheep flocks: lamb performance to weaning

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Application Improving ewe BCS has a positive impact on the weight of weaned lamb per ewe per year.

Introduction Ewe BCS is a highly repeatable management tool farmers can use to assess flock condition at key times during the annual production cycle. Identifying the stages of the ewe's annual production cycle where BCS has the greatest impact on litter size and lamb growth rate to weaning is a key objective of this research.

Material and methods This is interim data from a four year project utilising data collected from three commercial farms (total ~3,000 ewes) in England. Ewe BCS and live weight data were collected on the farm using digital weight scales and trained BCS assessor. Data were collected at weaning, mating, scanning, lambing (BCS only at lambing) and 8 weeks post lambing. Lamb weights were recorded at 8 weeks post lambing and at weaning (~12 weeks) on all farms and birthweight recorded on one farm. All ewes were identified with an electronic identification tag (EID) in their ears enabling the BCS score and weight to be linked to each ewe. Lambs were tagged with EID at birth and linked to their dams. All data were collected and entered into an EID reader, synchronised to an EID software computer programme by Bluetooth. A summary of the data was downloaded from the EID software programme into Excel. Data were checked and analysed for the effect of ewe BCS and live weight on litter size at scanning, lamb weight at 8 weeks and lamb weight at weaning using Gentstat statistical software. Data were analysed on an annual basis (weaning to weaning) and comparisons made with previous years' results. At the end of the project, the four year dataset will be analysed to look at longer term trends of BCS on lamb weight to weaning.

Results BCS improved with successive seasons on all three farms during the project and continues to improve.

Table 1 Summary of ewe BCS and weight effect on twin lamb weight at weaning in 2014 lambing season on three farms

Description	Farm 1	Farm 2	Farm 3		Farm 1	Farm 2	Farm 3
Co-factors							
Lamb weight at 8 weeks	×	×	×				
Ewe BCS				Ewe weight			
Weaning				Weaning			
Mating	×			Mating			×
Scanning			###	Scanning		×	###
Lambing	×		×				
8 weeks				8 weeks	×	×	×
Weaning				Weaning		×	×
Change weaning - mating				Change weaning - mating			
Change mating - scanning		×		Change mating - scanning		×	
Change scanning - lambing				Change scanning - 8 weeks			
Change lambing - 8 weeks	×						
Change 8 weeks - weaning	×			Change 8 weeks - weaning			×

X – significant P<0.005 ### - data not available

Conclusion Ewe BCS is an important KPI for commercial farms and has an impact on lamb weight through to weaning (in addition to impacts on litter size at scanning, not presented in this summary). The impact of ewe BCS and weight can be consistent on all three farms at some points during the year e.g. ewe weight at 8 weeks. However, we see some differences also where BCS and weight have a significant impact on lamb performance to weaning on one or two farms but not across all three. Analysis of all four years' data will be done at the end of the project (2017).

Acknowledgements The authors gratefully acknowledge the data collected by the three farms taking part in the project and project funding from AHDB Beef & Lamb.

Effect of nutritional restriction in mid-pregnancy on the response of ewes to additional protein supply in late-pregnancy and lactation

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Application Ewes with a low BCS show the greatest response to additional MP supply in late pregnancy and lactation.

Introduction Over the last few years genetic selection for higher productivity has increased lamb birth weight and milk production, such that current feeding standards (AFRC, 1993) may underestimate MP requirements (Robinson, 2001). Various studies have investigated the effect of additional MP supply, above requirements, on ewe and lamb performance. However, limited benefits have been demonstrated. In most studies, ewes were in good body condition score (BCS) when the study commenced, and body reserves may have provided a 'nutrient reservoir' to ensure that foetal growth and milk production were not compromised. A greater response to additional MP supply might be expected if ewes are in poorer condition (Dawson, *et al.*, 1999). The objectives of the study were to investigate the effect of nutritional restriction in mid-pregnancy on the response of ewes to additional MP supply in late pregnancy and early lactation.

Material and methods 48 twin-bearing (Suffolk x Mule) ewes (LW 77.5 kg; BCS 3.00) were allocated by parity, LW and BCS at day 70 of gestation into two groups, and fed to achieve a mean BCS of either < 2.5 (L) or > 3.0 (H) prior to housing. All ewes were individually housed from week -6 *pre-partum* to week +4 *post-partum* and offered straw *ad-libitum*, together with one of two concentrates formulated to supply either a low (LP) or high (HP) level of MP. Both concentrates were formulated to supply similar levels of ME, FME and ERDP, but different levels of CP and DUP. Concentrate LP supplied 180 and 28 g/kg DM CP and DUP, whereas concentrate HP supplied 212 and 56 g/kg DM CP and DUP. Concentrates were fed to provide a rising plane of nutrition in late pregnancy and 3.0 litres of milk in lactation (AFRC, 1993), with the LP diets supplying 1.0 and 0.8 of MP, and HP diets supplying 1.25 and 1.0 of MP requirements during pregnancy and lactation. Ewe LW and BCS were recorded weekly, with colostrum and milk yield being estimated at 16 hours and 21 days *post-partum*. Lambs were separated from the ewes, which were injected with 1 ml oxytocin and machine milked until the udder was empty. The procedure was then repeated 4 hours later and secretion rate calculated. Litter weight and litter growth rate were recorded. From week +4 *post-partum* ewes were group housed within each treatment and performance monitored until week +8 *post-partum*. The experiment was analysed by ANOVA as a 2 x 2 factorial design.

Results *Pre-partum*, ewes on treatments L had a higher BCS loss than those on treatment H, and ewes offered concentrates HP had higher LW gains than those offered concentrates LP. *Post-partum*, ewes on treatments H had a higher BCS loss and higher litter growth rates than those on treatments L. Ewes offered concentrates HP had higher colostrum yields, litter weights and litter growth rates than those offered concentrates LP (Table 1).

Table 1 Effect of nutritional restriction in mid-pregnancy and level of protein supply on ewe and lamb performance.

	Low CS (L)		High CS (H)		SED	Probability		
	LP	HP	LP	HP		BCS	Protein	Int
<i>Pre-partum (-6 to -2)</i>								
LW change (kg)	10.10	13.20	9.50	11.30	1.250	NS	0.008	NS
BCS change	-0.60	-0.54	-0.40	-0.21	0.159	0.020	NS	NS
<i>Post-partum (0 to +8)</i>								
LW change (kg)	-9.02	-9.55	-10.06	-10.18	1.867	NS	NS	NS
BCS change	-0.90	-0.71	-0.96	-0.95	0.104	0.050	NS	NS
Colostrum (l/day)	2.46	3.59	2.65	3.16	0.414	NS	0.009	NS
Milk (l/day)	2.89	3.26	3.05	3.19	0.243	NS	NS	NS
Litter weight (kg)	9.26	10.44	9.26	9.74	0.505	NS	0.027	NS
Litter gain (g/day)	495	579	545	619	20.4	0.004	<0.001	NS

Conclusion Nutritional restriction had no effect on litter weights, but reduced litter growth rates. Additional MP increased litter weights and litter growth rates. The response to additional MP was greatest in ewes subjected to nutritional restriction.

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Interactive effects of by-pass protein supplementation and parasitism on ewe performance

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Application Periparturient supplementation of ewes with digestible undegradable protein (DUP) may temporarily increase ewe body weight and boost lamb weaning weight in the presence of parasite challenge.

Introduction There is a large body of evidence supporting the view that periparturient DUP supplementation increases ewe performance and reduces the level of parasitism (Houdijk *et al.*, 2012). However, it was recently observed that in the absence of parasite challenge, DUP supplementation did not increase litter weight gain (Houdijk *et al.*, 2015). This led to the hypothesis here tested that DUP supplementation and parasite challenge interact on ewe performance.

Material and methods Twin-bearing Dorsett-mated Dorsett crosses, with mean body weight (BW) of 59.6±0.8 kg and condition score (CS) of 3.14±0.05 at day₋₄₈ (day₀ is parturition), were housed individually and dewormed. Then, half of the ewes were dosed until turnout (day₁₂) with 10,000 *Teladorsagia circumcincta* larvae every Mon-Wed-Fri (Par) and the other half with water (Sham), whilst feeding *ad libitum* hay and 100 g/day commercial concentrates (n=24). From day₋₂₁ until day₁₂, ewes were fed at 0.9 times energy 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 in the concentrates to increase DUP levels from 25 (LP) to 52 (HP) g/kg (n=24). From day₁₂, ewes grazed one of eight 0.5 ha previously-grazed grass-clover pastures (six ewes and their 12 lambs each), where HP ewes were supplemented with a high DUP pellet (45% RaPass[®] and 45% SoyPass[®]) at 400 g/head/day until day₅₇. Housed ewes were assessed regularly for BW, CS, and serum BOHB. Lambs were weighed at birth and day₁₂. During grazing, ewes and lamb BW, and ewe CS and faecal egg counts (FEC), were taken on day₃₆, day₅₇ and day₁₀₅ (weaning). Data were analysed using 2×2 ANOVA, with ewes and plots as experimental units before and after day₁₂, respectively.

Results Experimental parasite exposure reduced ewe BW until turn out but led to increased ewe BW at weaning (Table 1), which coincided with lower FEC in Par ewes than Sham counterparts at day₅₇ and weaning. Treatments did not affect CS, which gradually reduced to 2.30±0.06 at turn out and then increased to 2.91±0.12 at day₁₀₅. Both DUP supplementation and parasite exposure increased serum BOHB at day₋₁₂ (Table 1). DUP supplementation increased periparturient ewe BW throughout supplementation and litter weight at turnout through weaning, though more pronounced for Sham ewes (Table 1). The FEC of Par ewes was greater than that of Sham ewes on day₃₆ (P<0.001), but smaller on day₅₇ (P<0.001) and day₁₀₅ (P=0.071). DUP supplementation reduced Par geometric FEC by ~40% on day₃₆, but this interaction was directional at best (P=0.130).

Table 1 Experimental treatment effects on ewe BOHB, ewe and litter body weight, and ewe FEC post turnout.

	Day	Treatment combinations				s.e.d.	P-values		
		Par/LP	Par/HP	Sham/LP	Sham/HP		Parasitism	Feeding	Interaction
Ewe BOHB (mmol/l)	-12	0.65	0.76	0.48	0.58	0.07	0.001	0.033	1.000
Ewe BW (kg)	-4	63.9	64.4	65.2	65.2	0.76	0.038	0.554	0.510
	0	54.3	55.2	56.4	55.5	1.10	0.117	0.925	0.251
	12	55.6	56.9	57.3	58.8	1.11	0.027	0.079	0.963
	36	54.4	57.5	55.2	58.2	1.69	0.551	0.015	0.963
	57	54.8	56.8	54.9	56.5	1.56	0.920	0.100	0.858
Litter BW (kg)	105	51.2	51.6	50.1	48.4	1.75	0.089	0.595	0.398
	0	7.8	7.7	7.8	7.5	0.33	0.341	0.740	0.844
	12	14.0	15.1	14.2	15.2	0.44	0.760	0.002	0.903
	36	27.2	26.7	24.4	30.4	1.28	0.623	0.004	<.001
Ewe FEC (log epg)	57	37.7	37.7	34.4	41.4	1.50	0.771	0.002	0.002
	105	52.9	53.1	50.7	57.3	1.92	0.449	0.017	0.023
	36	2.57	2.37	1.82	1.99	0.16	<.001	0.885	0.130
	57	1.60	1.77	2.29	2.31	0.23	<.001	0.565	0.635
	105	0.97	1.59	1.57	1.78	0.30	0.071	0.059	0.332

Conclusion This data support the view that in the presence of worm challenge, DUP supplementation may boost ewe performance. The unexpected temporal effects of experimental challenge on ewe BW and FEC indicate that ewes were exposed to natural infection post turnout, and that previous worm exposure may assist a faster return to immunity as lactation progresses.

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The sheep skeletal muscle proteome: the effect of weight loss in three ovine breeds of different origin using label free proteomics

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Application Several proteins (apolipoprotein A-IV, immunoglobulin V lambda chain, ferritin heavy polypeptide 1, and serpin peptidase inhibitor clade H member 1) were proposed as putative biomarkers of tolerance to SWL. The expression of these proteins can serve as an indicator of individual tolerance levels to SWL and used in sheep selection strategies in drought prone regions.

Introduction Seasonal Weight Loss (SWL) is the most pressing issue in animal production in the tropics. To counter SWL, farmers use supplementation with commercial feeds, expensive and difficult to implement or use animals that have an ability to withstand pasture scarcity, such as the Damara (Almeida, 2011). Identification of markers of tolerance to SWL through proteomics will lead to increased stock productivity being of relevant interest to animal production. With this work, we aim to establish protein markers of tolerance to SWL in meat producing sheep from 3 breeds that lose weight and condition to different extents during SWL periods and hence with different levels of tolerance to SWL: Merino (not tolerant), Damara (tolerant) and Dorper (intermediate).

Material and methods Samples of gastrocnemius muscle were collected from the carcasses of animals used in an animal assay, in which there were two nutritional groups per breed: underfed and control groups, as described (Scanlon *et al.*, 2013). Nine samples per category, fifty-four samples from 54 different animals in total (9*6 categories) were used. One hundred milligrams of muscle samples were added to 500 µl of ammonium bicarbonate (AMBIC) 50 mM, urea 8M, thiourea 2M buffer, containing 1 tablet Complete Mini EDTA-free cocktail and homogenized. The samples were centrifuged and supernatant removed. Per sample, 15 µg of proteins were digested with trypsin using a filter aided sample preparation protocol (FASP) (Wisniewski *et al.*, 2009), and desalted. Peptides were analysed on an LTQ-Orbitrap Velos mass spectrometer. Protein identification and label free quantification analysis were performed using Mascot and Progenesis software. In total 685 proteins were identified and used for quantification. For protein quantification, 4 experimental designs were used: A) Damara Control group vs Restricted group (9 vs 9 samples); B) Merino Control group vs Restricted group 2 (9 vs 9 samples); Dorper Control group vs Restricted group (9 vs 9 samples); D) Control groups vs Restricted groups (27 vs 27 samples). Per comparison, a protein was considered as differentially expressed if a minimum two peptides per protein were quantified, the ANOVA p value was below 0.05 and the fold change was higher than 2.

Results Data analysis resulted in 685 proteins identified with ≥ 2 peptides (FDR: 3.5%). Looking at the comparisons between groups, we identified as differentially expressed the following number proteins for each of the experimental designs: A) two proteins; B) seven proteins; C) five proteins; D) four proteins. To our knowledge, this is the first description of sheep muscle proteome. Moreover, we compare, within three sheep breeds, two experimental nutritional conditions: control group vs. restricted groups. In a global comparison of Control vs. Restricted we identified four differently expressed proteins. Within each breed several proteins were found. Interestingly, in the Dorper breed, three of the five proteins are in the global comparison independently of breed. For the Merino two of the seven proteins are also in that list, and for Damara one of the two is also in that list.

Conclusion Results suggest that the four proteins we identified in D experimental design - apolipoprotein A-IV, immunoglobulin V lambda chain, ferritin heavy polypeptide 1, and serpin peptidase inhibitor clade H member 1 - might be interesting candidates as markers of tolerance to SWL. Apo A-IV is known to play a role in the regulation of food intake; immunoglobulin V lambda chain is involved in inflammation; ferritin heavy polypeptide 1 is the major intracellular iron storage protein in prokaryotes and eukaryotes. serpin peptidase inhibitor clade H member 1 is a heat shock protein that plays a role in collagen biosynthesis as a collagen-specific molecular chaperone.

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Understanding the development of ovine footrot

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Application Footrot is reported to account for over 80% of lameness in sheep in the UK and is a major welfare concern. In addition the disease is economically significant, costing the UK sheep industry approximately £80 million per annum.

Introduction Footrot is an infectious dermatitis of the ovine interdigital skin caused by *Dichelobacter nodosus*. *Fusobacterium necrophorum* is closely associated with footrot but its role in disease initiation and persistence is not fully understood. *F. necrophorum* is assumed to be present in sheep faeces and on pasture, but there is little evidence for the continuous presence of *F. necrophorum* at either site. It has however been detected on healthy feet and in mouths of sheep. The aim of this study is to determine at which sites in the environment of sheep *F. necrophorum* can be detected, and how changes in load of *F. necrophorum* at these sites are associated with health and disease in feet.

Material and methods Two longitudinal observational trials were conducted sampling sheep (feet, mouth and faeces) and their environment (soil and grass) over time. In the first trial (Trial A) a subset of 5 ewes and 5 lambs were sampled every 2 weeks for 4 visits. At each visit the feet of each animal were examined and scored for both interdigital dermatitis (ID) and footrot on a scale from 0-4. Each foot and the mouth of the animal were swabbed. Soil (n=22), grass (n=6) and faeces (n=10) samples were collected from the pasture grazed by the animals. Local climate data for the trial period was also obtained. DNA was extracted from samples and quantitative PCR (qPCR) used to measure load of *F. necrophorum*. In the second trial (Trial B) a group of 40 healthy ewe lambs were sampled weekly over a period of 20 weeks. The methodology was similar to Trial A, except that faeces samples were collected directly from sheep rather than from pasture, soil temperature and soil moisture were measured at each visit, and soil and grass were also sampled 10 days before the start of the trial. A subset of 878 samples from Trial B was analysed initially using an *F. necrophorum* specific qPCR. This included all soil and grass samples (n=640), plus samples from 3 sheep which had footrot and 2 sheep which only had healthy feet throughout Trial B. These were 172 foot swabs, 43 mouth swabs and 43 faecal samples. Preliminary statistical analysis was conducted using a Chi square test ($P < 0.05$).

Results *F. necrophorum* was detected in feet, mouths, soil and grass on at least one occasion but not in faeces collected directly from sheep (Table 1). The distribution of positive samples was not random ($P < 0.01$); there were more positive samples than expected by chance in feet and mouths in Trial A, and in feet in Trial B. The detection levels of *F. necrophorum* in feet, mouths, soil and grass also differed significantly between the two trials ($P < 0.01$). Further statistical analysis will be conducted following completion of laboratory analysis of further samples.

Table 1 Frequency of detection of *F. necrophorum* in different sample types in Trial A and Trial B

Sample type	Samples with detectable <i>F. necrophorum</i>	
	Trial A	Trial B
Foot swabs	50% (n=152)	25% (n=172)
Mouth swabs	80% (n=38)	5% (n=43)
Faeces	2.5% (n=40)	0% (n=43)
Soil	8% (n=88)	1% (n=462)
Grass	4% (n=24)	0% (n=178)

Conclusion The prevalence of *F. necrophorum* appears to vary between the two trials, potentially due to different levels of disease in the flocks, and different climatic conditions. *F. necrophorum* is detected on sheep pasture at low frequency. Contrary to prior belief, the faeces of sheep were not a significant reservoir of *F. necrophorum*. Investigation of the patterns of detection over time will inform on when in the disease process feet, mouths and the environment become positive.

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A longitudinal study of factors associated with acute and chronic mastitis and their impact on lamb growth rate on 10 suckler sheep flocks in Great Britain

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Application The results from this study provide evidence that acute and chronic mastitis impact productivity and should help inform flock management decisions in relation to reducing mastitis. The study also suggests areas for further research.

Introduction Acute mastitis (AM) in ewes can lead to sudden death, loss of an affected udder half, chronic intra-mammary infection detected as intra-mammary masses (IMM), raised somatic cell count or full recovery. AM has economic costs for farmers in treatment costs, costs of replacement ewes and reduced income from loss of lambs or poor growing lambs. AM is also a significant welfare concern as it is a painful disease. Studies in suckler sheep have reported larger litter size and previous mastitis among the risk factors for AM (Waage and Vatn, 2008) while work in dairy sheep has included looking at udder conformation in relation to udder health (Casu *et al.*, 2010). A common practice among suckler sheep farmers is to check the udder of each ewe at the end of lactation or before the start of the breeding season and ewes with IMM may be culled. The impact of this practice is unknown; possible hypotheses include that it reduces onward transmission of bacterial strains causing mastitis and reduces the number of slow growing lambs in a flock. The aim of this study was to investigate ewe risks for and inter-relationships between AM, IMM and udder conformation and their impact on lamb growth rate.

Material and methods A 2-year prospective, longitudinal study of 10 suckler sheep flocks in Great Britain was run to identify factors associated with acute mastitis (AM) and chronic mastitis, and their impact on lamb growth rate. Data collection occurred from November 2012 to July 2014. Each flock was visited twice each year, approximately 4 weeks before lambing and approximately 9 weeks into lactation, for two years and all ewes present at a visit were examined. There were 7021 examinations in total. At the examinations, data were collected on intramammary masses (IMM; a marker for chronic mastitis), udder conformation, teat lesions and body condition. Data on cases of AM, ewe nutrition, litter size, lamb weight and general flock management were obtained from farm records and interviews. Factors associated with AM, IMM in pregnant and lactating ewes, and lamb daily live weight gain were explored using mixed effect multivariable models.

Results AM was reported in 2 – 3% of ewes each year and IMM were detected in 4.74% of ewes in pregnancy and 10.92% of ewes in lactation. Key factors associated with an increased risk of AM are presented in table 1. Further results on IMM and lamb daily live weight gain will be presented.

Table 1 Key factors associated with increased risk of acute mastitis.

Variable	Category	OR	Lower 95% CI	Upper 95% CI
Pregnancy protein	Adequate	Ref		
	Underfed	4.05	1.44	11.35
Non-traumatic teat lesions	None	Ref.		
	At least 1 teat	2.09	1.07	4.09
No. lambs rearing	1	Ref.		
	≥ 2	2.65	1.49	4.72
Teat angle	5	Ref.		
	4	3.99	2.05	7.79
	3 – 1	4.68	1.36	16.16
Litter DLWG		0.03	0.01	0.18

Conclusion We conclude that inadequate nutrition is an important cause of mastitis in suckler ewes. AM, IMM, poor udder conformation and teat lesions all reduce lamb daily live weight gain and so impact on productivity.

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Teat-end hyperkeratosis in dairy ewes and its predisposing factors

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Application Vacuum level was directly related to the prevalence and severity of teat-end hyperkeratosis in dairy ewes. The use of lower vacuum levels could help towards the reduction of teat-end hyperkeratosis prevalence in dairy sheep flocks.

Introduction Although teat-end hyperkeratosis is a condition well known and described in dairy cows, relative information is scarce in dairy ewes. The objective of the present study was to assess teat-end hyperkeratosis in dairy ewes raised under Mediterranean conditions.

Material and methods A random sample of 28 dairy sheep flocks was used. The average flock comprised 229±146.0 milking ewes with an average milk yield of *ca* 273±71.7 kg/lactation period. All flocks were visited by the same veterinarians that assessed milking procedure using a designated recording sheet. Records included use of gloves by milkers, pre-stripping, clusters removal after vacuum cessation and post-milking disinfection. Technical characteristics of the milking parlour and relative equipment were also assessed in terms of their functionality and general condition. A designated equipment (Exendis Milking System Analyser, PTV) was used to measure vacuum level at teat-end, pulsation rate and pulsation ratio in 3 randomly selected milking units of the milking parlour. The vacuum level in the vacuum pump was also recorded; the difference of vacuum level between clusters and vacuum pump was calculated. In each flock, a random sample of 20% of the milking ewes was subjected to a detailed teat-end examination for the assessment of hyperkeratosis using the 4-scale evaluation method described by Mein *et al.* (2001). Evaluation was performed immediately after cluster removal. The problem was considered serious when scores 3+4 and/or 4 were above 20% and 10%, respectively. Data were analysed using univariate analysis of variance with SPSS 22, statistical software. Vacuum level, difference of vacuum level between clusters and vacuum pump, pulsation rate, pulsation ratio and clusters removal after vacuum cessation were transformed into categorical variables and used as fixed effects.

Results Average prevalence of hyperkeratosis score, at teat-level, is shown in Table 1. The analyses at flock level showed that 39% of the flocks (11/28) had a serious problem of teat-end hyperkeratosis. In 5 out of the 11 flocks scores 3+4 were assigned to 30.2% of teat-ends; from them in 14.5% of cases the hyperkeratosis score was equal to 4. The other 5 flocks had scores 3+4 assigned to 23.9%, whereas in 1 flock, score 4 was assigned to 12.5% of teat-ends. The effect of vacuum level on teat-end hyperkeratosis is shown in Table 2. In flocks where the vacuum level of the milking parlour was >40 kPa a significantly ($P<0.05$) higher percentage of teat-ends scored 3+4 was found. The effects of other factors assessed were not significant.

Table 1 Prevalence (%) of hyperkeratosis score 3+4

Score	Mean	min	max
1	69.3	41.7	94.7
2	15.2	3.8	37.5
3	9.9	0.9	25.0
4	5.2	0	18.8

Table 2 Vacuum level (kPa) effect on teat-ends (%) scored

Vacuum level			
≤40	>40	s.e.d.	P
7.8	17.5	4.39	0.038

Conclusion A high prevalence of teat-end hyperkeratosis in dairy ewes was observed; nearly 40% of the examined flocks had a serious problem. In most cases, the higher percentage of teat-ends scored 3+4 was observed in flocks milked with higher vacuum level. The latter suggested that teat-end hyperkeratosis in dairy ewes may be closely related to the functionality of the milking parlour, particularly to the level of vacuum applied.

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Introducing a Targeted Selective Treatment worming approach on a hill farm using Electronic Identification of lambs

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Application Targeted worming of lambs can reduce risk of anthelmintic resistance in hill sheep farming without compromising productivity. It is also a worthy application of the use of compulsory electronic identification in lambs.

Introduction There is growing concern about anthelmintic resistance on sheep farms, as previous worming strategies are increasingly failing. Although resistance more commonly occurs on lowland farms, hill areas are also affected. TST is a refugia-based approach to lamb worming, where only a proportion of animals are treated, based on their actual *vs* expected weight change. This approach relies on individual identification of animals, which is possible using electronic identification (EID) tags, which were made compulsory in 2010 for UK sheep. Such an approach could potentially turn what was often considered as a burden to farmers into a valuable management tool. The TST approach has been successfully tested on lowland farms by Moredun Research Institute (Busin *et al.*, 2014). The aim of this study was to investigate the feasibility of a TST approach using EID technology in a hill farming environment, where animals are weighed and monitored less often.

Material and methods This research was based at SRUC's Hill & Mountain Research Centre, Kirkton/Auchtertyre hill farms in the western Highlands of Scotland, over 3 consecutive years (2013-2015). The farms carry 1200 hill ewes and 22 hill cattle, on 2200 ha. Most of the land is rough grazing, with only 230 ha of improved and semi-improved pastures. In this study, 900 hill ewes (600 Scottish Blackface and 300 Lleyn) have been allocated to two management systems balanced for age, live weight, litter size and sire. The first group were managed conventionally (control – CON), the other with a Precision Livestock Farming (PLF) management protocol, around the use of EID technology. In the PLF management approach, all lambs (n=1183 over 3 years) were subject to the TST. Lambs were weighed monthly after 8 weeks of age (June) – namely in July, August and September - and drenched only if they did not reach their individual target weight, which was calculated using the “Happy Factor” algorithm developed by Greer *et al.* (2009), based on pasture availability. Wormer doses were also based on animal weight (to the nearest 10 kg). The treatment stopped once the lambs left the pasture for finishing indoors (October). Lambs in the CON system (n=1129 over 3 years) were wormed using a whole flock approach, based on pooled faecal samples, by litter size groups. If the faecal egg count (FEC) was >500 eggs/g, all lambs in that group were wormed (based on the heaviest animals in that group); if the count was lower, the lambs were not wormed.. Final (September) lamb weight data were analysed in Genstat (16th edition) using a Linear Mixed Model analysis with breed, sex, management system and litter size as fixed effects (year as random effect). Proportion of lambs dosed was analysed with a chi-square test.

Results Over the three years, the proportion of lambs dosed during the summer months was significantly ($P<0.001$) lower in the PLF than in the CON (0.40 *versus* 0.77). This equated to a 44% saving in the amount of wormer used over the three years between the PLF and the CON (16.5 litres *versus* 29.6 litres respectively). For 100 lambs, the average worming costs per year over the three years were £13 for the PLF and £71 for the CON (which included the FEC costs). Although breed, litter size and sex had significant effects on final lamb weights, with the Lleyn ($P=0.03$), the twins ($P<0.001$) and the male lambs ($P<0.001$) being heavier, the management systems did not ($P=0.08$).

Conclusion It is feasible to introduce a TST approach in a hill farm environment; it reduced the amount of anthelmintic products used on hill lambs without compromising lamb final weights. TST has the potential to slow down anthelmintic resistance in hill sheep flocks, and avoid all the associated negative impacts on farm economics and efficiency and the environment. Provided hill farmers have access to an automated EID reader on a weigh crate, the TST approach could also greatly reduce farm labour.

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Investigating growth and nutritional characteristics of *Tenebrio molitor* reared on diets of varying quality

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Application Determining the impact of different quality feeds on insect growth will aid the assessment of their viability as a sustainable alternative source of protein.

Introduction Increased *per capita* meat consumption highlights the global importance of livestock and fish as protein sources for humans (Steinfeld *et al.*, 2006). The growing population requires expansion of the livestock production sector. One of the limitations is the accessibility of protein sources. Insects are a potential alternative source of protein. In fish, due to high palatability, yellow mealworm larvae could replace up to 60% of dietary fishmeal at 26% inclusion without affecting the growth or feed utilisation of African catfish (Ng *et al.*, 2001). To be sustainable this feed source should itself utilise feeds that do not compete with alternative sources of protein. This study sought to determine the variation in growth rate and nutrient deposition in yellow mealworms (*Tenebrio molitor* larvae) consuming different feeds.

Material and methods Six week old yellow mealworms were kept at 28°C fed on a wheatbran (WB) (crude protein 167.4 g/kg, crude fat 30.1 g/kg, Gross Energy 17.91 MJ/kg) or a sow feed (SF) (crude protein 174.8 g/kg, crude fat 18.8 g/kg, Gross Energy 17.73 MJ/kg) with 10 groups of 50g batches of mealworms per feed. They were weighed on days 0, 5, 11, and 15. Complete batches of mealworms were killed by cold exposure (-20°C) once the first mealworm had entered pupation. Mealworm batches were prepared by oven-drying over 25 hours at 100°C for proximate nutrient analysis; crude fat (Soxhlet ether extraction), crude protein (combustion analysis by nitrogen/protein analyser) and energy (bomb calorimetry). Treatment groups were compared using generalised linear regression (Genstat), errors are standard deviation and significance was accepted at $P < 0.05$.

Results Over 15 days of the treatment period, (which ranged from 14–23 days), the SF fed mealworms grew at 3.5 ± 0.4 g/day compared to 2.8 ± 0.4 g/day on WB ($P < 0.001$) (Figure 1). There were neither significant effects of feed on the time to first pupation or on the total weight of mealworm larvae batches on each feed. At first pupation the mealworm batches fed on SF had lower crude protein content ($P < 0.05$) than WB fed, whilst for crude fat this was reversed ($P < 0.001$) (Table 1). The energy content of the meal worms was not significantly different ($P = 0.063$) (Table 1).

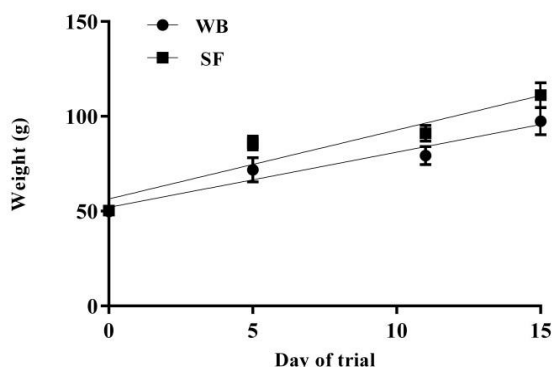


Figure 1 Effect of different feeds on *Tenebrio molitor* larvae growth. Wheat bran (WB) days 0–12 (n=10), day 15 (n=9). Sow feed (SF), days 0–12 (n=10), day 15 (n=6).

Table 1 Gross nutritional composition of dry *Tenebrio molitor* larvae fed wheat bran (WB) or sow feed (SF) at the point of first pupation.

	WB	SF	Significance (P)
Protein (g/kg)	564±26	491±37	<0.05
Fat (g/kg)	271±5	309±7	<0.001
Energy (MJ/kg)	26.26±0.15	26.67±0.21	NS

Conclusion The metabolisable energy in WB is likely to be less than SF due to a higher fibre content therefore this may explain the lower fat content of the mealworms on WB, despite it having a higher fat content. In addition to this WB animals had greater protein content than those fed SF. These experiments indicate the growth rate and composition of *Tenebrio molitor* can be influenced by feed composition.

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Heavy metal uptake and DNA integrity in *Cyprinus carpio* as biomarkers of freshwater pollution

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Application The results of this study could form the basis to bio-monitor freshwater pollution by using heavy metal and Comet Assays in aquatic organisms such as fish.

Introduction Fish is one of the most common aquatic organisms worldwide with superior nutritional significance. Enhanced fish production can be one strategy to fulfil the nutritional requirements of a rapidly growing human global population. In developing countries many river systems have been contaminated with the mixture of domestic effluents and industrial discharges along with other pollutants which have been adversely affecting human health and aquatic ecosystems. Thus heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and diversity of aquatic organisms. As pollution related stress can alter DNA integrity, it is important to assess this to establish the consequences of pollutant exposure on organisms. Therefore, this study aimed to explore the genotoxic influence of heavy metals on the liver of *Cyprinus carpio* sampled from the River Indus in Mianwali district, Pakistan by using single cell gel electrophoresis (SCGE =Comet assay).

Material and methods This completely randomised study involved two polluted sites (Shabaz Khel= S2 and Ballo Khel= S3) and a relatively less polluted site (Kukranwala=S1 as the control) that were about 30 kilometers apart from each other along the River Indus in Mianwali. A total of 81 samples of *Cyprinus carpio* were collected by using nine fish samples of similar size (approx. 1000g) as replicates from three locations of each site. Replicated water samples were also collected from each of these three sites. The concentration of selected heavy metals (As, Cr, Cu, Mn and Pb) in water and fish livers were analysed by using a Varian Vista-MPX CCD Simultaneous ICP-AES machine (Varian Inc, Australia). The DNA integrity in fish liver was assessed using Comet assay. The cells were scored by Comet Assay IV software (Perceptive Instruments Ltd). The data were statistically analysed by using ANOVA in Minitab software version 17 to test the effect of sampling sites on the metal uptake and DNA integrity of fish livers. Tukey's post-hoc test was used to compare the means for different sites at P<0.05.

Results Table 1 shows that the heavy metal concentrations in river water were within the safe limits except arsenic. However, the heavy metal concentrations in fish liver were many folds higher than the WHO recommended levels for food fish. Table 2 shows more DNA damage at polluted sites indicating the oxidative stress in fish species (P<0.05) due to heavy metal accumulation. The order of heavy metals and DNA damage was S3>S2>S1.

Table 1 Mean concentration of heavy metals in fish liver (mg/kg), river water (mg/l), maximum permissible levels (MPL) of heavy metals in food fish and water suitable for aquatic life according to WHO (2006) and P-values.

	S1		S2		S3		WHO MPL in Fish	WHO PML in Water	P-Value
	Liver	Water	Liver	Water	Liver	Water			
As	1.83	0.011	2.66	0.014	2.76	0.015	0.1	0.01	0.001
Cr	0.24	0.001	5.19	0.001	5.52	0.002	0.05	0.05	0.001
Cu	41.16	0.013	76.96	0.025	81.24	0.053	30	2	0.001
Mn	3.95	0.006	6.56	0.008	10.11	0.009	0.01	0.5	0.001
Pb	8.14	0.003	23.49	0.006	35.57	0.008	2.0	0.01	0.001

Table 2 Comet Assay based means \pm SD of head and tail length (μ m), head and tail intensity (%) and extent tail moment (arbitrary units) in fish.

Sites	Head Length	Tail Length	Head Intensity	Tail Intensity	Extent Tail Moment
S1	21.5 \pm 1.68 ^a	10.3 \pm 0.6 ^c	88.8 \pm 3.5 ^a	1.7 \pm 0.21 ^c	15.1 \pm 1.2 ^c
S2	21.0 \pm 1.7 ^a	19.4 \pm 2.1 ^b	74.1 \pm 3.1 ^b	25.9 \pm 2.1 ^b	367.5 \pm 33.8 ^b
S3	17.1 \pm 1.4 ^b	75.1 \pm 5.6 ^a	44.2 \pm 4.2 ^c	41.5 \pm 5.2 ^a	4236.7 \pm 110.6 ^a

(mean with different letters in the same column differ significantly)

Conclusion This study suggests that the fish in the study area were living in a stressful and genotoxic environment as evident by the DNA damage and elevated levels of heavy metals in fish tissues. This is a cause of concern.

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Do puppies from “puppy farms” show more temperament and behavioural problems than if acquired from other sources? Using CBARQ to assess

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Application The information can be used to educate potential owners and discourage them from acquiring puppies from sources where the puppies' welfare is not paramount.

Introduction Puppies purchased from on-line or commercial breeding establishments (UK “puppy farms”) may have increased morbidity and mortality (Kennel Club, 2013; McMillan *et al*, 2013), but there is no research into the effect on subsequent behaviour. Our aim was to explore the effect of source on puppies' temperament and behaviour as an adult dog.

Materials and methods We used the validated Canine Behaviour And Research Questionnaire: CBARQTM (see Hsu and Serpell, 2003) developed for owners / behaviourists to report typical responses of their dogs to common stimuli in their natural environment. Answers to CBARQ questions (Likert scales e.g. 0 never – 4 always) were used to produce a mean score for a behavioural category e.g. owner-directed aggression; separation-related behaviour.

The first survey included 101 questions to generate CBARQ behavioural categories and 38 questions on health and indicators of a “less responsible breeder”. This was distributed via internet dog forums for owners of any breed to complete.

The second survey focused on 3 popular small breeds: Pugs, Jack Russells and Chihuahuas, fewer behavioural categories and eleven questions relating to where the puppy was acquired to give an indication of the responsibility of the breeder (age puppy was purchased; where the puppy was purchased [e.g. small breeder, pet store, puppy farm]; was the mother seen; were there any concerns for the mother/puppies; how many litters of puppies were available; was the owner responsible and caring towards the dogs; were the puppies housed in suitable accommodation; were the puppies in the owner's house; were the puppies with the mother; were health/pedigree documents seen). These were categorised as responsible (R) or less responsible (LR) depending on the response (e.g. if puppies were sold at less than 7 weeks this was LR). An overall categorisation was given where if more than 4/11 responses to the above questions were LR the overall categorisation was LR. The survey was distributed via internet breed forums, dog charity tweets and conforms to British Psychological Society ethical guidelines for internet research. All data was tested for normality (Shapiro Wilk) and homogeneity of variance (Levene's test). Differences in behavioural categories were analysed using Mann-Whitney U tests for non-parametric data and independent samples t-test for parametric data.

Results and discussion Survey 1 (67 usable questionnaires) reported that 93% of respondents were able to see the dam, but 16% did not see dam-puppies interaction, this had been proposed as an indicator of whether the puppy came from a commercial breeding establishment. The only significant finding for the CBARQ behavioural categories was where respondents reported concern for the puppies' welfare at the source, their dogs subsequently had increased stranger directed aggression (n=6; mean 1.04; SE 0.61 compared to where no concern was reported n=54: mean 0.26 SE 0.09 P<0.05).

The second survey had 307R and 163IR usable questionnaires Amalgamated breed data showed dogs from R sources had significantly lower (better) median behaviour scores than IR for most behavioural categories: Stranger Directed Aggression 0.3, 0.6 P<0.001; Owner Directed Aggression 0, 0.13 P<0.001; Dog Directed Aggression 0.5, 1.13 P<0.001; Dog Directed Fear 0.75, 1.0 P<0.05; Chasing 1.7, 2 P<0.01; Stranger Directed Fear 0.3; 0.8 P<0.05; Non Social Fear 0.67, 0.83 P<0.01; Separation Related Problems 0.5, 0.8 P<0.001; Touch Sensitivity 0.5, 0.8 P<0.001; Excitability 2, 2.3 P<0.01. There was no difference in Attachment 2.3, 2.5 P=0.31 or Energy 2, 2.5 P=0.21. For Trainability IR was slightly but significantly better than R (2.13, 2.25 P<0.01). Further analysis is required by breed and full analysis by breed for individual indicators of responsible breeders. Asking about behaviour before source may reduce potential bias in future surveys.

Conclusion Dogs from less responsible breeders do not score as well in reported behavioural categories as dogs acquired from sources which had more indicators of being a responsible breeder.

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Investigating health risks of different pesticide residues in *Channa marulius*, water and sediments from the River Indus

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Application This study may form the basis to formulate standards to regulate the safety of fish meat by developing intervention strategies to maintain water quality and protect the health of fish and people fish consuming.

Introduction Environmental contamination of aquatic systems is a very serious problem worldwide. Numerous deleterious effects on aquatic organisms can occur when agricultural, industrial and commercial chemicals discharge into the aquatic environment. While pesticides are needed to control weeds and pests in agricultural farming, residual pesticides can emigrate from treated fields to the air and water and so may adversely affect relevant organisms. This is mostly true for aquatic environments where pesticides are absorbed by aquatic organisms like fish and can cause damage to fish health and the eating quality of fish for human beings. Fish can be used for environmental monitoring because they can accumulate the contaminants directly from diet and water. Therefore, this study aimed to involve replicated samples of *Channa marulius* (giant snakehead or sole) to monitor the extent and level of the presence of different pesticides in fish muscles from different sites of the River Indus in Mianwali District of Pakistan for potential health risks to the consumers.

Material and methods This completely randomised study involved three sites around the River Indus where Kukranwala (KW=S1) was designated as relatively low polluted and other two sites Rokhri (RK=S2) and Ballo Khel (BK=S3) which were medium and heavy polluted sites, respectively. These sites, 45 km apart, were used as the commercial fish sale points. Neighbouring farmers regularly use newly introduced pesticides to control insects and other pests in order to get better crop production in the study area. Therefore, this study aimed to determine the presence of pyrethroids (cypermethrin, lambda cyhalothrin, deltamethrin), carbamates (carbofuran) and neonicotinoids (imidacloprid, acetamiprid, thiacloprid, thiamethoxam and 6-choloronicotinic acid) in *Channa marulius* alongside samples of river water and sediments. A total of 81 samples of *Channa marulius* were collected by using nine fish samples of similar size of approximately 1000g each as replicates from three locations of each site. Replicated water and sediment samples were also collected from each of these three sites. The standards and samples of fish muscles, river sediments and water were analyzed for pesticide residues by using reverse phase HPLC with UV-Visible detector using standard procedures. Identification of pesticides was carried out by comparing sample peak relative retention times with those obtained for standards from Dr. Ehrenstorfer (Augsburg, Germany). The areas of sample peaks were compared with standards used to quantify the presence of different pesticides. The data were statistically analysed by using ANOVA in Minitab software version 17 to test the effect of sampling sites on the pesticide residues in fish, water and sediments. Tukey's post-hoc test was used to compare the means for different sites at $P < 0.05$.

Results Initially nine pesticide standards were run for deltamethrin, lambda-cyhalothrin, cypermethrin, imidacloprid, acetamiprid, thiacloprid, thiamethoxam, 6-choloronicotinic acid and carbofuran through HPLC but three pesticides representing pyrethroids and carbamates were detected in fish muscles and sediments from the selected sites of the Indus River. No neonicotinoids were detected in any sample of this study. Table 1 shows that pesticide residues were not detected in fish and sediments sampled from the low polluted site (S1) whereas, residues of deltamethrin, carbofuran and cypermethrin were detected in fish muscles and sediments sampled from polluted sites of the River Indus. No pesticide residues were detected in water of any sampling sites. Deltamethrin and Cypermethrin concentrations were within the permissible levels.

Table 1 Mean concentration (($\mu\text{g/g}$ wet weight \pm SD) of deltamethrin, carbofuran and cypermethrin in muscles of *Channa marulius*, sediments and water sampled from selected sites (S1, S2 and S3) of the Indus River and FAO-WHO (2013) maximum permissible levels (MPL).

Pesticide	<i>Channa marulius</i>			Sediments			Water	FAO-WHO
	S1	S2	S3	S1	S2	S3	S1/S2/S3	MPL ($\mu\text{g/g}$)
Deltamethrin	ND	0.05 \pm 0.001	0.28 \pm 0.01	ND	0.21 \pm 0.03	0.32 \pm 0.03	ND	0.5
Carbofuran	ND	0.61 \pm 0.03	0.95 \pm 0.02	ND	0.07 \pm 0.002	0.08 \pm 0.002	ND	0.1
Cypermethrin	ND	0.14 \pm 0.02	0.17 \pm 0.002	ND	0.18 \pm 0.03	0.20 \pm 0.003	ND	2

(ND= not detected)

Conclusion This study revealed that pesticide residue level of carbofuran in fish samples were above the maximum limits at the polluted sites and could thus be a source of pesticide transfer to humans via fish consumption. However, a preliminary risk assessment indicated that the daily intake of detected pesticides by the fish-consuming people around the Indus River is low and does not present an immediate risk.

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Does self-control training in puppy classes influence subsequent impulsivity in dogs?

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Application Dog trainers and behaviourists can assist in managing impulsivity and thus some behavioural problems in dogs by providing early intervention to owners with potentially highly impulsive puppies.

Introduction Impulsivity in domestic dogs is an underlying trait of many behaviour problems, including aggression (Zulch and Mills, 2012). Human research supports self-control training to improve self-control (e.g. in anger management), but there is no canine research on its efficacy despite existing training programmes (e.g. tasks where dogs are trained to wait patiently for food) and anecdotal evidence. This study aimed to determine whether a self-control training programme in puppies had an effect on their impulsivity using the Dog Impulsivity Assessment Scale (DIAS, Wright *et al.*, 2011), an owner-reported psychometric tool validated for puppies over 27 weeks old. The DIAS has a total score and scores for Factors 1: self-control (F1); 2: aggression due to impulsivity (F2) and 3: responsiveness to the environment linked to trainability (F3). For F1 and 2, a high score indicates undesirable high impulsivity whereas a high Factor 3 score indicates the dog is more responsive and trainable.

Material and methods Puppies ($n=58$) of various breeds were six cohorts of a puppy training/socialisation course, (age <20 weeks at the start). The course was 1hr/week for six weeks (full attendance was not always achieved), indoors, with owners present, comprising basic obedience exercises (using positive reinforcement). Each cohort was randomly assigned to either control (C) ($n= 10, 8, 10$) or treatment (T) ($n= 10, 10, 10$). On week one the puppies' frustrated behaviours were recorded (barking and pulling on the lead towards other puppies or people) using scan zero-one sampling every two minutes for 60 minutes (total scores were a proportion of all sample intervals). Groups had an intervention on weeks two, three, four and five: controls were taught a trick (bow; crawl; spin; play dead) and treatment taught "self-control" exercises (floating food bowl; ignore what is on the floor; manners at the door; ignore that dog). After the course owners were posted and emailed a DIAS questionnaire when puppies reached 27 weeks old. Informed consent was obtained. The study adheres to ASAB Guidelines (2012). Differences between treatment groups were analysed using independent samples T-tests. Tests of association between behaviours in week 1 of the course and subsequent DIAS score used Spearman Rank correlation; Pearson correlation was used to compare associations between scores for DIAS Factors (SPSS 21).

Results No differences in barking and pulling in week 1 between treatment groups (T: 0.2 [SE 0.04] C: 0.17 [0.03] NS) suggest puppies had been randomly assigned. Only 26 usable questionnaires were returned (13 T; 13 C; puppies attending follow-on classes with self control activities were excluded from the main analysis). There were no overall differences in total DIAS (T: 0.54, C: 0.55); F1 (T:0.51, C:0.52); F2 (T:0.32, C:0.31) suggesting no effect of the treatment on self-control or impulsivity-related aggression. Factor 3 is approaching significance (T:0.75, C:0.67, $P=0.09$) with the treatment group reportedly more responsive and trainable. Not all puppies received 4 self-control interventions due to attendance. Exploratory scatterplots suggest that DIAS score improved with increasing self-control interventions during the 6 weeks course. When DIAS data was included from puppies attending the follow-on classes it did not alter the DIAS score, suggesting early intervention may be more effective. Table 1 shows significant correlations with barking and pulling in week 1 with subsequent DIAS score. Table 2 reflects Wright *et al.* (2011) within-DIAS correlations, providing further validity of the DIAS tool.

Table 1 Frustrated behaviours in week 1 correlated to DIA scores of control group (Spearman Rank)

Factor/Total	DIAS	F1	F2	F3
Pulling	0.20	0.31	0.12	-0.58*
Barking	0.43	0.55 ^T	0.07	-0.33

$n=12$ ^T $P<0.10$; * $P<0.05$

Table 2 Scores correlated (Pearson)

Factor/Total	F1	F2	F3
DIAS	0.89***	0.35*	-0.15
Factor 1		0.43*	-0.36*
Factor 2			-0.34*

$n=35$; * $P<0.05$; *** $P<0.001$

Conclusion These results provide initial support that self-control training in puppy classes might prove beneficial for assisting impulsive individuals enhance self-control. Barking or pulling on the lead in a frustrating situation for puppies may be early proxies for high impulsivity and as such enable behaviourists to suggest early interventions to build self-control, thus avoiding later problem behaviours developing. Number of self-control tasks may be associated with better self-control and early intervention (first puppy classes) may be better than later (follow-on puppy classes), but further research is recommended to confirm these suggestions.

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Do Staffordshire Bull Terriers experience more problem behaviours when acquired from rescue shelters than other sources – using CBARQ™ Canine Behaviour And Research Questionnaire

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Application The results of this research could be promoted through rescue charities to educate potential owners and improve the re-homing rates of rescue shelter SBTs rather than potential owners getting dogs from breeders.

Introduction A study has shown that dogs acquired from rescue shelters are more likely to display behavioural problems than those which have been acquired from breeders or another home environment (Wells *et al*, 2002). This may also be public perception thus reducing the chance of dogs being acquired preferentially from rescue centres than other sources. Another study has shown that the success of dog adoption from rescue shelters is not linked to behavioural problems but whether help is sought for behavioural problems (Diesel, *et al*, 2008). Our study focuses on one breed, the Staffordshire Bull Terrier (SBT), an over-represented breed in UK rescue centres. We use internet mediated research using the validated Canine Research And Behaviour Questionnaire CBARQ™ (Hsu and Serpell, 2003) to explore behaviour of a population of SBT from rescue centres (including exploring the effect of duration of stay) compared with those SBT acquired from other sources.

Materials and method The CBARQ questionnaire was developed for owners / behaviourists to report typical responses of the dogs to common stimuli in their natural environment (owner report removes confounding factors in test situations). Answers on a Likert scale (e.g. 0 never – 4 always) are used to produce mean scores for behavioural categories e.g. owner-directed aggression; separation-related behaviour. In addition to the validated CBARQ questions, further information was sought regarding length of time in shelter, source (e.g. shelter; breeder and had at owners home since a puppy “permanent home”, foster home). The survey was distributed via internet forums, promoted via dog charity tweets, and conforms to the BPS ethical guidelines for Internet Mediated Research. All data was tested for normality (Shapiro Wilk) and homogeneity of variance (Levene’s test). Differences in behavioural categories were analysed using Kruskal Wallis for non-parametric data.

Results and discussion There were 421 usable questionnaires (271 from owners whose dogs had a permanent home since a puppy; 128 from owners whose dogs had come from a rescue shelter; 22 from owners whose dogs had come from a foster home). There was no difference between dogs from a permanent home, rescue shelter or foster home for the following CBARQ behavioural categories Stranger-Directed Aggression; Owner-Directed Aggression; Dog-Directed Aggression; Dog Rivalry; Chasing; Non Social Fear; Touch Sensitivity; Excitability or Attachment. There were significant differences in median scores for Dog Directed Fear (home: 0.25; shelter: 0.25; foster 1.0; $P < 0.01$); Stranger Directed Fear (home: 0; shelter: 0; foster: 0; $P < 0.05$); Separation Related Problems (home: 0.15; shelter: 0.38; foster 0.0: $P = 0.05$) and Energy levels (home: 2.0; shelter: 2.0; foster: 1.0; $P < 0.05$). The medians suggest that Shelter Dogs may have more separation problems than home dogs, however the figures for both are very low (n.b. 0 = no problem; 4 = serious problem), otherwise differences between home and shelter dogs are negligible. Foster dogs appeared to have higher Dog-Directed Fear (perhaps the cause of them being in foster homes), but lower separation problems and less energy. There were no significant differences in any CBARQ behavioural category for shelter dogs when categorised on length of time they had spent in shelter (less than one month, 1-6months, 6+months). Further details of this analysis will be presented.

Conclusion Staffordshire Bull Terriers from rescue centres perform as well as any Staffordshire Bull Terrier who has lived with their owner since a puppy in all CBARQ behavioural categories except perhaps separation problems, however the score for both groups is exceedingly low. We recommend conducting the study on other popular breeds found in shelters.

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Can activity assessment be used to detect oestrus in captive felids?

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Application Observational studies suggest that subtle changes in activity in felid species may be associated with oestrus. However, there is a lack of quantitative data from any felid species indicating that this occurs, and a number of potential confounding environmental variables have not been investigated. Research of felid activity may ultimately offer a method to improve oestrus detection and thus reproductive success in captive felids.

Introduction Oestrus is often difficult to detect in felids, but it can be associated with subtle increases in the expression of behaviours such as grooming, allogrooming, rubbing, rolling, scent-marking, vocalising, and locomotion. These behaviours appear to correlate with an increase in overall physical activity (OPA). Thus it may be possible to improve oestrus detection in felids by monitoring changes in the OPA associated with oestrus remotely via accelerometry. Accelerometers have been validated for monitoring the OPA of individual cats, and have since been used to study feline behaviour and daily activity patterns (Andrews *et al.* 2015; Lascelles *et al.* 2008; Piccione *et al.* 2013; Watanabe *et al.* 2005). The present study aimed to examine the relationship between OPA and reproductive state (anoestrus or oestrus) in outdoor colony-housed domestic cats (*Felis catus*), while also assessing the impact of environmental variables on OPA.

Material and methods Actical® ‘MiniMitter’ accelerometers (MiniMitter, Bend, OR, USA) were attached to the existing collars of 10 entire female domestic cats (1.8–10.1 years of age) at the Massey University Centre for Feline Nutrition (Lat. 175° 38′ E Long: 40° 22′ S Palmerston North, New Zealand). Activity data were collected from each cat over 34–43 consecutive days, during which time the behaviour of the cats was monitored daily to determine their reproductive state (anoestrus or oestrus). The activity data of the cats were then compared against concurrent temperature (°C), rainfall (mm) and humidity (%) data using analysis of variance (ANOVA). Seasonal changes in temperature were adjusted by calculating the difference between the observed and expected temperatures per day (i.e. residual temperature), with expected temperatures determined from regression analysis of temperatures *versus* date. The effects of reproductive state and day-of-the-week (given there was a reduced staff presence in the colony at weekends) on the activity of the cats, as a group and individually, were also examined using ANOVA. Regression analyses were then conducted to assess the relationship between the mean activity counts of the cats and binned residual temperature, rainfall and humidity. In addition, the effects of these environmental factors on the activity of individual cats were examined.

Results A total of 414 days of activity data were collected from the 10 cats, comprising 135 and 279 days of anoestrus and oestrous activity data respectively. Reproductive state consistently affected the activity of the cats ($P < 0.001$), with the cats being significantly more active during oestrus. The OPA of the cats was also significantly affected by temperature ($P = 0.014$), rainfall ($P < 0.006$) and humidity ($P < 0.05$). While there was considerably more variation between cats in response to these environmental factors, the results showed that OPA decreased with increasing residual temperature ($P = 0.015$), rainfall ($P = 0.010$; most pronounced when above 8 mm/day) and humidity ($P = 0.008$; most pronounced when above 80% rh). Day of the week did not appear to influence the OPA of the cats ($P = 0.214$).

Conclusion The most significant finding of this study is the influence of reproductive state on the OPA of cats, providing the first quantitative support for anecdotal observations that cats are typically more active during oestrus. Consequently, there is potential for accelerometry and activity assessment to be used to improve the real-time detection of oestrus in cats. While this may be of limited value for domestic cats, it is the first step towards developing this methodology for use on endangered non-domestic felids in which oestrus is difficult to detect behaviourally. Environmental factors such as residual temperature, rainfall and humidity also affected the OPA of colony-housed cats, but did so in a highly variable and complex manner that precludes a simple correction factor when assessing accelerometer data.

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Exploring the effectiveness of hydrotherapy to rehabilitate gait in dogs with joint problems

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Application In the absence of randomised control trials or industry guidance for dogs undergoing hydrotherapy this study starts to contribute data on the efficacy of hydrotherapy using gait analysis. It flags the need for more studies using dogs paired for condition, either receiving hydrotherapy or not, to quantify any extraordinary improvement over time.

Introduction Hydrotherapy, long established for humans, recently used in horses and increasing in dogs can be used to rehabilitate after surgery or manage chronic conditions such as osteoarthritis. Most dogs attending hydrotherapy are for therapeutic purposes referred by a vet as the ability to exercise bearing less weight has the potential to strengthen muscles and may enhance recovery. Despite referrals from vets, there is little data to evidence the beneficial outcome of hydrotherapy (Waining, *et al*, 2011). There is also no standard gait score descriptor as a tool to assess improvement. Defining normal gait with over 400 breeds has inherent difficulties. With the absence of data and tools for assessment there are no recommendations for best practice; guidance on schedules of treatment; assessments of improvement or recommendations on when to cease treatment. The aim of this study was to pilot an investigation of the efficiency of hydrotherapy in rehabilitating dogs with joint problems by comparing gait score pre and post hydrotherapy session over a 2 week period.

Material and methods The 10 treatment dogs suffering from joint problems (post-operative or chronic conditions: hip and elbow dysplasia; arthritis) were completing hydrotherapy for rehabilitation. The 5 control dogs had no joint injuries affecting their gait, attending on vets' recommendation for additional exercise. Dogs varied in breed and were categorised: small < 11kg; medium 11kg to 27kg and large > 27kg. Gait scores from each dog were recorded immediately before and immediately after a 30-40 minute hydrotherapy session by one of 2 trained scorers, once per week over 2 weeks (they were not blind to treatment). Gait score was assessed on a 5 point score indicating degree of lameness when walking a 12 m walkway and pain on manipulation of the joint (Table 1). Results were analysed using 2 sample t test for differences between sessions and Pearson correlation for age of dog and gait scores over both sessions (Minitab 1.6).

Table 1 The criteria used to gait score dogs

Degrees of Lameness	Degree of Pain
1 Stands and walks normally	No pain on joint manipulation
2 Walks without lameness (abnormal posture may be visible when standing)	Mild discomfort on joint manipulation elicited at full flexion or extension
3 Mild lameness when walking	Moderate discomfort on joint manipulation elicited at full flexion or extension
4 Moderate lameness when walking	Severe discomfort on joint manipulation elicited at full flexion or extension
5 Severe lameness when walking	Severe discomfort on joint manipulation elicited through full range of joint

Results The gait score pre- and post- session 2 showed significant improvement; and highly significant improvement between pre-session 1 and post-session 2. There were no differences in change in score between dogs categorised on size or gender (i.e. no size of dog had a better improvement). There was no correlation between age and change in gait score $r=0.097$ (NS).

Through reduced gait score there is the suggestion they dogs may experience less pain, in human research, patients who had undertaken hydrotherapy walked better and had lower pain scores than a control group (King *et al.*, 2012). Further research is needed in dogs.

Table 2 Mean score (SE) therapeutic dogs

Sessions	Pre-	Post-	
1	3.4 (0.4)	3.35 (0.4)	NS
2	2.8 (0.3)	2.45 (0.3)	P<0.05
1 to 2	3.4 (0.4)	2.45 (0.3)	P<0.001

Conclusion This study suggests hydrotherapy is beneficial in rehabilitating gait in dogs with joint problems, but more than one session is required before any beneficial effect occurs. Age, size and gender of the dog had no significant effect on the average improvement of gait scores between sessions one and two.

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An internet survey to explore differences in temperament and behaviour in neutered and entire Staffordshire bull terriers using Canine Behaviour And Research Questionnaire CBARQ™

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Application Information on the presence or absence of “personality” changes when dogs are neutered can inform owners in the decision to neuter their dog. Increasing neutering may help reduce dog overpopulation and abandonment.

Introduction Between April 2014 and March 2015, 102,363 stray dogs were handled by local UK authorities (Dogs Trust, 2015) costing over £57 million to UK welfare charities annually (GOV.UK, 2015). Low levels of neutering are associated with high levels of straying and dogs that are relinquished to shelters are also more likely to not be neutered (Diesel *et al.*, 2010). A reason for not neutering is the perceived change in personality. In previous reports this has been found in, for example, roaming and aggression, but little is known about the wider personality. Indeed Starling *et al.*, (2013) recommend further review of the recommendation for neutering to address behavioural problems as there is little research into the effect and recent studies have found conflicting results. Neutering is seen as a way to tackle over population. More information on the types of potential changes in personality, if any, may increase voluntary neutering. This study explores neutering in Staffordshire Bull Terriers using the validated Canine Research And Behaviour Questionnaire CBARQ™ (Hsu and Serpell, 2003).

Material and methods The CBARQ questionnaire was developed for owners / behaviourists to report typical responses of their dogs to common stimuli in their natural environment. Answers on a Likert scale (e.g. 0 never – 4 always) are used to produce mean scores for behaviour categories e.g. owner-directed aggression; separation-related behaviour. In addition to the validated CBARQ subscale questions, further information was sought regarding other indicators of personality e.g. playfulness with humans and the respondents rationale for neutering or not neutering. The survey was distributed via internet forums, promoted via dog charity tweets, and conforms to the British Psychological Society ethical guidelines for internet mediated research. The results between breeds were analysed using Kruskal Wallis, Mann Whitney U-tests using Bonferroni corrections for multiple pair comparisons (SPSS 21).

Results There were no differences in trainability between neutered males (mean 2.8, SE 0.1) and entire (2.4, 0.1, P=0.44) and neutered females (mean 2.4; SE 0.1) and entire (2.5, 0.1, P=0.77). There were no significant differences between neutered and entire females for Stranger Directed Aggression; Dog Directed Aggression; Dog Directed Fear; Dog Rivalry; Chasing; Stranger Directed Fear; Non Social Fear; Separation Related Problems; Touch Sensitivity; Excitability, Attachment or Energy (see Hsu and Serpell, 2003 for details of categories). Females appeared to differ significantly, though not meaningfully for owner-directed aggression (median 0, CI 0.0-0.0; median 0, CI 0.0-0.0; P<0.05). The score is very low for both. There were no differences between neutered and entire males' behaviour for Dog Rivalry; Chasing; Stranger Directed Fear; Non Social Fear; Separation Related Fear; Attachment or Energy. There were differences in Stranger~; Owner~ and Dog-directed aggression, Dog-directed fear, Touch Sensitivity and Excitability, suggesting entire dogs performed better for these categories. This was further analyzed creating a separate group for dogs who had been neutered to address behavioural problems. Table 1 shows that any significant differences were negligible.

Table 1 Median scores for Staffordshire Bull Terriers by gender

0 = low 4 = high	Entire Male N=88	Neutered Male n=144	Male Neutered for behav. probs. n=38
Stranger Dir. Aggression	0.0 _a	0.1 _b	0.2 _{a,b}
Owner Dir. Aggression	0.0 _a	0.0 _b	0.0 _c
Dog Dir. Aggression	0.3 _a	0.5 _b	1.5 _b
Excitability	1.8 _a	2.0 _b	2.3 _c

Medians with different subscripts differed at P<0.05 level (Bonferroni adjusted)

Conclusion There are no changes of consequence in bitch behaviour if neutered. Although there are some statistically significant changes in dog behaviour once neutered, these are very slight and not meaningful enough to deter a person from neutering. We suggest that neutering would not noticeably change desirable behaviours of a dog as a companion animal.

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Comparing welfare of dogs in rescue shelters and owner homes using a pragmatic location-based cognitive bias task

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Application This pragmatic test could be used for assessing cognitive bias in dogs and maybe other species.

Introduction Cognitive bias tasks, originating in human psychology, have been used to indicate welfare in rats, starlings, sheep, pigs, rabbits, and with difficulties in dogs (Burman *et al.*, 2008). It is an expanding research area as it measures the presence of positive emotional states. Quality of life is becoming recognised as important in animal welfare assessment. The rationale is that affective (emotional) state can influence cognitive processing: positive states associated with ‘optimistic’ biases; negative with ‘pessimistic’. Measuring an animals’ classification of ambiguous stimuli gives an indication of its affective state. The aim of the study was to determine whether dogs could be trained and assessed in a simple location-based cognitive bias task, and whether this could be used to compare affective state in dogs housed in rescue shelters and owner-homes. The hypothesis was that rescue shelter dogs would have more pessimistic bias.

Method Rescue shelter dogs (n=10) or owned dogs (n=10) varying in breed, age, sex, time in environment were trained on a “go/no-go” task using a small set of 3 drawers. Each dog had 1 hour training per day (two 15min sessions morning and afternoon). Figure 1 shows the dog was 6 metres from an assistant who opened the top drawer of a portable set of drawers. The experimenter (the same for each dog) said “go”, if the dog approached the top drawer within 60sec it received a food reward (5 repetitions per session i.e. 20/d). Latency of approach was recorded. The dog was considered successfully trained when it approached 16/20 occasions (binominal test, $P < 0.05$). The unrewarded bottom drawer cue was then introduced: 4 sessions per day as above but with 10 random cue presentations per session (rewarded or unrewarded sequence determined by tossing a coin until 10 of each cue). The top cue continued to be rewarded, opening the bottom cue was not rewarded. A “go” response was a pull on the lead to move forwards. The dog was considered able to discriminate when it reached 16/20 correct responses in the random sequence (“go” to top cues and “no-go” to bottom cues). The dog’s go/no-go and latency was then tested with a single ambiguous cue (opening the middle drawer, no reward). Dogs were trained and tested individually in a familiar field belonging to the shelter, or owners’ garden. Independent samples T-tests compared latency to respond to rewarded and unrewarded cues and time to reach training success criterion. Pearson correlation was used for associations between age and latency to approach; chi-square was used to determine differences in response to the ambiguous stimuli.

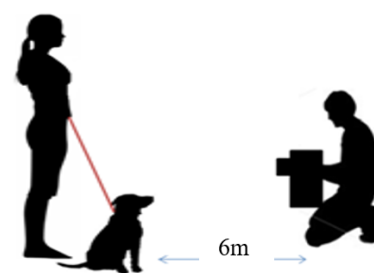


Figure 1 Training and test configuration

Results There was no difference in latency to approach the rewarded cue (rescue 12.5sec SE 2.4; owned 14sec SE 2.3 $P = 0.66$). Rescue dogs took longer to reach training criteria for the rewarded cue (mean 10days, SE 0.7) than owned (7days, SE 0.5 $p < 0.001$) and took longer to train to discriminate between rewarded and unrewarded cues (rescue mean 19.6days SE 0.3; owned mean 17.2, SE 0.7 $p < 0.01$). Table 1 shows no significant differences were found between the housing groups in interpretation of the ambiguous stimuli. Supporting this there was no significant difference between the groups in latency to approach the ambiguous stimuli (rescue mean 16sec, SE 3.5; owned 21, SE 3.7). Analysis using both groups found that older dogs took longer to approach the ambiguous cue ($r = 0.56$ $p < 0.001$). We suggest that either the shelter environment promotes a similar affective state to a home environment (in this shelter dogs have their own space, daily conspecific social contact and 1-3 walks per day) or the test situation is stimulating a positive affective state.

Table 1 The dogs’ classification of the ambiguous stimuli

	go	no-go
Owned		
observed	4	6
expected	5.5	4.5
Shelter		
observed	7	3
expected	5.5	4.5
Chi-squared	0.91	$p < 0.27$

Conclusion Dogs can be trained to this pragmatic cognitive bias task, as such it has potential for use in cognitive bias studies in dogs and perhaps other animals. The benefits include limited equipment, limited movement of animal or handling of animals. Refinements could include testing dogs in the first few days after arrival at a shelter (the hypothesis being they are more stressed), this could explore the sensitivity of the test. Further refinements include testing in the dog’s home pens or house and automating the training to remove any associations (positive or negative) with humans.

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Comparing the temperament of Newly Recognised Cross-Breed Dogs (Labradoodles and Cockapoos) and parent breeds using Canine Behaviour And Research Questionnaire

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Application Standardised temperament profiles for both Newly Recognised Cross-Breed Dogs (NRCBD) and existing breeds, such as those generated via the Canine Behaviour And Research Questionnaire (CBARQ™) will help align owners' desired temperament in a canine companion with that of a suitable breed.

Introduction Kennel Club (KC) breeds have "breed standards" defining aspects of appearance and temperament, thus potential owners can choose a breed based on a likely temperament. There are no such breed standards for NRCBD for breeders to breed towards and no temperament research has been conducted despite their increasing popularity. Behaviour perceived as a problem by the owner is a major reason for relinquishing dogs, when it may represent a misalignment between typical breed behaviour and naïve owner expectation. Some NRCBD have received negative media publicity, but there is no research to support or refute claims. It could also be argued that breed standards contain invalidated descriptors which make it impossible to compare breeds ('intelligent, biddable, a will to please, affectionate, full of life, exuberance, gay-spirited' are a selection from various KC breed standards). This study explores differences between Labradoodles and Cockapoos with their parent breeds. It uses the validated CBARQ™ (Hsu and Serpell, 2003) developed for owners / behaviourists to report typical responses of the dogs to common stimuli in their natural environment, for example separation-related behaviour, thus removing confounding factors in test situations.

Material and methods The link to the 101 question CBARQ (for details of this survey, its validation, reliability and calculations of behavioural category scores, see Hsu and Serpell, 2003) was distributed on-line via 14 breed-specific dog forums, generating 215 usable questionnaires. Answers were on Likert scales (0 never performs a behaviour to 4 always). The CBARQ tool generated behavioural category scores from related questions (see Table 1 for examples of categories). Differences in behavioural category scores between NRCBD and parent breeds were analysed using Kruskal Wallis and Mann Whitney U tests (only Bonferroni corrected significances are reported e.g. $P < 0.017$ or $P < 0.025$ is equivalent to significance at the $P < 0.05$ level, SPSS 21). The study was approved by the University's Animal Welfare Ethical Review Board and adheres to British Psychological Society's ethical guidelines for internet mediated research.

Results Table 1 is a summary of medians for all breeds where significant differences were found. Only the comparisons below between NRCBD and their parent breeds were significantly different: Poodles was more trainable than Labradoodles ($P < 0.017$). Labradoodles had higher familiar dog aggression/rivalry ($P < 0.017$) and were more excitable than Labradors ($P < 0.025$). Labradors engaged in coprophagia more than Labradoodles ($P < 0.017$). Labradoodles lead pull ($P < 0.017$), bark more ($P < 0.017$) and escape/roam more ($P < 0.017$) than Poodles. Cockapoos performed better than Cocker Spaniels for trainability; owner directed aggression; begging; food stealing; lead pulling; ($P < 0.017$), escaping/roaming ($P < 0.025$), but chased more than Poodles ($P < 0.017$).

Table 1 Median CBARQ scores for parent breeds and NRCBD (with 95% CI)

	Poodle n=53	labrador n=27	C.Spaniel n=37	Doodle n=39	Cockapoo n=59
0 = low 4 = high					
Trainability	3.13 3.00 - 3.25	3.13 2.75 - 3.38	2.82 2.50 - 3.00	2.88 2.63 - 3.00	3.07 2.75 - 3.14
Owner Dir. Aggression	0.00 0 - 0	0.00 0 - 0	0.13 0.00 - 0.25	0.00 0 - 0	0.00 0 - 0
Dog Rivalry	0.25 0.00 - 0.67	0.00 0 - 0	0.38 0.00 - 1.00	0.00 0.00 - 0.25	0.00 0.00 - 0.50
Separation Problems	0.38 0.13 - 0.50	0.00 0.00 - 0.25	0.25 0.25 - 0.50	0.25 0.00 - 0.38	0.13 0.00 - 0.38
Excitability	1.83 1.80 - 2.17	1.83 1.50 - 2.33	2.00 1.83 - 2.50	2.33 2.00 - 2.50	1.83 1.50 - 2.33
Chasing	2.00 1.67 - 2.50	1.75 1.50 - 2.50	2.25 1.50 - 2.67	2.25 1.75 - 2.75	2.63 2.25 - 3.00
Coprophagia	1.00 0.00 - 2.00	2.00 1.00 - 2.00	0.00 1.00 - 2.00	1.00 0.00 - 1.00	1.00 1.00 - 1.00
Begging	1.00 0.00 - 1.00	1.00 1.00 - 2.00	1.00 1.00 - 2.00	1.00 0.00 - 1.00	0.00 0.00 - 1.00
Food Stealing	1.00 0.00 - 1.00	0.00 0.00 - 2.00	1.00 1.00 - 2.00	1.00 1.00 - 2.00	0.00 0.00 - 1.00
Pulling on leash	1.00 0.00 - 1.00	1.00 1.00 - 2.00	1.00 1.00 - 2.00	2.00 1.00 - 2.00	1.00 0.00 - 2.00
Barking	1.00 1.00 - 1.00	0.00 0.00 - 1.00	2.00 1.00 - 3.00	1.00 0.00 - 1.00	1.00 1.00 - 1.00
Escape/roam	0.00 0.00 - 1.00	0.00 0.00 - 1.00	1.00 0.00 - 2.00	1.00 0.00 - 1.00	0.00 0.00 - 1.00

Conclusion Only small statistically significant differences were observed. When looking at the medians and CIs it can be seen that none of these traits would be problematic at an applied level and all breeds are potentially well-behaved companions, unlikely to indicate innate problem behaviours in an applied context. Generating standardised subscale reports for all breeds and NRCBDs would help owner decisions when considering acquiring one breed relative to another, as the invalidated emotive descriptors published by the breed clubs are not helpful when comparing breeds and CBARQ would be a useful tool for this.

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Survey of floor types used on Northern Ireland beef farms

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Application This study will provide a greater knowledge of the floor types beef cattle in Northern Ireland (NI) are most likely to experience, and give a better understanding of farmer's perception of welfare on these different floor types.

Introduction Much of the previous research, which indicated that animals accommodated on concrete slats had poorer welfare than those on straw-bedded systems, has been carried out using bulls which were intensively reared and finished on fully slatted flooring. This has led to some UK retailers having a lack of confidence in the welfare of beef cattle which have been reared and finished on concrete slats. However, European systems outlined above are in contrast to grass-based systems common in UK and Ireland, where cattle are only housed during the winter months and are grazed outdoors during the summer months. The majority of NI cattle are accommodated on concrete slats, which is perceived poorer for welfare. Unlike mainland UK (which mainly use straw bedded systems), NI only has sufficient straw to bed 20% of beef cattle (AgriSearch, 2003), making it a limited and expensive resource. The aim of this survey is to evaluate farmer's perceptions of beef cattle welfare on different floor types in NI.

Material and methods A stratified sample of 150 farms with 100 or more cattle intended for slaughter was selected from a total farm population of approximately 500 farms which were derived from the NI Agricultural Census (June 2013) data. A 30% proportional stratified sample was selected stratified by the number of cattle intended for slaughter into two size groups, 100-199, 200 + and also by the county of farm. These farm size groups were chosen as it has been demonstrated by the NI Agricultural Census (June 2013), that the majority of beef cattle are found in these categories, providing a better representation of the floor type beef cattle are finished on. An additional replacement sample was also selected to provide replacement farms for non-responding farms on the original sample. The survey contained 63 questions covering farmer's perception of welfare on different floor types, reasons for choosing current floor types and farmer's perception as to what they believed to be most important for animal welfare. The level of importance and rating of welfare was measured using the Likert scale, 1 represents the lowest rating and 5 the highest. A questionnaire and coding scheme was then devised containing 390 variables incorporating questions on aspects of beef farming and outline data was collected on the houses and the floor types used on each farm. Data was collected by a combination of farm visits and phone responses. A total of 100 farms provided data for the final sample. The final data was combined with the variable labels and categories to create a final SPSS dataset and data validation was carried out on the dataset. Summary descriptive analyses were carried out on all the data estimating the proportions and number of farms for all variables. Further statistical analyses were carried out to test selected hypothesis appropriate to the data types using IBM SPSS Statistics Version 22.

Results The majority of farms have more than one type of flooring to accommodate their cattle. The results show that 73% of farms have concrete slats, 45% rubber flooring and 43% a solid floor with bedding material. Farmers perceived that a greater frequency of slipping events, incidence of lameness, leg and tail injuries occurred in cattle accommodated on concrete slats and least in bedded systems. There was no significant difference between concrete slats and rubber (Table 1).

Table 1 Farmer's perceptions of beef cattle welfare on different floor types in Northern Ireland

Welfare assessment ¹	Floor type			Significance ²
	Concrete slats	Rubber	Bedding	
Slipping (%)	46.6 ^a	33.3 ^a	2.3 ^b	***
Lameness (%)	47.9 ^a	31.1 ^a	9.3 ^b	***
Leg injuries (%)	23.3 ^a	17.8 ^a	2.3 ^b	**
Tail injuries (%)	12.3 ^a	8.9 ^a	0.0 ^b	*

¹ Welfare assessment based on farmers perceived occurrence during last 12 months

² Chi-squared comparison test between concrete slats and bedding

^{a,b} Percentages in the same row with the same superscript do not differ significantly ($P > 0.05$).

Conclusion This survey indicates that farmers perceive the welfare of beef cattle to be highest on a solid floor with bedding material and lowest on concrete slats. However, these are perceptions and further research is required to clarify this.

Acknowledgements The authors gratefully acknowledge DARD for funding and the farmers who participated in the survey.

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Do ewes respond differently to the severity of pain experienced by their twin lambs after one is subject to a more painful procedure than the other?

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Application The use of analgesic treatments for painful procedures in young livestock animals could improve the dams' welfare as well as that of the lambs.

Introduction The sustainability of an agricultural system depends on its ethical justification. Of particular concern are the painful procedures on farm animals such as tail docking and castration in lambs. Studies have generally focused on the animals experiencing pain, but some studies indicate that animals may show empathy and can be stressed by observing the effects of procedures experienced by conspecifics (e.g. Howarth and Leach, 2015). Recent studies have shown that ewes show particular behavioural responses when their lambs have undergone painful castration procedures, and that they showed more maternal care the more severe the painful procedure, in the twins studied one was an untreated control (Futro *et al.* 2015; Hild *et al.* 2011). The study reported here, was to determine whether ewes would differentiate between pain produced by procedures of different severity, carried out on each of the twin lambs. Novel object/food approach tests were used, on the ewes, to determine changes in cognitive bias and to help determine if the procedures on the lambs (within the previous 30mins) had an effect on the ewe's affective state and thus her welfare.

Material and methods This study used North of England Mule ewes with twin Texel lambs. In 12 ewes one twin acted as a control lamb (experiencing handling but no procedure) and the other twin was subject to tail docking or castration using rubber rings (control v pain); In another 8 ewes one twin was subjected to tail docking and the other to either castration or castration and tail docking using rubber rings. Ewes and lambs were housed in straw-bedded individual pens (1.8m x 1.2m) in a commercial lambing shed. Behavioural observations (30 minutes, immediately post procedure) were made in situ, they included: point sampling of proximity (every 2 minutes using distance between ewe's head and lamb <10cm, 10-50cm or 50+cm) and continuous recording of the ewes' behaviours towards each lamb (contacts, sniffs, licks, glances and pawing). Lamb behaviour was continuously recorded over the 30 minutes focusing on the lesser observed and researched behaviours (Kent, personal communication, 2015) of tremble, lip curl, suckle, play and vocalise (after Molony *et al.* 2002). The novel object/food approach test was designed to be a pilot spontaneous cognitive bias test. The bucket was novel thus potentially fear inducing, but it smelled like food, so potentially have been rewarding, hence an ambiguous stimulus. The ewe's response of approach/not approach would assess her categorisation of the stimulus as positive or negative (respectively) and that this would reflect her underlying affective state (see Douglas *et al.* 2011).

Results No differences were found in the percentage of time ewes spent <10cm from lambs for either twins experiencing control *vs* pain ($P=0.105$) or for a lesser pain *versus* a more severe pain ($P=0.621$). The percentage of time ewes spent at <10cm from pairs of lambs with different treatments, was not different between control *versus* pain when compared with lesser pain *versus* more severe pain ($P=0.453$). There were more ewe behaviours directed towards the lambs in pain *versus* the untreated control lambs ($P=0.003$), but no difference in the ewe's behaviour towards a particular twin when one was experiencing a lesser pain and the other a more severe pain ($P=0.382$). No differences ($P=0.166$) were found, in the total number of ewe behaviours directed towards twin lambs, between the untreated control *versus* pain group and the lesser pain *versus* more severe pain group. The frequency of behaviours associated with pain was different between untreated control and lesser pain ($P=0.043$), but was not different between lesser and more severe pain ($P=0.794$). No differences were found in novel object/food approach test between control ewes with untreated lambs and ewes whose lambs had undergone painful procedures (10/15 control ewes and 5/10 ewes with treated lambs approached, chi square 0.69 $P=0.36$). Some ewes in each group were lame and this may have affected their responses to the novel object tests.

Conclusion Ewes focused more attention on the twin lamb in pain when one was in pain and the other not. There were no differences in the ewes' attentiveness when both lambs were in pain. No difference in attention by the ewes was found if the pain was more severe in one lamb than the other. This suggests that ewes either do not differentiate between the responses of the lambs to the two different treatments or that both treatments elicit the same (maximum) response. However, further refinements to analysis of the observations will be necessary before drawing this conclusion. Increased sample size and more rigorous selection criteria for ewes on health grounds is recommended if further research is to be carried out.

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Monitoring and modelling dust concentration and animal activity in broiler houses

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Application Using EyeNamic activity sensors as a proxy for monitoring dust concentration in broiler houses.

Introduction Precision Livestock Farming (PLF) technology provides continuous measurement of key production and welfare indicators on livestock farms, such as animal growth and activity, through image and sound analysis and other key sensors, with some applications developed into commercial products. Animal activity and dust concentration have been shown to be correlated (Demmers *et al*, 2010) and potentially this relationship can be used to manipulate the overall emission rate of dust from the broiler house. The aim of this study was to model the relationship between broiler activity, ventilation rate, broiler weight and measured dust concentrations.

Material and methods A mechanically ventilated broiler house (capacity 55.000 birds) was used for the experimental work. Dust concentration was measured below two (out of 18) ridge extract fan shafts using DustTrac DRX 8533 analysers (TSI Ltd). Ventilation rate was measured using three full size measuring fans (Fancom BV) fitted below the extract fans of ventilation stages 1, 2 and 3 (out of 6), as well as the duration each fan was operational. Broiler activity was measured using eYeNamic (Fancom BV), consisting of 4 cameras mounted equidistant in the ridge of the house. Light/dark cycle variations, including dawn/dusk for 30 minutes, were implemented and, in addition, natural daylight from side wall windows was used at variable intervals during the growing cycle. To model the dynamics of the dust concentration in relation to broiler activity, a transfer function modelling approach was used (Aerts *et al*, 2000). The proposed multiple input single output model (MISO) had two inputs, broiler activity and ventilation rate, and a single output (figure 1). Broiler weight was not yet used as a disturbance in the model. The transfer function model was used to estimate the order of the MISO model and the model parameters using two datasets, each comprising a full growing cycle. Further data sets will be used to validate the model.

Figure 1 Proposed MISO model for estimating the dust concentration from the broiler activity and ventilation rate.

$$y(k) = \frac{B_1(z^{-1})}{A_1(z^{-1})} u_1(k) + \frac{B_2(z^{-1})}{A_2(z^{-1})} u_2(k)$$

$y(k)$ = dust concentration $B(z)/A(z)$ = steady state gain
 $u_1(k)$ = ventilation rate k = discrete time step
 $u_2(k)$ = activity z^{-1} = backward shift operator

Results Dust concentration showed a regular pattern in relation to animal activity, with both dropping at the start and quickly rising at the end of dark periods (during the dark periods eYeNamic is unable to provide data on activity levels). The larger particles (PM₁₀ and total dust) were clearly increasing with the animal activity, whereas the smaller particles, PM₁, were reduced by increasing ventilation rate. Dust concentration and broiler activity were correlated at the start of growing cycle, with correlation coefficients of 0.59 and 0.60 for PM₁ and total dust respectively, but less so towards the end of the crop resulting in a poor overall correlation with coefficients of 0.36 and 0.39 for PM₁ and total dust respectively. The developed first order model estimated the dust concentration well, with separate values for the model parameters of the small and large particle sizes (Table 1).

Table 1 Parameters for the MISO model predicting the dust concentration from the broiler activity and ventilation rate.

	Average R ² _T	Steady State Gain (mean ± std)	
		Ventilation	Activity
PM1	0.97	-1.58 ± 0.53	0.5 ± 0.26
Total Dust	0.91	0.05 ± 0.046	0.82 ± 0.64

Conclusion Dust concentrations are not only clearly related to the level of broiler activity and ventilation rate, but can also be estimated, based on these variables, using transfer function models. The developed model provides an accurate estimate of the dust concentration (over various size fractions), which can be used to modify the overall dust emission of the building.

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Weight gain in calves weaned naturally compared with calves weaned by separation from their mother

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Application In an attempt to improve weaner calf welfare, the effects of weaning and housing methods on their daily weight gain were explored. Spring born suckler beef calves gained more weight between February and April when left with their mother and less when weaned by separation. Housed calves gained more weight between February and April than calves at pasture.

Introduction The losses through impaired performance suckler beef calves experience as a result of stress induced by the separation from their mothers are accepted by the industry and the veterinary profession (Enriquez *et al.*, 2011). Several methods have been investigated to ease the calf's adaption to life without its mother, with the main attention given to fence line weaning (Price *et al.*, 2003) and two step weaning (Haley *et al.*, 2005). As an additional alternative the consequences of natural weaning, without separation of cow and calf were the focus of this study. The hypothesis was that the calf's physical development would benefit if it remained with its mother and that this would happen at the expense of the cow's condition.

Material and methods Between February and April forty Aberdeen Angus cows and their calves were exposed to one of four treatments: cow and calf housed together, cow and calf at pasture together, cow and calf separated with calves housed and cows at pasture, and cow and calf separated with calves at pasture and cows housed. Pasture grass and hay for the housed animals was fed at libitum. Each animal received an equal amount of concentrates every day. All animals were under the same management and closely observed for disease. The groups were balanced for age (cows and calves), gender and breed (calves) and weighed twice: on the first day of the trial in February and on two days in April. The weight difference between the first and the second weighing was calculated for every animal and because of the differences in breeds, calf ages and durations between weighings, growth was expressed in percent weight difference from the first weight. An average weight difference was then established separately for calves and cows of every treatment group. Data were analysed using ANOVA.

Results Remaining with their mother had a highly significant effect on calf weight gain ($P < 0.001$). Being housed compared to pastured had a significant effect on calf weight gain ($P = 0.042$). Retaining her calf had a significant effect on cow weight loss ($P = 0.006$). Being housed compared to pastured had no significant effect on cow weight ($P = 0.88$).

Table 1 Average weight difference of cows and their calves in four different husbandry systems

Treatment Group	Calf weight difference (%)	Cow weight difference (%)
Together, housed	8.95 (SD 5.32)	0 (SD 4.4)
Together, pastured	6.29 (SD 5.36)	-0.89 (SD 3.26)
Separated, calves housed, cows pastured	3.15 (SD 3.74)	3.43 (SD 3.05)
Separated, calves pastured, cows housed	-3.58 (SD 7.62)	4.23 (SD 6.43)

Conclusion This trial indicates that suckler beef calves' weight gains benefit from remaining with their mothers to be weaned naturally. A weight loss was noted in the cows retaining their calves at pasture, but not when housed.

Acknowledgements The author gratefully acknowledges funding from Harley Farms (South)

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Risk factors for respiratory disease in dairy heifer calves on commercial farms in SE England

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Application Understanding risk factors for respiratory disease in dairy heifers can inform farm management to improve disease prevention. Reducing respiratory disease would improve calf health, welfare and performance.

Introduction Dairy heifer calves have high rates of contagious disease and mortality (Brickell *et al.*, 2009). Respiratory disease is known to be a common cause of death and long term reduced performance in heifer calves by delaying age at first calving and reducing the chance of completing a successful first lactation (Bach, 2011). The number of weeks with respiratory disease has been shown to have a long term impact on calf performance and can be used as an indicator of disease severity (Bach, 2011). There have been no studies of risk factors for respiratory disease on commercial UK dairy farms to inform farm management.

Material and methods Dairy heifers (n=492) were recruited at birth from 11 farms in SE England. In the first week of life a serum sample was taken to measure IgG by radial immunodiffusion. Calves were then checked weekly for disease from weeks 1-9 using the Wisconsin calf health scoring system (McGuirk, 2008). Calves with pyrexia and a sign of respiratory disease (score ≥ 5) were classed as having disease. IGF1 in blood was measured at weeks 1, 5 and 9. Husbandry factors assessed included type of housing and amount of milk fed. A generalised mixed model was used to calculate the odds of a calf having any respiratory disease and a linear mixed model was used to analyse the variable of disease duration.

Results About half the animals (266/492, 54%) had no recorded respiratory disease. The odds of having any respiratory disease were reduced by having higher IgG at recruitment (indicating improved passive transfer of immunity), by being housed in a fixed group rather than a continuous flow system and by being fed more milk solids in the second month of life. The effect of milk fed was, however, of marginal statistical and practical significance.

Affected calves (n=226) had between 1 and 6 weeks with disease. The model for the number of weeks for which a calf had respiratory disease explained a wider range of variation observed (Table 1). Holsteins were more at risk of respiratory disease than other breeds of calves. Increasing milk feeding had a large effect size: increasing milk feeding from the 1st to 99th percentile of the observed range predicted about 0.5 weeks less disease. Similarly, housing calves in fixed groups rather than a continuous flow system was associated with 0.5 fewer weeks of disease. IGF1 at 5 weeks was inversely correlated with disease duration.

Table 1 Risk factors associated with the number of weeks a calf has respiratory disease

Variable	Estimate	p value	
(Intercept)	1.76038	<0.001	***
Calf Breed: Holstein (n=349)	(ref)		
Calf Breed: not Holstein (n=143)	-0.27587	0.026	*
Estimated Milk Solids 35-63 days (kg)	-0.02151	0.04	*
IGF1 at 5 weeks (ng/ml)	-0.00221	0.04	*
Calf Groups: continuous flow	(ref)		
Calf Groups: fixed group	-0.50606	<0.001	***

* p<0.05, *** p<0.001

Conclusion Good passive transfer of IgG reduces respiratory disease incidence. Disease duration, which has more long term importance, is better reduced by improving calf housing and feeding. Holsteins appeared more susceptible than other breeds.

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Performance of winter-born Holstein Friesian heifer calves wearing calf coats during the milk-fed phase of rearing

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Application Calf coats can be removed from dairy heifer calves at 3 weeks of age without negative impact on their growth rates or health.

Introduction To meet the targets for first calving at 24 months, replacement heifer calves must grow at 0.7 to 0.8 kg/day (Margerison and Downey, 2005). Calves are homeotherms seeking to maintain body temperatures of 39°C. 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. Calf coats have been shown to reduce heat loss by 52% (Rawson *et al.*, 1989) and studies suggest that winter-reared dairy heifer calves wearing wool coats to 3 months of age have higher growth rates than those without (Loy *et al.*, 1999). Anecdotal evidence suggests that the use of calf coats is increasing; however, there are limited numbers of published research studies on the use of calf coats and limited justified guidance for calf rearers as to how long the calf needs to wear a coat. Our hypothesis was that removing coats from calves at 3 weeks of age would reduce growth rates when compare to calves wearing coats for the first 6 weeks of life. The aims of the present study were to assess growth rates and health in Holstein-Friesian heifer calves wearing coats for either 3 or 6 weeks from birth.

Material and methods The study was conducted from 26th November 2014 to 15th April 2015, in Shropshire UK. Within 12 hours of birth (day 0) 40 Holstein-Friesian heifer calves were allocated, according to birth weight and dam's parity, to one of 2 treatments. "C3" calves wore coats (Calf Jackets, Cosy Calf, UK) from birth until 3 weeks of age (n=20), "C6" calves wore coats from birth until 6 weeks of age (n=20). The coats were put on to calves once their coats were dry. Calves were removed from their dams at birth and fed 4 litres of colostrum. The calves were subsequently offered a further 2 litres of colostrum. From day 1 they were offered milk replacer (5.8 litres/day; 155g/l Advanced Superstart, 23% CP, 20% oil) from teated bottles in 2 equal feeds with *ad libitum* concentrate (HSE calf starter pellets, 18% CP). They were housed in individual, straw bedded, outdoor hutches (Calf-tel, Hampel Animal Care, US). Live weights were recorded at birth, 2, 3, 4, 6 and 7 weeks of age. Concentrate intakes were calculated from total concentrate offered each week minus concentrate refused. The incidence of respiratory and enteric disease were recorded in the two groups of calves over the duration of the study. Weather data were collected from an on-site weather station. The mean (\pm SD) weather data for the study period were – maximum temperature 9.2 (\pm 3.6) °C, minimum temperature 1.8 (\pm 3.3) °C, precipitation 1.6 (\pm 3.0) mm/d, wind speed 2.5 (\pm 1.8) m/s. Calf live weight, daily live weight gain and concentrate intakes were analysed using repeated measures analysis of variance in Genstat (16th edition). The incidences of respiratory and enteric diseases in the two groups of calves were compared using Chi-squared analyses.

Results There were no significant differences in the live weights or growth rates of the calves in treatment group C3 compared with those in group C6 at any of the time points measured. Live weights of C3 calves increased from 41.5 \pm 0.90 kg at birth to 69.5 \pm 1.56 kg at 7 weeks of age and those of C6 calves increased from 40.8 \pm 1.2 kg at birth to 68.2 \pm 1.52 kg at 7 weeks of age. Daily live weight gain from birth to 7 weeks of age was 0.64 \pm 0.02 kg/d in the C3 calves *vs.* 0.59 \pm 0.03 kg/d in the C6 calves (p=0.147). Concentrate intakes were 7.0 \pm 3.3 g/d *vs.* 19.2 \pm 6.5 g/d (NS) in C3 and C6 calves, respectively during week 1, increasing to 214.4 \pm 42.0 g/d *vs.* 163.6 \pm 6.5 g/d (C3 *vs.* C6 calves, respectively; NS) in week 4 and 656.7 \pm 80.4 g/d *vs.* 572.6 \pm 70.4 g/d by week 7 (C3 *vs.* C6 calves, respectively; NS). There were no significant difference in the number of calves treated for respiratory disease (4 of 20 *vs.* 3 of 20, C3 *vs.* C6 calves, respectively) or enteric disease (4 of 20 *vs.* 4 of 20, C3 *vs.* C6) during the study period.

Conclusion Reducing the period of time that young Holstein Friesian calves, born during the winter, wear coats from 6 weeks to 3 weeks after birth has no detrimental effects on growth rates through to 7 weeks of age or calf health. Concentrate intakes during the milk fed phase of rearing were also not significantly different.

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Short scrotum castration increases total weight gain of lowland lambs when compared to the conventional rubber ring method

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Application Short scrotum (SS) castration increases total weight gain of lowland lambs at no extra financial expenditure.

Introduction With estimations of global population increase, meat production must be increased to sufficiently supply demand and ensure livestock sustainability. The SS castration method ensures continued androgen production due to testicle retention and thus produces similar meat output to ram lambs (Wellington *et al.*, 2003), although previous literature has been conflicting. Similarly, heart girth composition is considered to be an indicator of good conformation in addition weight gain (Fourie *et al.*, 2002). Aside from this, SS castration poses several other potential benefits including increased welfare by reducing pain and increased meat quality. The present study compares the SS castration method and the conventional rubber ring (RR) method to see whether there is a difference in total weight gain of British lowland lambs.

Material and methods A total of thirty-three lowland lambs born on a small scale farm were allocated into treatment groups balanced for breed and weight, SS (n=17) and RR (n=16) respectively and castration took place before seven days of age under normal managerial farm practice. The two methods use the same materials, castration rubber rings and an elastrator, however SS lambs testicles were pushed into the abdominal cavity and the scrotum was removed, whereas RR lambs testicles were removed alongside the scrotum (Molony *et al.*, 2002). Throughout the 105 day trial, the lambs experienced the same nutrition, housing and social interaction opportunities and were weighed at birth, weaning and pre-slaughter. Weight measurements were collected via the use of weighing scales with various morphometric body measurements, such as heart girth, taken at each interval using a tape measure. Total weight gain and heart girth data were analysed using a T-test, assuming equal variances, conducted on GenStat (17th Edition), to compare average results between treatment groups.

Results SS lambs reached a higher total weight gain when compared to RR lambs ($30.7 \pm 0.96\text{kg}$ vs $26.9 \pm 1.38\text{kg}$; SS vs RR mean \pm SEM respectively, $P < 0.05$) and subsequently reached a higher finishing weight. On average SS lambs gained 3.8kg extra when compared to RR lambs (see figure 1), with the biggest increase in weight being between birth and weaning. Additionally, SS castrates heart girth was larger, when compared to RR castrates ($85.1 \pm 0.66\text{cm}$ vs $79.0 \pm 1.01\text{cm}$; SS vs RR mean \pm SEM respectively, $P < 0.001$) as highlighted in figure 2.

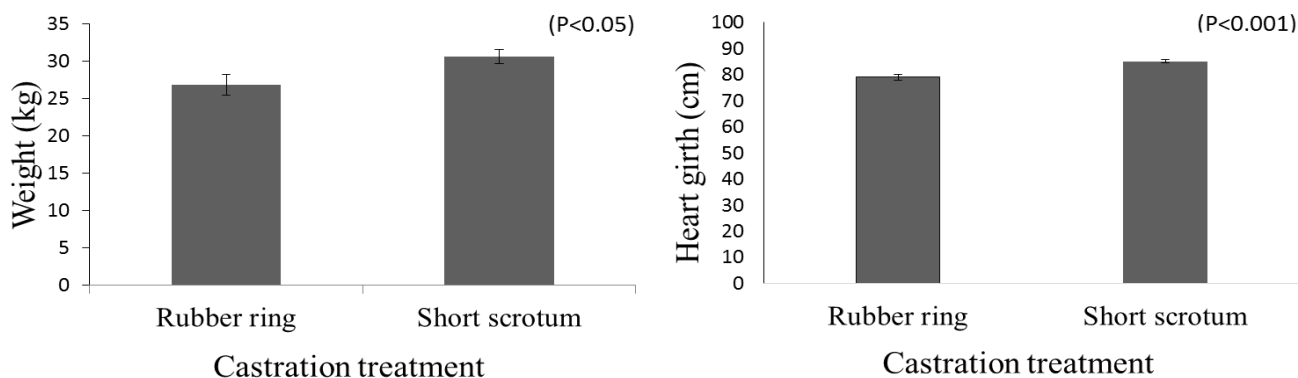


Figure 1 Effect of castration treatment on finishing weight **Figure 2** Effect of castration treatment on final heart girth size

Conclusion The SS method is an improved alternative to the conventional method due to its ability to increase total weight gain and increase heart girth, ensuring an improved production line for farmers, vital for the sustainability of the livestock sector, with no extra cost or materials. Furthermore, the practical convenience of the method allows for immediate industry implementation, although further research regarding sterility efficacy would provide a stronger foundation for industry use.

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Current commercial lamb tail-docking and castration practices, and a trial of 1) incidences of pain responses in lambs when docked and 2) cleanliness of fleece with docked or entire tails

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Application An understanding of current practices and the rationale for undertaking painful procedures can provide a useful context for research. Trial data can support researchers developing less painful alternatives.

Introduction Sustainability in agriculture includes the ethics of animal production and a significant issue is the painful tail-docking and castration of lambs. Research in lambs has shown pain of castration to persist for 40 days, in human vasectomies 8-15% experience permanent pain (O'Riorden, personal communication). Many studies of commercial lamb management were conducted years ago and may not reflect current practice, our survey provides an overview in the NE of England to determine if changes in management have evolved with, for example, better chemicals to manage flystrike; markets for entire lambs and whether this affects farmers' decisions to dock and castrate. This may help flag opportunities to reassess the need for these painful procedures, which may be based on un-challenged habit rather than evidence.

In addition to the survey, a pilot was carried out, on the effects of docking on fleece cleanliness. While avoiding painful procedures is preferable, if this is not possible, practical alternative methods have to be sought. Graham *et al.* (2002) found that 16% of lambs showed little evidence of pain in response to tail docking. They suggest this may be due to positioning of the rubber ring in the intervertebral space resulting in mechanical deformation of the nerve trunks. It is possible that new, more humane techniques could exploit understanding of this mechanism underlying the low response in these lambs. To help validate the aforementioned finding we recorded the proportion of non-responders in the commercial flock. We also recorded the time taken for the tail to fall off, again as a comparison for future research into alternative methods.

Material and methods A 16 question survey was conducted face-to-face on 16 sheep farms by the Farm Business Survey, NE. The recording of non-responders was through observation of pain related behaviours (after Molony *et al.*, 2002). April born (indoor, lowland farm, NE England) lambs (n=54; mule x Texel/Charolais/Suffolk) were just tail docked with rubber rings and point sampled for normal/abnormal posture (at 2min for 20min), a subjective assessment was also made of whether the lamb was experiencing pain of tail docking (responder/low responder). A trial assessed the cleanliness of Mule x Texel lambs' rears at 6.5 months (Nov 2015). Ewe and castrated rams were assigned to either dock (n=9) or tail (n=10), photos were scored 0 clean to 4 very dirty and analysed by MWU. The tails of Mule x Texel (n=11) were recorded on or off in 2 observations 7 days apart. The study was approved by the University Animal Welfare Ethical Review Board.

Results The respondents had an average 423 ewes (69% mules) with a 135% lambing percentage; 54% lowland, 30% upland; 15% hill; 82% lambed in April; 19% inside; 44% outside; 31% a mixture; 75% docked all lambs; 25% docked 50% or according to breed e.g. Swaledales had tails left on. Reasons for docking were 87% to keep lambs clean; 37% to prevent flystrike; 23% for appearance at auction. No farm used anaesthetic or analgesic for docking or castration; 95% use rubber rings, 5% used burdizzo. Flystrike incidence was 2.5%, fatalities 0.8% (mean of 3 farms reporting fatalities) 95% used pour on products to prevent flystrike 5% also stated dipping. Only 5% of farms sold lambs on contract (the rest through the mart) but did not know if buyers had a policy on entire tails or males. 81% clipped between 10-50% of their docked lambs because they were dirty. All farms castrated: 82% to avoid tugging ewe lambs; 18% due to other ram nuisance behaviour; 18% cannot sell if they do not castrate; 5% explained that they considered that ram lambs take longer to finish. 38% and 31% would consider not docking or castrating respectively stating: if buyer requested or lambs got away early. Objective measure analysis (100% time in normal posture between 10-20 minutes) found 23% (of 54) "low responders" whereas 39% were subjectively categorised as low-responders. 82% of tails fell off within 16-21 days. There was no difference in lamb cleanliness scores (mean rank tail: 10.3; docked: 9.7; MWU 42.5 P=0.47). Ad-hoc interview of farm manager also revealed no perceived difference in cleanliness or incidence of fly strike. Ad-hoc observations noted Suffolk crosses when dirty were much dirtier and had much longer tails compared to Texel- and Charolais-crosses and warrants investigation.

Conclusion All farms sampled routinely dock and castrate with no pain relief. Most farmers would not consider leaving tails / males entire, but a significant proportion would if buyers required it. No differences in cleanliness was found between docked or undocked lambs on one lowland farm in the NE England. "Low-responses" of lambs to tail docking, assessed subjectively, appear to under-estimate the incidence of pain behaviours when compared to objective measurement of behavioural events. If the number of "low/non responders" could increase to 100%, by refinement of the method of application of rubber rings, the welfare of the lambs would be improved.

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Effect of weaning system and percentage of East Friesian genotype on milk yield and lamb weight

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Application The adoption of an adequate weaning system and suitable genotype could improve the income of dairy sheep farmers through reaching good milk yields and adequate lamb weight.

Introduction The East Friesian (EF) breed has been introduced to several countries in Latin America to favour the development of the local dairy sheep industry in pure breed flocks and to improve milk production of local sheep breeds through crossbreeding. Some other exotic dairy breeds have been introduced with variable results in milk production and adaptability to environmental conditions (Angeles-Hernandez *et al.* 2015). Flock management practices have great influence in dairy performance. Amongst management practices weaning system is important. The aim of this study was to evaluate the effect of weaning system and genotype on milk production variables and lamb weight.

Material and methods A total of 553 weekly milk yield records from 59 confined multiparous crossbred EF × Pelibuey ewes with either 50% EF (EF50, n=35) or 75% EF (EF75, n=24) were used. Ewes were randomly assigned to one of three weaning systems: Traditional meat weaning (TRAD; n=18), where ewes were mechanically milked twice daily (0800 and 1500 h) after lambs were weaned at 60 days old. Intensive dairy system (INT, n=20), where ewes were mechanically milked twice daily after lambs were weaned at 48 h old. Mixed system (MIX, n=21), where ewes were mechanically milked once daily (0800) from day 31 after lambs were removed during the evening only (from 1500 h), and milked twice daily after lambs were weaned at 60 days old. Milk production (M) at time t was used to estimate maximum potential of secretion (MS), relative proliferation rate of secretory cell numbers during early lactation (GR) and relative decline in cell numbers as lactation progressed (DR) using a mechanistic model (Pollott, 2000): $M_t = (MS/(1+a*e^{(-GR*t-150)}))(2-e^{(DR*t-150)})$. MS, GR and DR were estimated for each individual lactation through nonlinear regression (SAS 2002). Lambs were weighed at weaning (W60). Effects of genotype (EF50, EF75) and weaning system (TRAD, INT, MIX) on milk production, length of lactation, MS, GR, DR and W60 were analysed through the using general linear model (SAS 2002).

Results Table 1 shows mean milk production parameters and lamb weaning weights. EF75 ewes had greater milk yields than EF50 ewes (74.96 vs. 57.61 l, respectively; P=0.05), which concurred with greater values of GR (0.186 vs 0.127; P=0.01) and smaller values of DR (-0.011 vs 0.0014; P=0.01), which determine major number of secretory cells available to milk synthesis. INT ewes had greater milk yields (78.54 l) than TRAD ewes (49.53 l) and MIX ewes (58.09 l; P=0.04). The TRAD system had the shorter lactation (21 days; P=0.001), which could explain its lower milk yields. The INT systems showed the largest lactations (70.53 days) followed by MIX (58.09 days). INT and TRAD had the higher values of MS (1.67 and 1.83 l, respectively). Lamb weaning weight was not affected by genotype or weaning system.

Table 1 Milk production parameters and lamb weaning weights for different genotype and weaning system.

	Weaning system (W)				Genotype (G)			P values	
	TRAD	INT	MIX	s.e.d	EF50	EF75	s.e.d.	W	G
Milk yield, l	49.53 ^b	78.54 ^a	58.09 ^b	7.46	57.61 ^b	74.96 ^a	9.08	0.04	0.05
Lactation length, d	21.00 ^c	70.53 ^a	45.68 ^b	3.23	46.41	52.76	4.04	0.001	0.29
MS, l	1.83 ^a	1.08 ^b	1.67 ^a	0.18	1.32	1.30	0.161	0.005	0.91
GR	0.144	0.095	0.154	0.02	0.127 ^b	0.186 ^a	0.016	0.07	0.01
DR	0.005	0.006	-0.005	0.005	0.0014	-0.011	0.004	0.22	0.09
Lamb weaning weight, kg	24.04	23.11	19.56	2.21	20.61	21.99	3.86	0.36	0.68

Conclusion Using sheep of 75% EF genotype showed acceptable results and could be a viable option to dairy sheep farms with similar characteristics to those of the present work. The INT weaning system showed the better milk yields without negative effects on lamb weight. Further research should explore the costs of implementation of the recommended systems.

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An investigation into the effect of feeding a *Saccharomyces cerevisiae* fermentation product to transition and finishing Scottish heifers

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Application Results from this study demonstrate the benefits of feeding a commercial *Saccharomyces cerevisiae* fermentation product to transition and finishing heifers fed a high cereal diet.

Introduction Optimising cattle performance and improving margins are important aspects when finishing cattle. The wide range of feed additives available and variable published results for these products would suggest there is a requirement for commercially relevant research to aid decision making by producers. Previous research (Marsh *et al.*, 2014) has demonstrated the significant benefits of feeding a commercial *Saccharomyces cerevisiae* fermentation product to intensively finished bulls. The objective of this experiment was therefore to investigate the effect of feeding Diamond V XP_{LS} on the physical and financial performance of intensively finished heifers in a Scottish situation. Diamond V XP_{LS}, (Diamond V, Cedar Rapids, IA, USA) contains unique metabolites generated from a proprietary technology of *Saccharomyces cerevisiae* fermentation.

Material and methods Fifty two Continental, Limousin, Charolais and Simmental heifers were blocked according to breed randomly assigned to one of two treatments, with two replicates in each treatment to give a total of 4 pens of 13 animals. At day 0, all animals were moved from a shared grass enclosure to handling facilities where they were weighed (477 kg average initial weight, s.e.d. 9.1 kg) and randomly allocated to straw bedded pens. All animals were fed the same total mixed ration, which progressed from a starter ration, including 26% barley (DM basis) to a finishing ration containing 77% barley (DM basis) over a 33 day transition period. Animals in the treatment groups also received 45 g/h/d XP_{LS} top-dressed and lightly forked into the feed. The experiment lasted 90 days after which time animals were selected for slaughter. The data were analysed using ANOVA.

Results Overall daily liveweight gain (DLWG) was significantly greater ($P < 0.05$) for XP_{LS} fed animals compared to control animals (1.56 kg/d v 1.40 kg/d). Furthermore, DLWG was significantly higher ($P < 0.05$) in the first 28 days of the experimental period for XP_{LS} fed animals (table 1).

Table 1 Mean daily liveweight gain

Period (day no.)	XP _{LS}	Control	s.e.d	P value
0-28	1.73	1.45	0.116	0.017
28-58	1.45	1.33	0.139	0.395
58-90	1.50	1.43	0.113	0.455
0-90	1.56	1.40	0.075	0.038

The DLWG advantage (0.16-kg/d) was worth 34 p/h/d (based on the value of each kg of live weight worth £2.12), minus the cost of the XP_{LS} at 7 p/h/d gives an advantage of 27 p/h/d. There was no significant difference between groups for feed use and therefore feed conversion ratio (kg feed/ kg gain) was improved for animals fed XP_{LS} (11.7:1 and 13.1:1). The improved performance effectively reduces feed cost/kg gain (inclusion of XP_{LS} cost) to £1.17 /kg LWG (from £1.26 /kg of LWG). This 9p difference can be applied to the typical 140-kg increase in live weights that the XP_{LS} fed animals made over the duration of the experiment. The improvements in DLWG with same feed intakes for animals fed XP_{LS} therefore reduced cost per kg gain by £12.60 /head. At the end of the experiment 20 animals were selected for slaughter. Thirteen of these animals were from the XP_{LS} fed, with equal numbers selected from each pen.

Conclusion Feeding XP_{LS}, a *Saccharomyces cerevisiae* fermentation product, to transition and finishing heifers in a Scottish situation resulted in increases in DLWG with improved feed conversion ratio by 11%. This equates to a financial advantage using costings prevailing at the time of the study. It is therefore concluded that the addition of Diamond V XP_{LS} is economically viable in cattle transition and finishing diets.

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The combined effects of including nitrate and increasing the lipid concentration within a silage-based diet on performance and efficiency of finishing beef steers

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Application Adding nitrate to the diet had negative implications for steer performance, but increased lipid concentration had no adverse effects. Feeding high lipid feedstuffs can be recommended provided its use is economically competitive.

Introduction Previous studies have shown that adding nitrate or increasing the concentration of dietary lipid in mixed forage and concentrate diets can reduce methane emissions from finishing beef cattle without adversely affecting animal performance or efficiency (Duthie *et al.*, 2015). However, information on the effect of combining dietary mitigation strategies is limited. The objective of this study was to investigate the combined effects of adding nitrate and increasing the dietary lipid concentration on steer performance and efficiency.

Material and methods The experiment was of a 2 × 4 factorial design consisting of two breeds (AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin) and 4 treatments (forage:concentrate (g/kg dry matter, DM): 557:443): (i) Control containing rapeseed meal as the main protein source which was replaced with either (ii) Nitrate (Calcinit, 18 g nitrate/kg diet DM), (iii) added lipid in the form of maize dark grains (acid hydrolysed ether extract (AHEE) increased from 25 to 37 g AHEE/kg diet DM) or (iv) Combined nitrate (18 g nitrate/kg diet DM) and lipid (36 g AHEE/kg diet DM). Steers (n=80) were group-housed in even numbers of each breed across 4 pens; each treatment was allocated to 1 pen. Individual dry matter intake (DMI) was recorded for 56 days, with feed offered *ad libitum*. Liveweights (LW) were measured weekly and ultrasonic fat depth (12th/13th rib) at the end (FD1) of test. Growth was modelled by linear regression of weight against test date, to describe average LW gain (LWG) and mid-test LW. Feed conversion ratio (FCR) was DMI/LWG. Residual feed intake (RFI) was calculated as deviation of actual DMI from DMI predicted from linear regression of actual DMI on LWG, Mid-LW and FD1. Statistical analyses were conducted using the mixed procedure of SAS (SAS Inst. Inc.) with the fixed effects of breed, nitrate and lipid and the random effect of pen (and slaughter batch for carcass traits).

Results Lipid did not adversely affect animal performance. However, addition of Nitrate resulted in poorer LWG (P<0.01) and reduced FCR (P<0.05) compared with diets not containing nitrate. AAx steers achieved greater LWG (P<0.01), but showed greater DMI (P<0.001), greater FD1 (P<0.01) and poorer RFI (P<0.01) than LIMx steers. Furthermore, AAx steers achieved poorer KO than LIMx steers (P<0.001).

Table 1 Effect of breed, nitrate and lipid on growth, feed intake and feed efficiency.

	Treatment				Breed		Significance		
	Control	Nitrate	Lipid	Combined	AAx	LIMx	Breed	Nitrate	Lipid
Age start (days)	413	414	413	414	417	411	NS	NS	NS
Mid-LW (kg)	546.3	542.5	538.0	533.9	542.0	538.6	NS	NS	NS
LWG (kg/day)	1.73	1.53	1.72	1.62	1.74	1.56	**	**	NS
DMI (kg/day)	11.78	11.43	11.75	11.44	12.15	11.07	***	NS	NS
DMI/LW(g/kg)	21.60	21.08	21.90	21.43	22.44	20.59	***	NS	NS
FCR (kg, kg)	6.85	7.52	6.89	7.20	7.02	7.20	NS	*	NS
RFI (kg)	-0.08	0.06	-0.02	0.03	0.24	-0.24	**	NS	NS
FD1 (mm)	8.40	8.86	8.81	8.26	9.13	8.05	**	NS	NS
Slaughter LW (kg)	685.9	674.7	674.7	677.8	689.2	667.4	*	NS	NS
Cold carcass weight (kg)	391.0	380.2	382.6	380.5	382.4	384.7	NS	NS	NS
KO (%)	57.0	56.4	56.7	56.2	55.5	57.7	***	NS	NS

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

Conclusion In contrast to previous work (Duthie *et al.*, 2015), adding nitrate to a mixed forage:concentrate diet had a negative impact on growth and efficiency of finishing beef steers. However, increasing the dietary lipid concentration did not adversely affect steer performance or efficiency and thus can be recommended for use within finishing cattle diets.

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Relationships between net feed efficiency (NFE) and mechanical measures of beef tenderness and cooking loss in finishing Stabiliser steers

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Application The results suggest that selecting for lower NFE and therefore more feed efficient Stabiliser beef cattle in future generations can be applied in commercial herds without adverse changes in beef tenderness or cooking loss.

Introduction Earlier work has shown that substantial variation in NFE exists within the Stabiliser cattle population in the UK (Hyslop *et al*, 2014) 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 additional traits such as beef eating quality that may be associated with low NFE animals. Consequently the objective of the current study was to examine the potential relationships between NFE measured in finishing Stabiliser steers and measures of mechanical beef tenderness and cooking loss in sirloin steaks obtained from the same individual steers following slaughter.

Materials and methods NFE was determined in finishing Stabiliser steers as described previously (Hyslop *et al*, 2014) during the 8-week period prior to commercial slaughter. 48 hours after slaughter, samples of sirloin steaks were obtained from the 12th/13th rib site from 113 Stabiliser steers across 4 batches, transported at below 5 °C to the JSR food laboratory, matured for 14 days and then stored frozen prior to mechanical assessment of tenderness using a TenderScot™ machine and loss of weight on cooking (% cooking loss). Both slice shear force (SSF) which simulates the force required (kg Force) to force a “blade” through a beef sample and the Mirinz bite test (MNZ) which simulates the force required (kg Force) to “bite” through a beef sample with a metal “tooth”, were measured. After thawing, beef samples were cooked to a constant internal muscle temperature of 71 °C, cut in a defined and consistent way with regard to muscle grain and then assessed for tenderness using SSF, MNZ & cooking loss % as described above. 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, SSF, MNZ & % cooking loss 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 either SSF, MNZ or % cooking loss between the three NFE groups. Linear relationships with NFE for each of these parameters also showed no significant relationships between NFE and any of the MEQ parameters studied (Figures 1, 2 and 3).

Table 1 NFE and MEQ in Stabiliser steers

	low NFE	mid NFE	high NFE	sed	Sig
NFE (kg/DMI/d)	-0.70 ^a	-0.07 ^b	+0.84 ^c	0.086	***
SSF (kg force)	14.1	14.5	14.1	0.83	
MNZ (kg force)	4.43	4.87	4.91	0.284	
Cooking loss (%)	21.3	21.9	22.8	0.91	

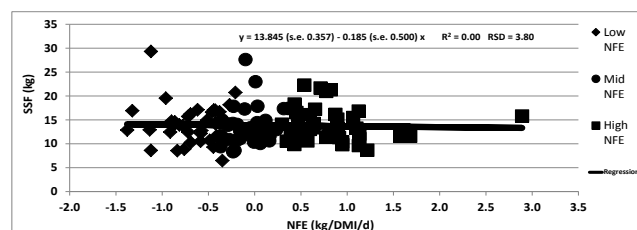


Figure 1 Relationship between NFE & SSF in steers

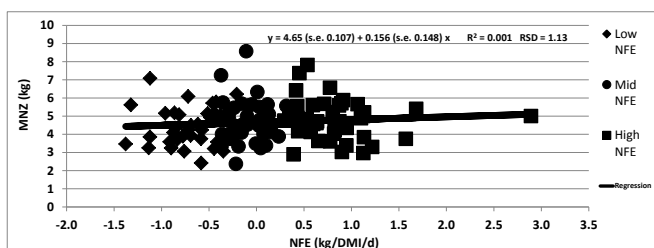


Figure 2 Relationship between NFE & MNZ in steers

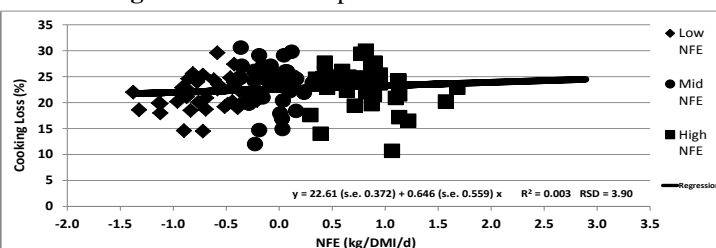


Figure 3 Relationship between NFE & % cooking loss in steers

Conclusion No significant relationships were seen between NFE and any measures of mechanical beef eating quality in Stabiliser steers regardless of differences 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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Contrasting finishing strategies for Holstein-Friesian bulls slaughtered at 19 months of age

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Application Greater carcass performance was observed for dairy bulls finished on concentrates *ad libitum* compared to bulls finished at pasture. Extending the finishing period for dairy bulls finished at pastures had no effect on carcass traits.

Introduction Previously a desktop study by Ashfield *et al.* (2014) reported that slaughtering dairy bull at 19 months of age was the most economic bull production system. It was concluded that the increased utilization of pasture during the second grazing season made the system more economical. Recent research suggested that dairy bulls finished at pasture had comparable carcass weights to bulls finished indoors but that fat score was significantly lower for pasture based finishing strategies (Murphy *et al.*, 2014). Consequently the objective of this study, carried out at the Teagasc Johnstown Castle Research Centre, Co. Wexford, Ireland, was to investigate alternative finishing strategies for Holstein-Friesian (HF) bulls slaughtered at 19 months of age.

Material and methods Data were available for 45 spring born HF bulls. Mean date of birth was 6 February 2014. Bulls were randomly assigned to one of three treatments; blocked by date of birth, farm of origin and weaning weight. Treatments included bulls finished indoors on concentrates *ad libitum* for 100 days (AL); bulls finished at pasture offered 5 kg of concentrate dry matter (DM) per head daily for 100 days (SPC) and bulls finished at pasture offered 5 kg DM of concentrate per head daily for 150 days (LPC). During the first season at pasture bulls were rotationally grazed and offered 1 kg DM of concentrate per head daily. Bulls were housed on 4 November and fed grass silage *ad libitum* and 1.5 kg DM of concentrate per head daily. On March 23, bulls returned to pasture for a second grazing season. The LPC group commenced finishing on 21 April and were adapted to 5 kg DM of concentrate per head daily in one feed, over a 10 day period. On 10 June AL were housed and adapted to concentrates *ad libitum* over a 21 day period. Straw was offered *ad libitum*. The AL group were split into pens of five. Fresh concentrates were offered daily with weigh backs completed twice weekly to estimate individual concentrate DM intake (CDMI). Simultaneously, SPC were adapted to 5 kg DM of concentrate per head daily as above. Pre and post grazing swards heights were 10.5 (s.d. 3.11) and 4.1 (s.d. 0.84) cm, respectively. Concentrates consisted of 800 g/kg barley, 140 g/kg soya bean meal, 40 g/kg molasses and 20 g/kg minerals. Bulls were weighed fortnightly. Post slaughter carcass weight, conformation and fat score were recorded. Data were analysed using PROC MIXED of SAS. Treatment was included as a fixed effect and live weight at the start of finishing was included as a covariate.

Results During the finishing period, CDMI was greatest for AL and lowest for SPC, LPC was intermediate ($P < 0.001$), 1133, 474 and 718 kg DM, respectively. Live weight at the start of finishing was lowest for LPC but greater for both AL and SPC ($P < 0.01$) (Table 1). However, average daily gain (ADG) during finishing was greater for AL than both SPC and LPC ($P < 0.001$). Slaughter ($P < 0.01$) and carcass ($P < 0.05$) weight were similar for SPC and LPC but greatest for AL. Treatment had no effect on kill-out proportion (KO) or conformation score (CS). Fat score (FS) was acceptable across all treatments but greater for AL than both SPC and LPC ($P < 0.001$).

Table 1 Effects of finishing strategy on animal and carcass performance

	AL	SPC	LPC	SEM	P-value
Start of finish (kg)	416 ^a	420 ^a	358 ^b	19.2	<0.01
Average daily gain during finish (kg/day)	2.12 ^a	1.36 ^b	1.36 ^b	0.887	<0.001
Slaughter weight (kg)	613 ^a	537 ^b	550 ^b	23.0	<0.01
Carcass weight (kg)	325 ^a	289 ^b	294 ^b	14.3	<0.05
Kill-out proportion (g/kg)	530	528	535	6.1	0.4816
Conformation score (1-15)	5.67	5.07	5.20	0.412	0.2579
Fat score (1-15)	7.40 ^a	5.53 ^b	5.00 ^b	0.377	<0.001

Conclusion Extending the finishing period at pasture did not affect animal or carcass performance. Although indoor finishing strategies had greater slaughter and carcass weight, FS and CDMI, pasture based finishing strategies had similar KO and CS with reduced CDMI.

Acknowledgements Financial support from the Research Stimulus Fund 11/SF/332 is gratefully appreciated.

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Optimal slaughter age of UK beef cattle to increase profitability and reduce greenhouse gas emissions

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Application Reducing days to slaughter will improve profits for farmers and increase cattle turnover on farm. Additionally this will reduce greenhouse gas (GHG) emissions which are likely to become an increasingly important factor as the government consider the introduction of an emissions tax.

Introduction Beef animals are most commonly finished on 24 month systems. The decision to intensively finish cattle or over winter on straw can mean the difference between profit and loss and is often influenced by the variation in feed and bedding costs. This study looked at the optimal age at slaughter for improved profit and reduced emissions accounting for slaughter weight, fat, conformation and season of birth.

Material and methods The analysis focused on purebred Holstein Friesians, Limousin-Holstein Friesian crossbreeds and purebred Limousin (106,059, 81,590 and 24,426 animals respectively) from seven UK abattoirs born between 2001 and 2013. Average slaughter age across all breed types was 715 days with a mean carcass weight of 325Kg. Carcass weight, conformation and fat class were used to calculate the value of the animal from price grids, with the base price set at £3.66/Kg. Penalties were applied to animals which missed the target weight range (260-420Kg) or were over 30 months of age. The cost of finishing was calculated based on breed, season born and finishing system. Finishing system was inferred from age at slaughter and further costs were accrued for each additional day spent outside of the finishing system. Concentrates were charged at £170/tonne, straw at £67/tonne and silage at £30/tonne. Other fixed costs were charged at £1.06/day in the summer and £1.18/day in the winter in order to account for seasonal costs associated with the number of days spent housed or at grass as a result of the animal's season of birth. The profit obtained from each animal was the difference between the value of the carcass and the total costs as described above. Cattle emissions for concentrates were estimated at 13.8g CO₂e/g/hd and 21.15g CO₂e/g/hd for forage.

Results Holstein Friesian cattle were slaughtered most frequently between 365 and 550 days. Whilst their peak carcass value was at 730 days the difference in slaughter value between 365 and 730 days was just £135 making them most profitable at 365 days (12 months) (Figure 1). Limousin cattle were slaughtered most frequently at 730 days and peak carcass value was between 400 and 430 days making them most profitable between 365 and 430 days (12-14 months) (Figure 1). Holstein Friesian-Limousin crossbred cattle were most frequently slaughtered between 700 and 800 days. Peak carcass value of these animals was reached at 800 days although the difference between 365 and 800 days of age was just £80 and therefore the most profitable age of slaughter was 365 days (12 months) (Figure 1). An additional advantage of the 12 month finishing system was the three fold decrease in emissions when compared with the current 24 month system (Figure 2).

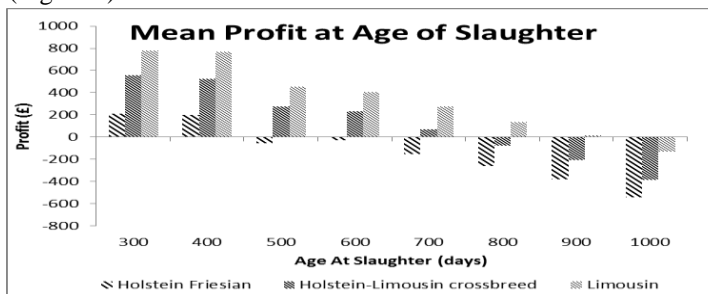


Figure 1 Profitability at slaughter age of different breed types

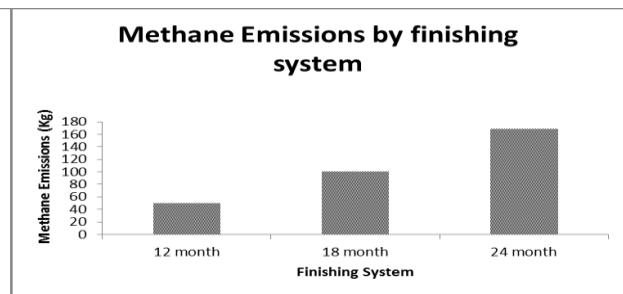


Figure 2 Methane emissions for each finishing system

Conclusion For all breed types the optimal time to slaughter is at 12 months in order to maximise profit and also to provide the opportunity to acquire more stock. This is of particular importance for dairy and dairy crossbreeds where profit rapidly becomes a loss with each additional day. Additionally reducing days to slaughter decreases GHG emissions which may potentially impact the profitability of beef in the future when charges are made for emissions.

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Performance of growing ongole crossbred cattle fed rations containing different levels of oil palm fronds silage

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Application Oil palm fronds (OPF) silage can be fed to growing ongole crossbred cattle (OCC) up to 60% on dry matter (DM) basis with the optimum level of 46%.

Introduction The OCC (crossing among zebu - *Bos indicus*, banteng - *Bos javanicus*, and other indigenous Indonesian breeds) are well adapted to harsh environments under hot and humid climate and low-quality feed to produce meat. In the dry season, natural pastures decrease in nutritive value and improved grasses cannot grow. OPFs are a by-product of the cultivation of oil palm trees; about 10.8 t OPF DM is yielded per year from each hectare of oil palm trees (Zahari *et al.*, 2003) with estimated fibre supply for about 6-7 animal units/ha/year. OPFs are a palatable, low-protein, high-fibre material with good feeding potential for ruminant livestock. Ensiling OPF is a promising alternative to conserve and utilize it as an efficient ruminant feed, which is not a usual phenomenon in Indonesia. The objectives of this trial were to find out the optimum level of feeding of OPF silage in OCC and its effect on their productive performance.

Material and methods Fresh OPF was wilted for 1-2 days before it was chopped to 1-2 cm lengths using a diesel-driven chopper. The chopped OPF was mixed with lactic acid bacteria (LAB) supernatant (3 ml kg⁻¹ OPF), 5% molasses and 3% rice polishing on DM basis. The whole material was then ensiled in the air tight fibre drum silos (\pm 100 kg capacity) for 30 days. Experimental treatments were T1: 30% OPF silage + 70% concentrate; T2: 40% OPF silage + 60% concentrate; T3: 50% OPF silage + 50% concentrates; T4: 60% OPF silage + 40% concentrate. Concentrate was composed of rice bran, palm kernel meal, soyabean meal, cassava pulp, molasses, salt and mineral mixture with 140-160 g crude protein (CP) and 650 – 700 g total digestible nutrients (TDN) per kg DM. Feeding trial was conducted according to randomly completely blocked design (RCBD) using 12 growing OCC (146.4 \pm 25.3 kg) for 90 days. Weights of the diet offered and refused were recorded daily. Each animal was weighed after every two weeks and the data were used to calculate daily weight gain (DWG). In the last 7 days of the experiment, weight of the faeces voided from each animal was recorded every morning and 100 g sample was taken together with the diet. These samples were later analyzed for DM, ash, CP, neutral detergent fibre (NDF) and acid detergent fibre (ADF). DM intake (DMI), nutrient digestibility and feed conversion (FC) was calculated. Data were subjected to analyses of variance based on RCBD and orthogonal polynomial test was performed on data showing significance among treatment means ($P \leq 0.05$) (SAS Inc., 2008), contrasts used were linear (L) and quadratic (Q).

Results There was no significant ($P > 0.05$) effect of treatments on DMD, OMD and CPD, but NDFD and ADFD changed quadratically ($P < 0.05$) as level of OPF silage increased in the diet (Table 1). Increasing the level of OPF silage resulted in a decreasing DMI (linearly, $P < 0.05$), meanwhile BWG and FC changed quadratically ($P < 0.05$). The relationship between different levels of OPF silage (X) with BWG and FC respectively followed these equations, $BWG = -9.79 X^2 + 8.63X - 1.05$; $R^2 = 0.70$, and $FC = 0.0073X^2 - 0.62X + 21.126$, $R^2 = 0.59$.

Table 1 Relationship between OPF silage levels with parameters

Items	OPF silage levels in diet (% DM)				Relationship
	30	40	50	60	
DMD	73.84	76.31	71.49	67.83	n.s. ($P > 0.05$)
OMD	76.99	77.46	73.17	69.87	n.s. ($P > 0.05$)
CPD	83.48	83.75	79.77	79.34	n.s. ($P > 0.05$)
NDFD	69.6	73.1	67.7	63.0	Q ($P < 0.05$)
ADFD	68.3	70.2	65.5	62.2	Q ($P < 0.05$)
DMI (kg h ⁻¹ d ⁻¹)	4.60	4.50	4.32	3.80	L ($P < 0.05$)
DWG (kg h ⁻¹ d ⁻¹)	0.714	0.740	0.929	0.636	Q ($P < 0.05$)
FC	6.44	6.08	4.65	5.97	Q ($P < 0.05$)

Conclusion OPF silages could be used as a component of OCC diet with optimum level of 46% DM.

Acknowledgements This study was funded by Ministry of Education and Cultures of the Republic of Indonesia through PUPT Scheme. The authors gratefully acknowledge UU ISS PTPN VI for providing research facilities.

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The effects of dietary nitrate addition and increased lipid concentration on methane (CH₄) and hydrogen (H₂) emissions from beef cattle are independent

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Application There was no interaction between added nitrate and increased lipid concentration on CH₄ emissions from steers fed a mixed forage: concentrate diet. Potential health effects associated with feeding nitrate could be reduced by combining lesser amounts of nitrate with increased dietary lipid content.

Introduction Globally, emissions from livestock production are estimated at 7.1 Gt CO₂-equivalent per annum, with beef production responsible for 2.9 Gt CO₂-equivalent (Gerber *et al.*, 2013). Methane emissions account for 44% of the total GHG emissions from beef production systems and are primarily affected by feed intake and quality. Previous studies have shown that adding nitrate or increasing dietary oil concentrations in mixed forage and concentrate diets lowered CH₄ emissions (Patra 2013; Troy, 2015). However there is little information on the effect of combining dietary CH₄ mitigation strategies. This experiment was designed to investigate the combined effects of adding nitrate or increasing dietary lipid on CH₄ and H₂ emissions from finishing beef cattle fed a mixed 500:500 forage:concentrate diet (DM basis).

Material and methods The experiment was of a 2 × 4 factorial design whereby two breeds (AAx, crossbred Aberdeen Angus; LIMx, crossbred Limousin) received a mixed forage and concentrate diet (forage:concentrate (g/kg DM): 557:443) with four CH₄ mitigation treatments (Control, Ctrl; Nitrate, Nit; Lipid, Lip; Combined, Comb): (Ctrl) containing rapeseed meal as the main protein source which was replaced with either (Nit) Calcinit, (19 g nitrate/kg diet DM); (Lip) maize dark grains (acid hydrolysed ether extract (AHEE) increased from 25 to 37 g AHEE/kg diet DM) or (Comb) nitrate (19 g nitrate/kg diet DM) and lipid (36 g AHEE/kg diet DM). Diets were fed *ad libitum*. Steers (18 per treatment; live-weight, 659 kg (SE 8.3)) were offered experimental diets for a minimum of 10 weeks prior to measurement of CH₄ and H₂ emissions using 6 open-circuit respiration chambers (Troy *et al.* 2015). Steers were assigned to chambers using a randomised block design in 13 weekly periods. Data were available for 71 steers and were analysed using general linear models with fixed effects of breed, nitrate addition and increased lipid and random effects of chamber and block.

Results Steers receiving treatments which included nitrate produced less CH₄ and more H₂ than those receiving treatments without added nitrate, when expressed on both a daily and intake corrected basis. Increasing the dietary lipid content had no significant effect on CH₄ or H₂ emissions. There were no significant interactions between nitrate and lipid on CH₄ or H₂ emissions. Animals receiving the treatments which included nitrate produced 2.1 g/kg DMI less CH₄ than those animals that did not receive nitrate, which equates to 45% of the theoretical CH₄ reduction potential given the amount of nitrate added.

AAx steers were heavier than the LIMx steers and had a higher DMI during the chamber period. Therefore, they produced more CH₄ on a daily basis. However, the LIMx steers produced more CH₄ when corrected for feed intake. Breed had no effect on H₂ emissions on both a daily and intake corrected basis.

	Treatment				Breed		Significance		
	Ctrl	Nit	Lip	Comb	AAx	LIMx	Nitrate	Lipid	Breed
CH ₄ (g/day)	246	219	238	210	242	215	***	NS	***
CH ₄ (g/kg DMI)	24.0	22.1	23.4	20.9	22.1	23.2	***	NS	*
H ₂ (g/day)	0.46	0.99	0.40	1.05	0.77	0.67	***	NS	NS
H ₂ (g/kg DMI)	0.04	0.10	0.04	0.10	0.07	0.07	***	NS	NS
DMI (kg/day)	10.4	9.8	10.2	10.2	11.0	9.3	NS	NS	***

There were no interactions between the nitrate and lipid treatments or between breed and nutritional treatments.

Conclusion There were no significant interactions between nitrate and lipid on CH₄ or H₂ emissions; thus the effects of adding nitrate and increasing lipid were independent of each other. LIMx steers produced more CH₄ than AAx steers on an intake corrected basis.

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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Genotypic relationships between methane emission and production traits in dairy cattle

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Application Obtaining genetic parameters for methane emission and production traits will give insight in opportunities for developing a long-term mitigation strategy via genetic improvement, whilst minimizing adverse effects on production traits.

Introduction Twenty percent of methane production in the UK comes from the dairy sector (Garnsworthy, 2004), of which 80% is formed during enteric fermentation (Vergé *et al.*, 2007). Reducing this amount will have a huge impact on the total amount of methane produced. When using breeding strategies to accomplish this, the effect will be cumulative and permanent over generations (De Haas *et al.*, 2011). However, this strategy will only be adopted by farmers if there are no adverse side effects on production traits. In this study the feasibility of breeding for lower methane production and the effect on production traits is examined.

Material and methods In this study, data from the Langhill herd (SRUC Dairy Research & Innovation Centre) are used. Methane emission data (ppm-m) on 212 Holstein-Friesian cows (818 observations) were available. At time of measuring, this herd was also part of a long term feed trial (a high forage vs a low forage diet) and originates from two genetic lines (a selection line for high milk yield and a control line). Methane measurements were made using a Laser Methane Detector (LMD; Chagunda *et al.*, 2009) in 2010, 2011 and 2014 with approximately 3 measurements per cow. Traits related to production were milk yield (MY), live weight (LWT) and dry matter intake (DMI) (50,752 observations). Data were analysed with the use of ASReml 3.0 (Gilmour *et al.*, 2009). A tetravariate linear mixed model was fitted on MY, LWT, DMI and methane emission, with week of lactation, genetic line, calving season, lactation number, diet, age, an interaction between lactation number and age, and record year as fixed effects, and animal as a random genetic effect. Since for MY, LWT and DMI repeated observations were available, this model overestimates genetic parameters. Therefore a trivariate repeatability model was fitted on these traits, with the same fixed effects (excluding record year) as for the tetravariate model and animal both as a random genetic and random environmental effect. Since the animals were part of a feed trial and originating from two genetic lines, the effect of the interaction between these two factors was also estimated.

Results Heritability estimates (s.e.) were 0.35 (0.13) for MY, 0.65 (0.13) for LWT and 0.17 (0.07) for DMI, in agreement with previous studies in this field. Genetic correlations between these traits were highly positive and significant, also as expected. Heritability of methane emission was 0.04 (0.02, $p=0.054$) and genetic correlations were -0.17 (0.25, $p=0.32$) with MY, -0.08 (0.23, $p=0.38$) with LWT and 0.20 (0.24, $p=0.28$) with DMI, but these were not significant. When looking at the four combinations of genetic line and diet, all four groups differed significantly for DMI ($p=0.00$), but for MY and LWT only the combination of control line with high forage diet was significantly different (higher for MY ($p=0.03$), lower for LWT ($p=0.01$)) from the other groups. For methane emission only the combination of selection line with high forage diet was significantly higher ($p=0.01$) compared to the other groups.

Table 1 Heritabilities (diagonal), genetic correlations (below diagonal), phenotypic correlations (above diagonal) and repeatabilities (column 4) for MY, LWT and DMI, triivariate analysis

	MY	p values	LWT	p values	DMI	p values	Rep	p values
MY	0.35 (0.13)	0.01	0.23 (0.06)	0.00	0.52 (0.03)	0.00	0.73 (0.02)	0.00
LWT	0.65 (0.18)	0.00	0.65 (0.13)	0.00	0.37 (0.04)	0.00	0.84 (0.02)	0.00
DMI	0.87 (0.13)	0.00	0.78 (0.17)	0.00	0.17 (0.07)	0.02	0.45 (0.03)	0.00

Conclusion Heritability estimated for methane emission suggests there is a genetic component to this trait, however small, which indicates there is potential for genetic selection. Due to the small number of observations on methane emission, and high variability of LMD measurements, correlations between traits were not significant, so no conclusion can be drawn about the direction of the correlations. The effect of a high forage diet fed to the genetic selection line indicates that although genetic selection can mitigate the amount of methane produced, environmental effects such as diet still play an important role in expression of the trait.

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Formulating diets for dairy cows to reduce total feed carbon footprint

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Application Diets can be formulated to meet dairy cow requirements for energy and protein that also reduce total feed carbon footprint.

Introduction Life-cycle analyses of greenhouse gas emissions from the global dairy sector (Gerber *et al.*, 2010) and ruminant supply chains (Opio *et al.*, 2013) highlight the significance of emissions of methane from enteric fermentation and nitrous oxide from feed production and from nitrogen in manure. Feed production and processing accounts for about 0.2 of total global carbon footprint of milk production (Opio *et al.*, 2013). Individual raw material feeds vary in their carbon footprints (Vellinga *et al.*, 2012) and the objective of this study was to test the hypothesis that diets for dairy cows could be formulated to meet requirements for energy and protein and at the same time reduce total feed carbon footprint.

Material and methods Diets were formulated for a 650 kg dairy cow nine weeks post-calving yielding 40 kg milk/d of 39 g/kg fat and 31 g/kg protein using Ultramix (AGM Systems Ltd, Romsey, UK) and Feed into Milk (Thomas, 2004) to meet requirements for metabolisable energy (ME) and metabolisable protein. A maximum constraint was imposed on total dry matter (DM) intake (23.5 kg/d), with minimum constraints on ratio of effective rumen degradable protein to microbial crude protein (1.0), rumen stability balance (+20) and proportion of total DM intake from forage DM (0.4). Values for carbon footprint (CFP) of raw material feeds were from Vellinga *et al.* (2012). Formulations were varied from a base diet of maize and grass silage, barley grain, oilseed meals and by-pass fat, with mineral supplementation held constant, to give the lowest feasible feed CFP for the whole diet (C1) or for the concentrate only (C2). Nitrogen use efficiency (NUE) was calculated using the model of Garnsworthy and Wilkinson (2012). Human-edible feed use was calculated according to Wilkinson (2011).

Results Diet C1 contained maize silage plus a high proportion of by-products of low individual CFP, and had a total feed CFP 0.4 lower than the base diet. Diet C2 had a total feed CFP 0.3 lower than that of the base diet. Both C1 and C2 contained soyabean meal of relatively high CFP (1056 g CO₂e/kg DM) because soya has relatively high concentrations of ME and crude protein and a more favourable ratio of digestible undegraded protein to CFP than other raw materials. There was little difference in NUE between diets. Proportion of human-edible feed was lowest for C2, intermediate for C1 and highest for the base diet.

Conclusion Diets can be formulated to meet animal requirements for energy and protein that also reduce total feed carbon footprint by using human-inedible by-products.

Raw materials (kg DM/d)	Base	C1
Maize silage	11.9	14.3
Grass silage	4.0	-
Barley grain	3.4	-
Moist distillers' grains	-	3.1
Sugar beet pulp	-	1.1
Breakfast cereal waste	-	3.8
Soyabean meal	1.0	1.0
Rapeseed meal	1.9	-
By-pass fat	0.4	-
Minerals	0.2	0.2
Total feed CFP (g CO ₂ e/kg milk)	239	142
NUE	0.37	0.35
Human-edible DM in total DM	0.19	0.16

Acknowledgement This study was supported by AHDB Dairy.

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Long-term changes in rumen volatile fatty acids (VFA) and archaea to bacteria (AB) ratios in cattle fed either high concentrate or mixed forage:concentrate diets with or without the addition of nitrate (NO₃)

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Application Rumen VFA concentrations and AB ratios established after adaptation to different basal diets and introduction of NO₃ as a treatment to mitigate methane emissions were stable for up to 5 months.

Introduction Many strategies to reduce methane emissions from ruminant livestock are initially successful but their long-term effectiveness can be limited by adaptation of the rumen microbiota. However, long-term monitoring of the rumen is rarely reported. Therefore, long term changes in rumen VFA and AB ratios when NO₃ was included in the diet are reported.

Material and methods Rumen samples were obtained during an experiment in which performance (Duthie *et al.* 2015) and methane emissions (Troy *et al.* 2015) of beef steers were measured. The cattle (n=14 per treatment) were offered *ad libitum*, either a high concentrate (C, 920 g concentrate/kg DM) or forage-based (F, 500 g concentrate/kg DM) basal diet without (Con) or with the addition of NO₃ (NO₃, 20 g/kg DM) giving 4 treatments, CCON, CNO₃, FCON and FNO₃. Rumen samples were obtained by stomach tube before NO₃ was added to diets C and F (week (W) -7), 7 days after addition of 5 g NO₃/kg DM to diets (W-3), 1 week after addition of 20 g NO₃/kg DM to diets (W0) and 4 (W4) and 8 (W8) weeks subsequently. Samples were also obtained when steers left respiration chambers (between W9 and W20) by stomach tube and by sampling rumen contents at slaughter (between W12 and W21). Rumen samples were strained through muslin and analysed for VFA and DNA extracted and analysed for total bacteria and archaea (Rooke *et al.* 2014). VFA, reported as acetate to propionate ratio (AP, mol/mol) and AB ratio (copy number basis) were analysed using a split plot analysis of variance in Genstat with main effects the 2 x 2 factorial arrangement of diet and nitrate and sample time as a split plot. Four sample points were considered ((i), during adaptation to NO₃, W-3; (ii) as the mean of W0, W4, W8 (W4 in Fig. 1); (iii) on leaving respiration chambers (Ch in Fig. 1) and (iv) at slaughter (SI in Fig. 1); data from W-7 were included in analysis as a covariate (AP, P<0.01; AB, P=0.07).

Results AP ratio (Figure 1a) was greater in forage- (F v C, P<0.001) and NO₃-fed (NO₃ v CON, P<0.05) cattle. AB ratio (Figure 1b) was greater in forage- (F v C, P<0.05) and less in NO₃-fed (NO₃ v CON, P<0.05) cattle. These differences were consistent between samples. Although AP and AB ratios changed with time (P<0.001), differences between times were quantitatively small for AP. However, AB ratios for Ch and SI samples were greater than W-3 and W4 samples.

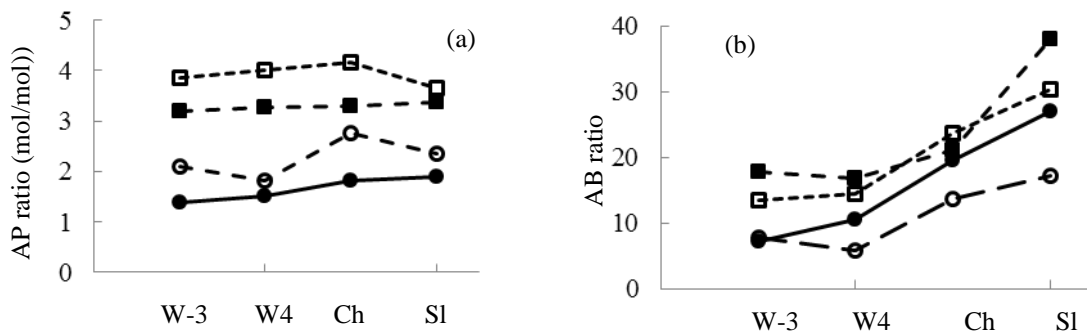


Figure 1 AP (a) and AB (b) ratios in rumen samples taken during and after adaptation of cattle to NO₃-containing diets. ●, CCON; ○, CNO₃; ■, FCON, □, FNO₃. SE of means were (a) for AP, 0.28 (n=13) and (b) for AB, 6.1 (n=9).

Conclusion The effects of basal diet and NO₃ on VFA patterns and AB ratios were consistent for up to 5 months. However, AB ratios were also influenced by sampling procedures.

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Prediction of enteric methane emissions from sheep fed fresh perennial ryegrass (*Lolium perenne*) using data measured in respiration chambers

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Application Grass intake, nutrient and energy contents and digestible organic matter concentration can be used to predict methane (CH₄) emissions from sheep. These models can contribute to develop robust national CH₄ inventories for sheep.

Introduction The IPCC Tier 1 default emission factor was used in UK to estimate enteric CH₄ production for sheep with no consideration of effects of animal and dietary factors (NAEI, 2015). There is an urgent need to develop more accurate emission factors specific to sheep and representative of the breeds and rearing systems employed in the UK sheep industry. The aim of the present study was to develop prediction equations of CH₄ emissions from sheep fed perennial ryegrass.

Material and methods Data used were collated from six experiments (from May 2012 to June 2014) with 82 growing lambs and replacement ewes (Highlander, Texel, Scottish Blackface and Swaledale) aged between 5 and 18 months, weighted from 24.5 to 62.7 kg and offered fresh perennial ryegrass only *ad libitum*. Fresh grass was harvested daily in the morning from the perennial ryegrass swards in the research farm at Agri-Food and Biosciences Institute (Hillsborough, Co. Down, UK). Grass contained on average (g/kg dry matter (DM)): ash 91, nitrogen (N) 28, neutral detergent fibre (NDF) 499, acid detergent fibre (ADF) 254, water soluble carbohydrates (WSC) 156 and gross energy (GE) 18.6 (MJ/kg DM). Animals were individually housed in pens and fed experimental diets for 19 days before being transferred to individual calorimeter chambers for a further 4 days with feed intake, faecal and urine outputs collected and CH₄ emissions measured for the final 3 days. Liveweight (LW) was measured at the beginning of the study and before entering and after leaving the chamber. Data were analysed using REML analysis to develop prediction equations for enteric CH₄ emissions using feed intake, grass chemical composition, LW and digestibility with effects of sex, breed and experimental periods removed. The regression procedure sequentially added terms to fixed model and only the predictors with a significant effect ($P < 0.05$) on the relationship were left in the models. The statistical programme used in this study was Genstat statistical package with a probability level of $P < 0.05$ for significance in relationships.

Results The prediction equations for CH₄ emissions are presented in Table 1. All relationships were significant ($P < 0.001$) and each predictor had a significant effect on the relationship ($P < 0.001$). There was a strong positive relationship between CH₄ production (g/d) and DM intake (DMI). Adding grass NDF, N and metabolisable energy (ME) concentrations and digestible organic matter (OM) in DM (DOMD) to this relationship increased prediction accuracy. Models using LW and grass DM, GE, OM and N concentrations as predictors performed satisfactorily. These equations can be used in commercial practice when DMI, DE and ME are not available. The negative relationship between CH₄/DMI (g/kg) and DMI indicated that when DMI increased by 1 kg/d, the predicted response in CH₄ production was a decline of 5.3 g/kg DMI.

Table 1 Prediction equations for methane emissions from sheep fed perennial ryegrass (n = 82)^{1,2}

Equations	SE	r ²
CH ₄ (g/d) = 16.7 _(0.74) DMI + 3.1 _(0.73)	2.63	0.86
= 18.3 _(0.62) DMI - 2.3 _(0.55) ME + 34.3 _(7.19) NDF + 260.6 _(41.3) N + 49.5 _(14.4) DOMD - 30.5 _(8.15)	2.05	0.92
= 0.34 _(0.059) LW + 151 _(28.5) DM + 24.5 _(3.70) GE - 463 _(69.9) OM - 1124 ₍₂₆₀₎ N - 23 _(58.5)	4.42	0.63
CH ₄ /DMI (g/kg) = -5.3 _(0.92) DMI + 25.8 _(0.90)	3.23	0.29
= -3.8 _(0.79) ME + 34 _(10.4) NDF + 375 _(58.3) N + 94 _(20.9) DOMD - 28 _(11.6)	3.02	0.41
= 7.9 _(0.74) DE - 7.3 _(0.65) ME - 2.7 _(4.63)	2.39	0.62

¹Values in subscript parentheses are SE;

²The units of parameters are kg/kg DM or MJ/kg DM except DMI (kg/d) and LW (kg).

Conclusion Methane emissions from sheep fed perennial ryegrass can be predicted by DMI and grass ME, NDF, N and DOMD concentrations. Models based on farm level data (e.g., liveweight and grass DM, GE, OM and N concentrations) were also developed and performed satisfactorily. Increasing 1kg DMI would decrease CH₄ yield by 5.3 g/kg DMI. These models can contribute to decrease the uncertainty in the development of CH₄ emission inventories of ryegrass pasture-based sheep industry. However, there is also a need for measurements with other forages (and with concentrates etc).

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Use of near-infrared reflectance spectroscopy to predict individual and total fatty acid content of perennial ryegrass (*Lolium perenne*)

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Application Offering a rapid and cheap high-throughput alternate method to gas chromatography (GC) for estimating forage fatty acid (FA) content which will aid in selection and breeding for this trait.

Introduction Near-infrared reflectance spectroscopy (NIRS) offers a rapid, non-destructive and low cost method for predicting chemical composition compared to traditional wet chemistry techniques (Corson *et al.*, 1999). Consequently, it has been widely adopted by the agricultural industry to provide accurate estimations of feed composition and particularly nutritional evaluation and improvement of forage (Shenk & Westerhaus, 1994). One forage trait attracting growing interest of late is FAs, due to potential for (1) increasing energy provision to ruminants, (2) improving FA profile of ruminant products, and (3) developing non-seed biomass oil crops. This study sought to establish and evaluate NIRS calibrations for prediction of individual and total fatty acid (TFA) content of perennial ryegrass.

Material and methods Four replicates of four Aurora x AberMagic F1 mapping and twenty 13th generation intermediate heading breeding population genotypes were maintained under poly-tunnel conditions. Plant material was harvested by hand to a height of ~5cm in July 2012, snap frozen, freeze dried and ground. FA methyl esters (FAMES) were extracted (Sukhija & Palmquist, 1988) and quantified via GC (CP-3800 with PAL Autosampler; Varian Inc, CA, USA) using a CP-select 100 m x 0.25 mm chemically bonded for FAME column (Agilent Technologies UK Ltd, Berkshire, UK). Peaks were identified and quantified using a 37 FAME standard and C23:0 internal standard respectively. NIRS analysis was carried out using a scanning monochromator (FOSS NIRSystems 6500; FOSS UK Ltd., Warrington, UK). Samples ($n = 96$) were scanned at 2 nm intervals from 400 - 2500 nm in reflectance mode with data collected using WinISI II (Version 1.02a; FOSS, Infrasoft International, PA, USA). Spectra were subjected to standard normal variate and detrend scatter corrections and 1st (1,4,4,1) and 2nd (2,6,4,1) derivative math treatments were evaluated. Outliers were identified as samples with a critical t-statistic of >2.5 or global-H value of >10 and removed from the dataset. Data from 1100 - 2498 nm were used to develop calibrations using modified partial least squares regression with group cross-validation using WinISI 4 (Version 4.6.8; FOSS Analytical A/S, Hilleroed, Denmark). Optimal calibrations were selected based on minimum standard error of cross-validation and maximum coefficient of determination.

Results FA and calibration results are presented in Table 1. The data used for calibration development showed coefficients of variation (CV%) ranging from 14.2 to 19.9 % for C16:0, C18:0, C18:1c9, C18:2n-6 and TFA while C16:1t3 and C18:3n-3 showed greater variation (31.9 and 26.8 %, respectively). Standard errors were generally low and all calibrations had good linearity with R² values > 0.8 and 1-VR values of > 0.7, except for C18:1c9 where R² was 0.53 and 1-VR was 0.29.

Fatty Acid	Math Treatment	n	(g kg ⁻¹ DM)				No. of Terms	SEC	R ²	SECV	1-VR
			Min	Max	Mean	SD					
C16:0	2 nd , (2,6,4,1)	92	2.64	5.21	3.93	0.560	5	0.160	0.92	0.189	0.88
C16:1t3	2 nd , (2,6,4,1)	94	0.13	0.71	0.37	0.118	7	0.030	0.94	0.037	0.90
C18:0	1 st , (1,4,4,1)	89	0.28	0.49	0.37	0.056	7	0.025	0.80	0.030	0.71
C18:1c9	1 st , (1,4,4,1)	95	0.39	0.78	0.58	0.084	6	0.058	0.53	0.071	0.29
C18:2n-6	2 nd , (2,6,4,1)	94	2.48	4.92	3.64	0.549	6	0.193	0.88	0.247	0.76
C18:3n-3	2 nd , (2,6,4,1)	92	6.28	20.67	12.74	3.411	6	0.711	0.96	0.853	0.94
TFA	2 nd , (2,6,4,1)	92	14.48	33.79	23.30	4.637	6	0.950	0.96	1.095	0.94

n , number of samples; SD, standard deviation; SEC, standard error of calibration; R², coefficient of determination for calibration; SECV, standard error of cross-validation; 1-VR, coefficient of determination for cross-validation.

Conclusion These results demonstrate that NIRS is capable of predicting individual and total FA content of perennial ryegrass, in agreement with previous investigations (Foster *et al.*, 2006). The accuracies of these predictions would suffice in screening plants for higher or lower FA content. However, further work to add more data encompassing a wider range of cultivars grown under a variety of conditions could increase calibration robustness with the potential to enable quantification of FAs through NIRS, which would facilitate plant selection and breeding for this trait.

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Prediction of nitrogen excretion in sheep offered fresh-cut perennial ryegrass (*Lolium perenne*)

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Application Nitrogen (N) excretion in sheep can be predicted from N intake (NI) or liveweight (LW) plus grass dry matter (DM) and N concentrations. Prediction of N output is essential to reduce the environmental impact of sheep industry.

Introduction Nitrogen excretion from sheep production systems is a considerable source of nitrate and nitrous oxide responsible for groundwater pollution and global warming. The European Union introduced the Nitrates Directives (European commission, 1991) which aims to minimise the nitrate pollution of groundwater and surface water arising from agricultural sources. Therefore, there is increasing interest to estimate N output in sheep production systems. The aim of the present study was to develop prediction equations for N excretion in sheep fed perennial ryegrass.

Material and methods Data used were collated from six experiments (from May 2012 to June 2014) with 82 growing lambs and replacement ewes (Highlander, Texel, Scottish Blackface and Swaledale) aged between 5 and 18 months, weighted from 24.5 to 62.7 kg and offered fresh perennial ryegrass only *ad libitum*. Fresh grass was harvested daily in the morning from the perennial ryegrass swards in the research farm at Agri-Food and Biosciences Institute (Hillsborough, Co. Down, UK). Grass contained on average (g/kg DM): ash 91, N 28, neutral detergent fibre (NDF) 499, acid detergent fibre (ADF) 254, water soluble carbohydrates (WSC) 156 and gross energy (GE) 18.6 (MJ/kg DM). Animals were individually housed in pens and fed experimental diets for 19 days before being transferred to individual metabolism crates in individual calorimeter chambers for a further 4 days with N intake and output measured. Liveweight (LW) was measured at the beginning of the study and before entering and after leaving the chamber. Data were analysed using REML analysis to develop prediction equations for faecal, urine and manure N outputs using N intake, grass chemical composition and LW with effects of sex, breed and experimental periods removed. The regression procedure sequentially added terms to fixed model and only the predictors with a significant effect ($P < 0.05$) on the relationship were left in the models. The statistical programme used in the present study was Genstat statistical package with a probability level of $P < 0.05$ for significance in relationships.

Results The prediction equations for N excretion in sheep fed perennial ryegrass are presented in Table 1. All relationships were significant ($P < 0.001$) and each predictor had a significant effect on the relationship ($P < 0.001$). The best single predictor for N output in faeces, urine and manure was N intake, and the r^2 value for prediction of manure N output was greater than those for faecal and urine N excretion (0.86 vs. 0.70 and 0.77, respectively). Grass DM and N content and animal LW instead of N intake were also used to predict N outputs because N intake may not be available in commercial practice. The models performed satisfactorily and indicated N excretions in faeces, urine and manure were positively related to animal LW and grass DM and N content, respectively. However, prediction of N output in urine using LW, DM and N content produced greater r^2 value than those in the prediction models of faecal and manure N.

Table 1 Prediction equations for nitrogen excretion in sheep fed perennial ryegrass (n = 82)¹

Equations	SE	r^2
Faecal N (g/d) = 0.20 _(0.015) NI(g/d) + 1.4 _(0.41)	1.66	0.70
= 0.11 _(0.032) LW(kg) + 0.06 _(0.014) DM(g/kg) + 0.32 _(0.061) N(g/kg DM) - 16.7 _(3.93)	2.55	0.32
Urine N (g/d) = 0.41 _(0.026) NI(g/d) + 2.4 _(0.70)	2.87	0.77
= 0.25 _(0.048) LW(kg) + 0.08 _(0.021) DM(g/kg) + 0.86 _(0.094) N(g/kg DM) - 35.1 _(6.01)	3.90	0.58
Manure N (g/d) = 0.62 _(0.028) NI(g/d) + 3.8 _(0.78)	3.18	0.86
= 0.36 _(0.073) LW(kg) + 0.14 _(0.033) DM(g/kg) + 1.18 _(0.142) N(g/kg DM) - 51.7 _(9.14)	5.92	0.51

¹Values in subscript parentheses are SE.

Conclusion Nitrogen intake is the best single predictor for N excretion in sheep fed perennial ryegrass. Animal liveweight and grass dry matter and N content together can be used to predict N output when N intake is not available in the commercial practice.

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

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Silvopastoral systems for sustainable animal production

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Application Three-level silvopastoral systems, developed in the tropics and sub-tropics, can be more sustainable than monoculture pasture systems and can be considered by producers for development in some temperate environments.

Introduction A system or procedure is sustainable if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning and morality of action. A production system might be unsustainable because of: inefficient usage of world food resources; adverse effects on human health; poor welfare of animals; harmful environmental effects such as low biodiversity or insufficient conservation; unacceptable genetic modification; not being “fair trade” in that producers in poor countries are not properly rewarded; or damage to rural communities. Any of these inadequacies could result in the quality of the product being judged as poor. Three-level plant production systems, that include pasture, shrubs with edible leaves and trees that may also have edible leaves are examples of silvopastoral systems. The production of leaves and other material that can be eaten by the animals is much greater on three-level silvopastoral than on pasture-only systems. Production animals, including cattle and sheep, may consume the plant material directly, or tree and shrub leaves may be cut and fed to ruminants, pigs, poultry or farmed fish.

Material and methods In five studies in Colombia and Mexico, a series of fields were planted with grasses such as *Cynodon plectostachys* and the leguminous shrub *Leucaena leucocephala* at 8,000 or 10,000 ha⁻¹ together with *Guazuma ulmifolia* in one of the studies. Trees of several species, or trees as live fences, mainly with edible leaves were left growing or planted in or around the fields. These three-level silvopastoral systems (SSP) were compared with “improved pasture”: monoculture fields planted to produce grass only (M) on the same farm. Beef or dairy cattle, 27 in some studies and 8 in others, were rotated through either the SSP or M fields. Field size varied but stocking density and duration in field were adjusted according to food availability with a mean of four days in each field. There were also comparisons of farms that used only SSP or only M.

CH₄ and N₂O were measured following the IPCC (2006) protocols and *in vitro* studies of *Cynodon/ Leucaena* mixtures.

Results Some examples of results from these tropical and sub-tropical (higher altitude) studies are shown in Table 1. Soil water-holding and production of herbage dry matter, plant protein and cattle were better on SSP. No added nitrogen was needed on SSP and, since meat production was greater on SSP, methane per tonne of meat was lower. Populations of predators of ticks and flies were greater on SSP so there was less disease transmission. Body temperature in sunny conditions was <4C lower on SSP, and cattle welfare was better because of reduced disease, more shade and more choice for the animals. In comparisons of farms, the biodiversity was much greater on SSP than on M. Worker satisfaction was better on SSP.

Table 1

	“Improved” monoculture (M)	pasture	Three-level silvopastoral (SSP)	system
M.E. Mcal ha ⁻¹	56.9		70.2	
Crude plant protein tonne ha ⁻¹	2.5		4.1	
Milk per cow kg day ⁻¹	3.5		4.1	
Meat kg ha ⁻¹ year ⁻¹	183		821	
Methane tonne of meat ⁻¹	208		128	
Bird species	24		75	
Anaplasmosis % of herd	25		<5	
Fights % difference	+37			
Social licking % difference			+46	
Social interactions in shade % difference			+57	

Conclusion Developments in temperate silvopastoral systems, suitable for UK use, are discussed. Two-level or three-level silvopastoral systems should be considered to replace one-level systems for animal production.

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Developing-world livestock projects: gender equality as the key to sustainability and resilience to climate change

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Application Re-orientation is proposed of livestock development projects towards an explicit gender-equality focus, coupled with the utilization of thrifty and adapted local breeds. We contend that this will contribute to resilience to climate change and to sustainable rural development.

Introduction Development agencies have for many years recognized the importance of livestock systems for rural livelihoods, but over the last 60 years projects have had a mixed and often disappointing history. Lessons have been learned, one of which has been the importance of a gender perspective, and gender issues are now stated as being “mainstreamed” in much policy research and development (references to all quotations: Chanamoto and Hall, 2015). In relation to climate change, it is also argued that there has been a problematic emphasis on technical and infrastructural adaptive strategies (Tschakert, 2007). We suggest that the goals of agricultural development, poverty alleviation, climate change resilience and gender equality can be achieved by an explicit focus on gender in livestock projects, with adoption of animals of locally adapted genotypes.

Material and methods The study proceeded as a literature review on gender equality theory and practice, combined with a review of the constraints operating in village-based livestock systems in the developing world (Chanamoto and Hall 2015). Reports and discussion documents produced by the following organizations were found to be especially relevant: FAO, IDS, IFAD, IFPRI, IIED, ILRI, Mallam Dendo, ODI, UN WomenWatch, World Bank.

Results Resilience of livestock systems to climate change has in our view two key dimensions: the gender dimension, and the animal dimension. Examination of gender roles, gender analysis of livelihoods, and gender-specific aspects of climate change, fosters a critical approach to the traditional roles-based understanding of gender relations, which has not challenged entrenched inequalities. While technical developments may increase production, the result can be additional labour burdens for women, and improvements in (for example) human nutrition do not automatically follow. Rural women are also predicted (UN WomenWatch, 2009) to be heavily challenged by the effects of climate change, because of the specific difficulties they face in adopting mitigation strategies. The animal dimension to resilience is acknowledged to be at least partly defined by the adaptations of local breeds and important studies suggest local breeds under improved management are at least equal, perhaps superior, on the household/systems level, to crossbreeds. Gender-transformative livestock projects can be designed; we suggest the traditional approach (which has been, at best, gender-neutral) may disempower women further. Large-bodied, expensive animals like cattle will probably usually be unsuitable. The participation of women in the selection of species and breeds is of paramount importance. Sheep and goats, which can survive and thrive on crop residues, and support artisanal activities such as handicrafts, could be particularly suitable, and a focus could be on increasing the survival of young animals (juvenile mortality being 27-28% in traditional systems in sub-Saharan Africa). The often-scorned donkey, which makes “a great contribution ... to the daily life of rural people, especially women” would be a strong candidate. Animal health services and husbandry advice will need to be presented in an accessible and gender-sensitive manner. While the Community Animal Health Worker model may be applicable, account will need to be taken of many animal diseases and injuries being in cash terms not worth treating. However, local knowledge can be expected to contribute, following the principle of capitalizing on the capabilities and strength of the poor (Heffernan, 2004).

Conclusion We recommend elevating the treatment of gender issues from the periphery to the centre of project design. We argue that the advancement of gender equality, coupled with resilience to climate change, should be the guiding philosophies and that local, adapted livestock breeds are of profound relevance.

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Developing tools to quantify sustainability of intensive and extensive ruminant farming systems in sub-Saharan East Africa

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Application This project informs regional development and individual stakeholders by rapidly assessing farms and proposed intervention strategies from the minimal available data for trade-offs in economic & environmental sustainability.

Introduction Developing economies throughout the world are striving to achieve increases in livestock productivity to meet demands of burgeoning populations. Significant variation between the prevalent smallholder or subsistence production techniques and a lack of available data makes quantification of economic and environmentally sustainable development strategies impractical. The aim of this study was to modify and apply the CLEANED project framework by Notenbaert *et al.* (2014) for rapid assessment to compare intensive and extensive subsistence farming systems within Tanzania.

Material and methods Utilising the CLEANED project framework an iterative process of conceptual testing was undertaken. Principal data sources were the FEAST report by Mangesho *et al.* (2013) and ILRI/CCAFS (2012) IMPACT Lite. These datasets were compiled with participatory interviews, regional farming practice surveys and comprehensive on farm investigations representative of the predominating smallholder systems being intensive (IN) upland mixed crop livestock and extensive (EX) traditional agro-pastoral production. Generating IN and EX system specific baselines of the current production practices sustainability. Input data related to systems typical agroecology, crop cultivation, land management, livestock productivity and management, livestock manure and feed basket. Employing the IPCC Tier 2 values to input data with energy requirement, feed quality, and production yield allowed for rapid assessment of farm-level GHG emission, N and soil organic carbon balance for IN and EX systems baselines. Distinct intervention scenarios were applied to IN and EX production baselines on stakeholder preference and present development strategies. These encompassed greater nutritional provision, 'improved' cattle production genetics and increased veterinary services. The sampling of milk and forage from sites included three in Lushoto upland mixed-crop region representative of IN production system and at two EX Maasai agro-pastoralist holdings in Handeni savannah region. This involved pooled and replicate sampling of all forage at point of feeding (n=19) alongside replicate milk samples of all individual stock (n=20) from both regions and pooled samples from Lushoto milk collection points (n=4). Forage quality was analysed for NDF, ADF, ADL, MADF, ME, DOMD, protein, PUFA's, OM and DM. Milk was characterised for fat, protein, DM, acidity and PUFA's composition.

Results The modelled enteric fermentation was highlighted as the greatest contributor to GHG emissions in IN (71%) and EX systems (80%) baselines. GHG emissions when considered as kg CO₂-eq ha⁻¹ were 1168.89 for the IN and 604.45 for the EX system baseline but as kg CO₂-eq kg Fat & Protein Corrected Milk (FPCM)-1, 2.04 for IN and 2.61 for EX system baseline. The nitrogen balances as kg N ha⁻¹ were -49.87 in the IN and +77.50 in the EX system baseline.

Table 1 N balance (kg N ha⁻¹) and GHG % change from baseline (kg CO₂-eq kg FPCM-1) for modelled scenarios

	Nutrition		Genetics		Veterinary	
	N balance	GHG % change	N balance	GHG % change	N balance	GHG % change
Intensive	-49.9	-5.83	-49.1	+9.11	-49.4	-6.03
Extensive	+77.6	-15.18	+5.9	-43.53	+74.2	-12.69

Modelled scenarios generally provide reductions in GHG emissions per litre milk with economic benefits relative to baseline practice. There is beneficial positive N balance per ha of the EX and N balance deficit for IN systems scenarios.

Conclusion Larger EX system herd sizes and communal grazing are major drivers in model differences. Milk and forage quality results provided detailed comparison of inherent differences of IN and EX systems (data not shown). Highlighting limitations for practicalities of modelled intervention scenarios with cultivation and holding capacity constraints, in EX systems current practices are principally dictated by environment and culture. Advancements to IN and EX subsistence farming should incorporate rudimentary interventions so that sustainability of development scenarios can be assessed against true baseline productivity. Greater inclusion of real-world data is required to improve model predictive accuracy.

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Holistically assessing dairy farm eco-efficiency by combining Life Cycle Analysis with Data Envelopment Analysis

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Application This study demonstrated a modelling framework facilitating the measurement and analysis of dairy farm eco-efficiency in much more depth than previous assessments.

Introduction Eco-efficiency is essential for sustainable dairy farming as it reflects a farm's ability to minimize environmental impacts (EIs) by producing at least the same output (physical or value added). It is typically expressed by multiple partial ratios (EIs per some functional unit, e.g. unit milk), which can be problematic because (i) incommensurability between them can complicate decision-making, (ii) substitution possibilities between EIs are ignored, and (iii) allocation of EIs to several outputs (e.g. milk, meat, crops) is challenging. The objective of this study was to demonstrate a 'global' eco-efficiency modelling framework overcoming issues (i)-(iii) by feeding EIs derived from Life Cycle Analysis (LCA) data to the productive efficiency method Data Envelopment Analysis (DEA).

Material and methods DEA (Charnes *et al.*, 1978) is a multiple-input, multiple-output efficiency measurement method calculating single, aggregated, farm-specific efficiency indices by assessing the whole production system, including EIs. With DEA allocation of EIs to specific products is unnecessary because the farm is assessed as a multiple-input, multiple-output entity. DEA constructs a best-practice frontier consisting of the best-performing farms in the sample and all other farms are benchmarked against it. Each farm receives a score between 0-1. A score of 1 means that a farm is on the frontier, i.e. it does not have to make any adjustments in its inputs and/or outputs and/or EIs to become efficient. A score less than 1 indicates that the farm under evaluation is below the frontier and should make adjustments to its inputs/outputs/EIs to become efficient. DEA is not affected by the variables' different measurement units (e.g. land use in ha, milk in kg). The variables are seen as multiple criteria and are weighted by an endogenous, 'data-driven', weighting scheme. This study used the DEA model Range Adjusted Measure (RAM; Cooper *et al.*, 1999) that is able to simultaneously minimize EIs and maximize outputs for each farm. This is unlike previously used models where choice of orientation (EI minimization or output maximization) is necessary. Another advantage of RAM over other DEA models is that it can be used to rank farms in terms of eco-efficiency scores. Hence, non-parametric rank tests (Kruskal-Wallis test and Dunn's test) can be employed to test for significant differences in terms of eco-efficiency score ranks between different farm groups. An additional DEA methodology (Brockett and Golany, 1996) was employed to correct farm eco-efficiency scores for eco-'inefficiency' attributed to managerial factors. Removal of managerial inefficiencies allowed for detecting differences in eco-efficiency between farms solely attributed to the uncontrollable factor 'region'. This was another novelty of this study, unlike previous dairy studies combining LCA with DEA. The modelling framework was demonstrated with LCA data (Charroin *et al.*, 2005) from 185 French specialized dairy farms grouped by region (West France, Continental France) and feeding strategy (<10%, 10-30% and >30% maize silage in the total forage area), regardless of region. The LCA data were derived from a partnership involving the Chambers of Agriculture (France), the French Livestock Institute, and voluntary participation of farmers (Inosys Réseaux d'Élevage). The five LCA-derived EIs used were non-renewable energy, land use, eutrophication, acidification and global warming potential. The three outputs were milk, meat and crop production.

Results West farms ranked higher, on average, than Continental farms in terms of eco-efficiency scores. This difference was significant ($P < 0.05$) only after removing managerial inefficiencies. Mean eco-efficiency score ranks were significantly higher ($P < 0.05$) for farms with <10% and 10-30% maize silage in the total forage area than farms with >30%, before removing managerial inefficiencies. After doing so, no significant differences were identified between feeding strategies.

Conclusion The proposed framework can significantly improve dairy farm eco-efficiency assessments compared to previous studies based on partial LCA ratios by combining LCA with an alternative DEA method. The capacity of the proposed framework to measure and understand eco-efficiency, especially between different farm groups, makes it a promising multiple-criteria tool supporting sustainable dairy production.

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The economic impact of animal breeding research: a case study of dairy cow fertility index adoption

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Application Research dissemination. The case-study is one of six cases in the IMPRESA EU Framework VII project which have been conducted with the aim of identifying aspects to support the improvement of efficiency in agronomic research and creating new indicators for increased European monitoring of Research Impact Assessment.

Introduction Ensuring maximum impact from public funded agricultural research is increasingly important, in response to grand global challenges but also diminishing resources for science resulting from fiscal austerity. We use a case study of the MAFF-funded Link project to create a Dairy Fertility Index (FI) (2001-2006), exploring the pathway from research through dissemination to its overall socioeconomic consequences, to illustrate the process and to indicate how impacts can be improved.

Material and methods The case study used documentary analysis, interviews with key informants and stakeholder workshops, guided by a standard protocol developed for use in parallel studies in four other EU countries, but with scope for adaptation to national needs. Key players in research, funding and extension bodies were interviewed to develop an account of the process from original investigation through to its adoption by farmers. Workshops to investigate farmers' awareness of the research impact pathway were held in south-west England, Scotland and Northern Ireland; in Wales, a questionnaire-based survey of farmers was used. A final workshop was held with researchers to confirm the research narrative. Audio recordings of discussions were transcribed, analysed and interpreted, and findings were verified by participants in the study.

Results Early adoption of the research output was achieved through the involvement of commercial breeding companies in the research. They had access to FI results within two years of the project starting, and were able to remove bulls with poor fertility results from the market. After FI results were published, farmers identified discussion groups as being most valuable as sources of information. They also valued the farm vet, and early adopter farmers. In Northern Ireland and Scotland, College-linked advisory services were used as sources for verification of husbandry approaches or innovations covered in the press. In England and Wales, which lack a permanent structure of advisory services, farmers were more likely to trust commercial representatives as sources of information, even though they are aware of a vested interest problem. Despite the Fertility Index first being published in 2005, farmers first reported awareness of it around 2010. The Calving Interval (CI) is used as a proxy measurement of fertility, and NMR results suggest this stabilised in 2008 and started to decline in 2010. The final workshop confirmed the relative importance and influence of different stages of the impact pathway, and highlighted potential forces which could, and indeed attempted to, prevent the research project from going ahead.

Conclusion Without being aware of the work, farmers benefitted at an early stage from the research due to the involvement of the commercial breeding companies. Despite barriers to the research, including absence of demand from farmers, lack of conviction of vets, a lack of interest by commercial companies and the levy body, there were sufficient countervailing factors. The project went ahead due to the considerable efforts of key academics and personnel at MAFF, together with the existence of pre-competitive research funds, the legacy of public good remits; success in adoption and awareness was largely due to the eventual engagement by the levy body. The project produced a Fertility Index, now incorporated into the national breeding goals of the Profitable Lifetime Index (£PLI), and had beneficial impacts, including a proof of concept. The concept has since been used for progress on mastitis, lameness and longevity and is now being used for indexing the resistance to bTB. While private sector engagement is required to produce industry-relevant information, ownership of the data is key, and the current institutional infrastructure to maintain the FI is fragile.

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An economic analysis of production costs on a group of Irish suckler beef farms participating in a knowledge transfer programme

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Application For suckler beef farms, concentrate costs as a percentage of total costs (TC) were higher for farms located in the west of Ireland and on suckler farms finishing progeny to beef. Veterinary costs as a percentage of TC were higher on suckler farms selling progeny as weanlings or yearling cattle.

Introduction Suckler beef production systems remain one of the lowest income enterprises in Irish agriculture with many beef farms loss-making when EU and national subsidy payments are excluded from revenue (Hennessy and Moran, 2015). Previous research (Taylor *et al.*, 2015) has shown no regional or production system differences in net margin on a per hectare (ha) or per livestock unit (LU) basis for suckler beef farms. However, this analysis highlighted differences in the cost structure of farms which has an important influence on profit. Therefore, the aim of this present study is to further examine the influence of region and production system on the relative importance of various cost categories when expressed relative to TC for Irish suckler beef farms and to quantify the relationship of these costs to profitability.

Material and methods Financial records of 38 farmer participants in the BETTER (Business, Environment and Technology through Training, Extension and Research) beef farm programme during the period 2008-2014 were obtained and analysed. Farms were assigned to one of four regions (north-east; NE, north-west; NW, south-east; SE, south-west; SW) and one of three production systems (Beef, Live, Mixed) as described by Taylor *et al.* (2015). Data were analysed as a factorial combination of region and system with year adjustment to allow for changes in conditions from one year to the next. A repeated measures model was fitted to model correlations across years using the GLIMMIX procedure in SAS 9.4 (SAS, 2014).

Results Concentrate costs as a percentage of TC were greater on NW and SW farms than SE farms ($P<0.05$) and higher on Beef farms than Mixed farms which in turn were greater than on Live farms ($P<0.001$). Veterinary costs as a percentage of TC were significantly greater on Live farms than on Beef and Mixed farms ($P<0.01$). There was a tendency for FC as a percentage of TC to be greater on SE farms than on SW farms ($P=0.055$). Concentrates as a percentage of TC had a moderate, positive correlation with net margin (NM) per ha ($r=0.32$, $P<0.001$) and per LU ($r=0.32$, $P<0.001$). Other variable costs and FC as a percentage of TC showed a moderate, negative correlation with NM per ha ($r=-0.31$ and $r=-0.38$, $P<0.001$, respectively). FC were also moderately, negatively correlated with NM per LU for TFC only ($R=-0.37$, $P<0.001$).

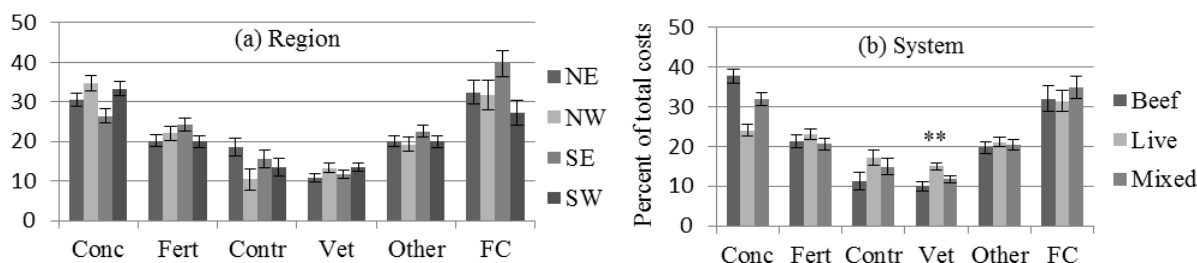


Figure 1a & 1b Regional and system analysis of cost categories expressed as a percentage of total costs

¹Conc= Concentrates, Fert=Fertilizer, Contr=Contractor, Vet=Veterinary, Other=Other variable costs (straw, transport, levies), FC=Fixed costs

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, ^x $P=0.055$

Conclusion The higher percentage of costs due to concentrate feeding on NW, SW and Beef farms is likely to have a moderately positive effect on NM per ha and per LU. However further research would need to be carried out to determine the extent of its benefit and the threshold beyond which positive returns diminish. Despite veterinary costs as a proportion of TC being higher on Live farms, its low correlation with NM would suggest it has little impact on farm profitability. The tendency for higher FC as a percentage of TC on SE farms, coupled with its negative correlation to NM implies that these farms have the opportunity to increase profitability should FC as a percentage of TC be reduced. However, these costs are likely to have occurred due to finishing system changes as a result of the knowledge transfer program and are expected to decline naturally as assets depreciate.

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Chemical composition and performance of chicks fed residues from the extraction of chemicals from distillers' grains

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Application The residue from the extraction of components of distillers' grains for industrial use still has value as an animal feed, and may be similar in nutritive value to distillers' grains and rapeseed meal.

Introduction The production of wheat distillers' grains (WDG), currently used as livestock feed) is forecast to rise significantly with the increased use of wheat for bioethanol production. This provides a source of material of a scale and consistency that is feasible to exploit for the production of materials for industrial applications. However, the latter will still leave a final residue and its value for feeding livestock has to be retained if the wheat-to-ethanol process is to maintain its positive greenhouse gas balance. A sequence of processes to extract some of the lipid, protein and carbohydrate fraction of WDG for industrial use has been developed at a laboratory scale. The objective of this study was to determine what effect this process has on the chemical composition and nutritive value of the final residual material.

Material and methods WDG, without the addition of solubles, were subjected to a series of procedures in which first lipid was extracted, using supercritical CO₂, then protein was extracted, using sodium hydroxide and ethanol, before finally the remaining carbohydrate fraction was extracted using steam explosion and fermentation with yeast. Fresh WDG (FDG) and a reconstituted product derived from dried WDG with solubles that was washed and then freeze dried (RDG) were compared with their respective residues following extraction (EFDG and ERDG). These four products were analysed for amino acids, crude protein, ether extract and neutral detergent fibre (NDF). They were further evaluated in a feeding trial with chicks reared from 1-10 d of age. Eight replicate pens of chicks (two male Ross 308 chicks per pen) were fed one of five diets (40 pens in total). Diets were balanced for metabolisable energy, lysine, methionine and threonine, and contained 80 g/kg (as fed) of one of the test feeds (FDG, RDG, EFDG, ERDG or rapeseed meal, RSM). RSM was used as a control on the basis of having similar crude protein content. Feed intake, growth (1-10 d), feed conversion ratio and protein efficiency ratio was calculated on a pen basis. The effect of feed source (FDG, RDG, EFDG, ERDG, RSM) on measures of chick performance were determined by orthogonal contrasts of: (1) CON *cf* DG; (2) extracted *cf* original; (3) fresh *cf* reconstituted; (4) interaction between (2) and (3).

Results There was a loss of arginine and cystine, but an increase in the concentration of threonine and ether extract when the products were extracted (Table 1). Extracted products resulted in higher intakes (P=0.004) although the mash diets used resulted in generally low intakes. No other significant differences between treatments were observed (Table 2).

Table 1 Chemical composition (g/kg DM) of the four distillers' grains products.

	FDG	EFDG	RDG	ERDG
Ether extract	92	134	45	60
Crude protein	289	300	413	430
Neutral detergent fibre	899	709	680	703
Lysine	11.7	10.8	8.5	9.7
Methionine	5.5	6.0	5.9	7.3
Cystine	7.3	1.9	7.9	2.3
Threonine	10.9	12.4	11.2	13.7
Arginine	17.7	11.4	17.0	9.7

Table 2 Chick performance (1-10 d) when fed diets containing distillers' products or rapeseed meal

	RSM	FDG	EFDG	RDG	ERDG	SEM	P for contrast:			
							1	2	3	4
Feed intake (g/bird)	203	194	208	194	214	6.5	0.693	0.004	0.832	0.617
Growth (g/bird)	168	160	177	160	179	7.4	0.519	0.060	0.840	0.945
Feed conversion ratio	0.910	0.921	0.942	0.935	0.929	0.0027	0.505	0.802	0.991	0.664
Protein efficiency ratio	1.69	1.82	1.89	1.77	1.78	0.133	0.375	0.663	0.419	0.718

Conclusion The extraction of potentially valuable components from WDG results in a product that has some potential as an animal feed, which is equivalent to that of unextracted distillers' grains. However, it is still a fibrous feed, and so will probably prove more suitable in ruminant diets rather than in starter broiler diets.

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Effect on haematological parameters of broilers fed graded levels of toasted African yam bean seed meal for the first 5 weeks of growth

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Application Toasted African yam bean seeds with protease enzyme supplementation in the diets of broiler starter diets showed no deleterious effect on haematological parameters and can easily reduce competition between man and poultry.

Introduction The conventional protein feedstuffs are continually competed for by man to meet up their ever increasing protein requirements, thus resulting in escalating cost of the conventional protein sources. The replacement of expensive conventional feed ingredients with cheap locally available and non-human competitive substitutes in feed formulation offers a veritable means of reducing the total feed cost of poultry production (Umoren *et al.*, 2005).

Material and methods The experiment was carried out at the Poultry Unit, Teaching and Research Farm, Oyo State College of Agriculture and Technology Igbo-Ora, Oyo State, Nigeria, latitude 7°15'N and longitude 3°30'E with average annual rainfall of 1278mm and average temperature of 27°C. African yam bean seeds were procured at Bodija market while other ingredients were obtained from a reputable feed mill in Ibadan, Oyo state, Nigeria. Beans were toasted by using frying pan of 74.5cm by 38.00cm and placed on firewood as source of heat and evenly distribution of heat was attained in 3 to 5 minutes when beans turned to crispy to touch. Chemical compositions were determined by using A.O.A.C, 2010 while metabolizable energy was calculated as described by Pazenga (1985). Protease enzyme was used for the experiment and was used at 150g/1000kg of feed. Five diets were formulated having percentage crude protein ranging between 20.58(%) to 22.00(%) while energy ranged between 3005.40ME (kcal/kg) to 3076.42ME (kcal/kg). Treatment one was corn-soybean based (control) while toasted African yam bean seed meal was added to treatments 2, 3, 4, and 5 at 10, 20, 30 and 40% nutrient to nutrient inclusion rate respectively to replace soybean meal. A total of 200 unsexed day-old Marshal (R) strain broiler chicks were obtained from Obasanjo Farm Ltd., Igboora, Oyo State, Nigeria. The brooder house was cleaned, washed, disinfected and fumigated a week before the arrival of the birds. A total of 195 birds were used for the study. The birds were randomly allotted to each of 5 dietary treatments and were replicated 3 times with 13 birds per replicate in a completely randomized design (CRD). The birds were fed *ad libitum* with the experimental broiler starter feed from day old for 5 weeks. Litter was regularly turned and changed as at when due. All other vaccines and drugs were administered as at when due. At the end of the 5th week, one bird per replicate per treatment was randomly selected for hematological parameters. Blood samples were collected using sterilized needle and syringe through the wing vein of the animal into vials containing Ethylene Diamine Tetra Acetic Acid (EDTA) bottles and were transported to laboratory for hematological assay. Data collected were subjected to analysis of variance (SPSS, 2012). Means were separated by using Duncan multiple range test of the same software.

Result There were no significant ($P>0.05$) differences in packed cell volume, haemoglobin, red blood cell, white blood cell, lymphocytes, heterocytes, monocytes, eosinocytes and basophils while significant ($P<0.05$) different was observed on platelets. The non-significant ($P>0.05$) values obtained for haematological indices of experimental broiler starter in this study concur with Uko *et al.*, (1998) and showed that these broiler starters were well nourished.

Conclusion Toasted African yam bean seed meal with protease enzyme supplementation can be used to replace soybean meal up to 40% inclusion level in broiler finisher without adverse effect on health status of the birds.

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Assessment of *Xylopia aethiopica* (Negro Pepper) as growth promoter using broilers

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Application 2.5g *Xylopia aethiopica* per kg diet improved growth at starter and finisher phases, while 5g/kg improved growth only at the finisher phase. Total feed intake was improved by 10g/kg at the starter phase but similar to control at finisher phase. Final live weight was negatively affected by 10g/kg at starter and finisher phases due to poor feed: gain ratio. *X. aethiopica* reduced bacteria load of the feed.

Introduction Plant resources with bioactive substances such as spices have been suggested as possible growth promoters in antibiotics-free diets for broilers (Al-Harhi, 2000). Black pepper, garlic, thyme and ginger have been reported to positively influence broiler growth (Damme, 1999). Search for more natural bioactive compounds should continue to augment the known ones. Though widely used in human nutrition and medicine, not much has been reported about the nutritional importance of *X. aethiopica* in farm animals. It is a tree whose different parts are used as spice by man for food and medicine (Omodamiro *et al.*, 2013). The aim of this study was to determine the effect of *X. aethiopica* on growth performance of broilers and its antibacteria activity in feed.

Material and methods Ripe fruits of *X. aethiopica* were harvested and dried under the sun. Thereafter, they were grinded. Total bacteria load of the feed was determined by mixing 0.0, 2.5, 5.0, 7.5g and 10.0g *Xylopia* respectively with 1.0kg of the basal diet. 5.0g of each diet was then incubated at 37°C for 24 hours. Serial dilution method was used to determine the total bacteria load. 150 day old broiler chicks were allotted to 5 dietary treatments (T) of 30 birds each, replicated thrice on completely randomized design. Each replicate had 10 birds. A corn-soybean basal starter and finisher diets were formulated. 0.00, 2.5, 5, 7.5 and 10g/kg diet of the spice was each added to a portion of the basal diet to form T1, T2, T3, T4 and T5 respectively. The experiment which was conducted in deep litter, open-sided rearing house, lasted for 56 days (28days each for starter and finisher). Feed and water were provided *ad libitum*. The starter feed had 22.10% CP, 2880KcalME/kg and finisher diet 20.05% CP, 2901 KcalME/kg. Kerosene stove was used to increase the temperature of the house (35-30°C) for the first 21 days. Vaccination against Newcastle and Gumboro diseases was carried out. Data collected were analyzed by One Way analysis of variance using SPSS software at 5% level of significance.

Results The *X. aethiopica* was antibacteria showing dose dependent action. Total bacteria load in the feed was reduced from $3.6 \times 10^4 - 1.7 \times 10^4$ cfu. Tables 1 and 2 indicate that final live weight and weight gain were improved by 2.5g/kg at the starter phase ($P < 0.05$), while 10g/kg gave higher feed intake, but poor feed: gain ratio. At the finisher, 2.5g and 5g/kg improved growth while 7.5g and 10g/kg reduced the weight and 10g/kg had poor feed: gain ratio.

Table 1 Effects of dietary *X. aethiopica* addition (g/kg diet) on feed intake and live weight gains in starter broilers

Parameters	T1(0.00)	T2 (2.5)	T3 (5.0)	T4 (7.5)	T5 (10)	SEM values
Initial live weight	44.12	43.72	43.56	43.68	45.20	4.01
Final live weight	743 ^b	800 ^a	750 ^b	714 ^b	664 ^c	48.72
Daily weight gain	24.96 ^b	27.01 ^a	25.23 ^b	23.94 ^b	22.14 ^c	1.35
Total feed intake	1289 ^b	1389 ^{ab}	1370 ^{ab}	1405 ^a	1418 ^a	114
Feed: gain ratio	1.84 ^b	1.84 ^b	1.90 ^b	2.10 ^a	2.29 ^a	0.16

Table 2 Effects of dietary *X. aethiopica* addition (g/kg diet) on feed intake and live weight gains in finisher broilers

Parameters	T1(00)	T2(2.5)	T3(5)	T4(7.5)	T5(10)	SEM values
Initial live weight (g)	743 ^b	800 ^a	750 ^b	714 ^b	664 ^c	48.72
Final live weight (g)	2205 ^b	2336 ^a	2332 ^a	2125 ^c	1935 ^d	78.50
Daily weight gain (g)	51.1 ^b	54.9 ^b	56.6 ^a	50.4 ^b	45.4 ^c	4.86
Total feed intake (g)	4595 ^{bc}	4711 ^{ab}	4735 ^a	4559 ^c	4549 ^c	152
Feed : gain ratio	3.21 ^b	3.07 ^b	2.99 ^b	3.23 ^b	3.58 ^a	0.35

Conclusion It is concluded that 2.5g/kg *X. aethiopica* improved growth and sanitized the feed and could be used as a potential and novel feed additive in diets of broiler chickens.

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Effect of dietary powdered *Echinacea purpurea* and *Tribulus terrestris* on performance, haematology and some serum parameters of broilers

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Application Dietary powdered *Echinacea Purpurea* (EP) and *Tribulus terrestris* (TT) improved performance and some haematology indices in broilers; therefore EP and TT can be used in broiler diets as additives.

Introduction Previous studies revealed that no single alternative exists with the effects comparable to antibiotics; therefore, finding more efficient alternatives or combinations of different alternatives for maintaining health and improving performance of poultry and other livestock species is needed. Antioxidant, growth promoting (Landy *et al.* 2011) and immune stimulating (Nasir and Gashorn, 2010) effects of EP have been reported. TT has positive effect on some blood parameters and haematology (Christev *et al.* 2011). Present study was planned to survey the effects of powdered EP and TT on performance, haematology and some serum parameters of broilers.

Material and methods A total of 480 one-day-old broiler chicks (Ross-308) were randomly assigned to the 8-treatments, each with 4 replicates with 15 broiler chickens in each replicate. The treatments were as follows: i. Basal diet (control). Other treatments were basal diet + ii. antibiotic (5 mg Virginiamycin /kg diet), iii. EP (20 g/kg diet) and TT (10 g/kg diet) powder, iv. EP (10 g/kg diet) and TT (20 g/kg diet) powder, v. EP (20 g/kg diet) and TT (20 g/kg diet) powder, vi. EP (10 g/kg diet) and TT (10 g/kg diet) powder, vii. EP (10 g/kg diet) powder, viii. TT (20 g/kg diet) powder. The basal diets were formulated to meet the nutrients requirements of broilers. Birds were allowed free access to feed and water during the 42 days of experiment. Growth performance of broilers was evaluated by recording the daily body weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) during the 42 days of experimental period. Mortality was recorded as it occurred and was used to adjust the total number of birds. At 21 and 42 days of age, two broilers from each replicate of treatments were randomly selected, and blood samples were taken by puncture of the brachial vein for blood parameters and haematology analysis. All the data were subjected to ANOVA (at P<0.05 and P<0.01 levels) using the General Linear Models procedure of SAS software. The mean differences among different treatments were separated by Duncan's multiple range tests.

Results No significant treatment effect was observed on FCR, white blood cells, lymphocytes, haematocrit, cholesterol and glucose. DFI in viii treatment and DWG in iii, iv, v, vi, vii, and viii treatments was significantly (P<0.01) higher than other treatments. Significantly (P<0.05) higher neutrophils in treatment iii and monocyte in treatment viii were observed. Total protein, immunoglobulin G (IgG) and immunoglobulin M (IgM) contents were significantly (P<0.05) higher viii, viii and vii than other treatment groups, respectively.

Table 1 Effect of EP and TT on performance, blood parameters and haematology of broiler chicks

Items	Treatments								SEM	P
	I	ii	Iii	Iv	v	vi	Vii	Viii		
DFI (g/day)	128.7 ^{AB}	123.8 ^B	128.3 ^{AB}	139.9 ^{AB}	126.5 ^B	138.8 ^{AB}	134.8 ^{AB}	143.6 ^A	2.63	0.001
DWG (g/day)	55.4 ^{AB}	51 ^B	57.1 ^A	57.4 ^A	58.9 ^A	59.3 ^A	61.1 ^A	59.3 ^A	1.25	0.001
Neutrophil	15 ^B	21 ^{AB}	24 ^A	20 ^{AB}	14.25 ^B	19.5 ^{AB}	16.75 ^B	15.5 ^B	2.23	0.020
Monocyte	2.5 ^C	3.5 ^{ABC}	3 ^{BC}	3.7 ^{AB}	4 ^{AB}	3.5 ^{ABC}	4 ^{AB}	4.2 ^A	0.39	0.013
Total Protein	2.9 ^C	3.8 ^{ABC}	4.3 ^{AB}	3.3 ^{BC}	2.7 ^C	4.9 ^A	3.6 ^{ABC}	4.6 ^A	0.63	0.031
IgG	1.47 ^{BC}	1.28 ^C	1.42 ^C	1.40 ^C	1.47 ^{BC}	1.44 ^C	1.71 ^{AB}	1.75 ^A	0.052	0.021
IgM	0.19 ^{BC}	0.20 ^{BC}	0.22 ^{AB}	0.20 ^{BC}	0.18 ^C	0.23 ^{AB}	0.24 ^A	0.23 ^{AB}	0.008	0.019

Conclusion The results of current study show that effects of immunostimulating phytochemicals like EP and TT are observed, in terms of performance and neutrophil, monocyte, total protein, IgG and IgM counts if birds are reared under optimal environmental conditions.

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Comparison of bioavailability of different commercial sources of choline for broiler chickens

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Application An evident concept is that choline bioavailability is <100% in corn-soybean meal diets, but choline is essential for prevention of fatty liver and perosis in poultry.

Introduction It has long been known that chickens fed corn-soybean meal diets containing choline in excess of the NRC (1994) requirement, still need supplemental choline to achieve maximum growth (Marvel *et al.* 1943). The aim of the present study was to evaluate three commercial sources of choline (product of Belgium (CHO1), China (CHO2) and Germany (CHO3) for broiler chicken.

Material and methods Two hundred female broiler chickens (Ross 308) reared in a 42 days trial. Ten dietary treatments included three dietary added levels of choline (400, 800 and 1200 mg/kg) to the basal diet (contained 862 mg choline/kg). Birds received experimental feed *ad libitum*. Treatments replicated 4 times each included five chickens in a completely randomized design. Performance (body weight gain and feed conversion ratio), carcass characteristics (breast, thighs, liver and abdominal fat fractional weight), liver fat content, different fatty acids (palmitic acid, oleic acid, linoleic acid, palmitoleic acid : stearic acid) in liver fat content and also bone physical properties were compared statistically. Data were analysed using the GLM procedure of SAS. Duncan's multiple range test was used to determine differences among treatment means at $P < 0.05$. Relative bioavailability values of choline were estimated using CHO2 as the standard source by slope ratio comparison from multiple linear regressions (Little *et al.*, 1997).

Results Performance, carcass and bone characteristics had no significant difference between sources of commercial choline. Relative bioavailability of mentioned sources of choline did not show significant difference (Table 1).

Table 1 Relative bioavailability values (RBV) of choline sources (CHO1 and CHO3) in comparison with reference source (CHO2) based on slope ratios from linear regressions of feed conversion ratio, liver fat content, palmitoleic acid:stearic acid and bone strength on added levels of choline to basal diet at 42 days of age

Dependent variable	Choline source	Slope	SE of slope	RBV*	Intercept	R ²	Linear regression (P-Value)
Feed conversion ratio	CHO1	0.00003	0.00002	1	1.56	0.09	0.49
	CHO2	0.00002					
	CHO3	0.00003					
	CHO2	0.000004					
Liver fat content (%DM)	CHO1	0.001	0.001	1	13.75	0.03	0.85
	CHO2	0.001					
	CHO3	-0.001					
	CHO2	-0.001					
Palmitoleic acid : Stearic acid	CHO1	0.00003	0.00007	1	0.15	0.13	0.33
	CHO2	-0.00005					
	CHO3	-0.00008					
	CHO2	-0.00008					
Bone strength (N)	CHO1	0.08	0.04	1	116.05	0.20	0.13
	CHO2	0.09					
	CHO3	-0.002					
	CHO2	0.02					

*If the slope of reference source and the test source were not significant ($P > 0.05$), the RBV was reported by "1" and it means that the availability of choline in reference and test sources were the same.

Conclusion It is not necessary to add excess sources of choline to corn-soybean meal diets with low levels of metabolizable energy (ME) and crude protein (CP), because of same performance ($P > 0.05$) of birds which received different levels of choline. The re-evaluation of choline requirement of broiler chickens offered corn-soybean meal diets with high levels of ME and CP is recommended to achieve optimum performance.

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Effect of heat stress on growth performance and rectal temperature of broiler chickens

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Application Available data indicate that the average temperature of the earth is increasing and this is a threat to food security especially in the tropics. Adaptability of birds especially broiler chickens to high ambient temperature has become very important in ensuring food security.

Introduction Heat stress has been found to have various negative effects on liveability, production performance, immune functions, and disease susceptibility in poultry (Cahaner *et al.*, 2008; Chen *et al.*, 2004). This is likely to worsen due to climate change. The first step in solving the problem of heat stress is to look at the effect of heat stress on the growth performance of chickens. This experiment was conducted to find out the effect of heat stress on rectal temperature and growth performance of broiler chickens.

Material and methods Forty eight Cobb 500 unsexed broiler chickens were used for the study (Cobb Vantress, USA). The birds were two weeks old and the experiment lasted for two weeks. They were randomly allocated to two treatments (Heat stress, 36°C versus No Heat stress, 25°C) with twenty four birds under each treatment. Each treatment had four replications with six birds in each replicate. Birds for each replicate (6 birds) were kept in single cages (L=60.96cm, W=20.32cm and H= 30.48cm) with feed (21% CP and 3100kcal ME/kg) and water administered *ad libitum*. The following parameters were measured: feed intake, body weight, body weight gain, feed conversion ratio and rectal temperature. Analysis of variance (ANOVA) was performed using the general linear model procedure of SAS Version 9.4 (2015) at P<0.05.

Results Feed intake and body weight gain were higher (P<0.001) in broilers that were not on heat stress compared to those that were on heat stress (36°C). Feed conversion ratio (FCR) and rectal temperature were however not significantly different (P>0.05) between the two treatments (Table 1).

Table 1 Effect of heat stress on rectal temperature and growth performance of broiler chickens

Treatments/Parameters	Feed intake*, g	Weight gain*, g	FCR, feed/gain	Rectal Temp., °C
Control	4750.50	3314.25	1.43	39.17
Heat stress	2990.25	2026.00	1.48	39.08
SEM	13.37	48.048	0.0036	0.14

*Feed intake and weight gain were totals for six birds within two weeks. Means compared at P<0.05

Conclusion Heat stress reduced feed intake and body weight gain in this study but did not influence rectal temperature and feed conversion ratio (FCR).

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Effect of gelatinization of fermented cassava meal on its hydrogen cyanide content, haematological and serum biochemical indices of finisher broilers

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Application Effect of gelatinization of fermented cassava on the hydrogen cyanide content and its dietary effect on blood parameters of finisher broilers were carried out. Fermentation followed by gelatinization of cassava tubers resulted in hydrogen cyanide (HCN) free meal if peeled and 50mg/kg if unpeeled. Unpeeled and fermented cassava meal deteriorated PCV and serum globulin, total protein and glucose. This may necessitate a longer period of fermentation of unpeeled as cassava peels are known to contain more HCN than the pulp (Udedibie *et al.*, 2004).

Introduction Maize is the major source of energy in poultry rations. It is however getting increasingly scarce and costly due to over dependence on it for other uses such as staple food in Nigerian. The need for a search for other energy sources as alternatives to maize has led to the investigation on the potentials of cassava as energy source in livestock feed. Presence of HCN in cassava put a limit to its use. Processing of cassava has been advocated to reduce HCN effect. This study focused on fermentation followed by gelatinization as a method of processing cassava tubers for use as alternative to maize and its effect on the haematological and serum biochemical indices of broilers.

Material and methods Fresh cassava tubers of bitter variety were procured from a local market and divided into two batches. One batch was peeled while the other was unpeeled. Samples of both batches were analyzed for HCN content by Picrate paper method (Bradbury *et al.*, 1999). The samples were cut into similar sizes and separately fermented for 4 days in plastic vats containing water under atmospheric temperature. The water was removed at the end of the four days. The fermented samples were spread in the sun to dry. They were milled using a hammer mill with 2mm sieve. The two samples were subjected to gelatinization process and dried. The samples were subjected to HCN analysis according to Bradbury *et al.* (1999). Three soyabean – fish meal- based experimental diets were made and fed for four weeks. Control (T1) contained maize, while T2 and T3 contained only peeled and unpeeled fermented gelatinized cassava meals respectively in equal proportion with maize. Each treatment contained 40 day old Anak chicks, replicated four times with 10 birds each on completely randomized design (CRD). One bird was selected from each replicate for blood analysis. Blood was collected through the vein on the wing into a bottle containing anticoagulant for haematological analysis and the bottle for serum biochemistry did not contain anticoagulant. Data generated were subjected to analysis of variance (ANOVA). Means were compared using Duncan New Multiple Range Test (DNMRT) at 5% level of significance.

Results Peeling, fermentation and gelatinization reduced HCN to zero level compared to raw and peeled (500ppm) and unpeeled fermented (50ppm). Unpeeled and fermented significantly ($P < 0.05$) reduced PCV, total protein, globulin and glucose (Tables 1 and 2). Wbc, Rbc, albumin, Hb and cholesterol were not negatively affected.

Table 1 Effect of processed cassava on haematology of finisher broilers

Parameters	T ₁ (Control)	T ₂ (PFGC)	T ₃ (UFGC)	SEM
WBC ($\times 10^4/\text{mm}^2$)	6.40	4.9	5.00	0.10
HB (g/dl)	10.00	8.93	9.87	0.28
RBC ($\times 10^6/\text{ul}$)	9.18	8.3	8.68	0.22
PCV (%)	30.57 ^a	29.77 ^a	25.77 ^b	0.97

Table 2 Effect of processed cassava on serum protein, glucose and cholesterol of finisher broilers

Parameters	T ₁ (Control)	T ₂ (PFGC)	T ₃ (UFGC)	SEM
Total protein (g/dl)	4.10 ^a	3.90 ^a	3.40 ^b	0.13
Albumin (g/dl)	1.63	1.67	1.70	0.09
Globulin (g/dl)	2.47 ^a	2.30 ^a	1.70	0.13
Glucose (g/dl)	39.67 ^a	36.33 ^a	14.33 ^b	5.70
Cholesterol (mg/dl)	64.00	57.67	58.00	7.31

Conclusion Peeling and fermentation improved the quality of cassava meal, could replace whole maize in broiler diet.

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Comparison of bioavailability of different commercial sources of dicalcium phosphate for broiler chickens

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Application Environmental and economic concerns regarding phosphorus (P) in broiler production pledge a reduction in dietary P provision. However, to maintain growth performance and bone mineralisation, optimisation of P utilisation appears crucial.

Introduction In broilers, P is involved in many essential functions, representing a key element in the maintenance of growth performance and bone mineralisation (Le´tourneau-Montminy *et al.*, 2010). Thus, it must be of major concern that current economic and environmental pressures leading to an unavoidable decrease in dietary P supply represent a threat to animal productivity. Then it is necessary to find P sources with high levels of bioavailability. The aim of the present study was to determine the bioavailability of P from six commercial sources of dicalcium phosphate (DCP2: 17.75% P; DCP3: 17% P; DCP4: 17% P; DCP5: 18.5% P; DCP6: 17% P; DCP7: 18.06% P) relative to standard source (P1=Phosphoric acid 85%, contains 26.87% P), for broiler chicks.

Material and methods A total of 440 1-d-old Ross-308 female broiler chickens were randomly allotted to 1 of 4 replicate cages (5 chicks/cage) for each of 22 treatments in a completely randomized design involving seven P sources and 3 levels of added P (0.15, 0.30, or 0.45%) plus a control diet containing 0.15% available P. Feed and water were available *ad libitum* for an experimental phase of 42-d. Data were analysed using the GLM procedure of SAS. Duncan's multiple range test was used to determine differences among treatment means at $P < 0.05$. Relative bioavailability values of P were estimated using P1 as the standard source by slope ratio comparison from multiple linear regressions (Little *et al.*, 1997).

Results Body weight gain (BW), average daily feed intake, FCR, carcass and breast fractional weight, alkaline phosphatase (ALP), tibia characteristics (length (L), ash content, bone strength (BS)) were different significantly, between P levels and sources at 42d. Based on slope ratios from the linear regression of BW, FCR, ALP, BS, L and ash%, on added levels of available P, the bioavailability of P in source no. 2, 3 and 7 were the same, relative to phosphoric acid.

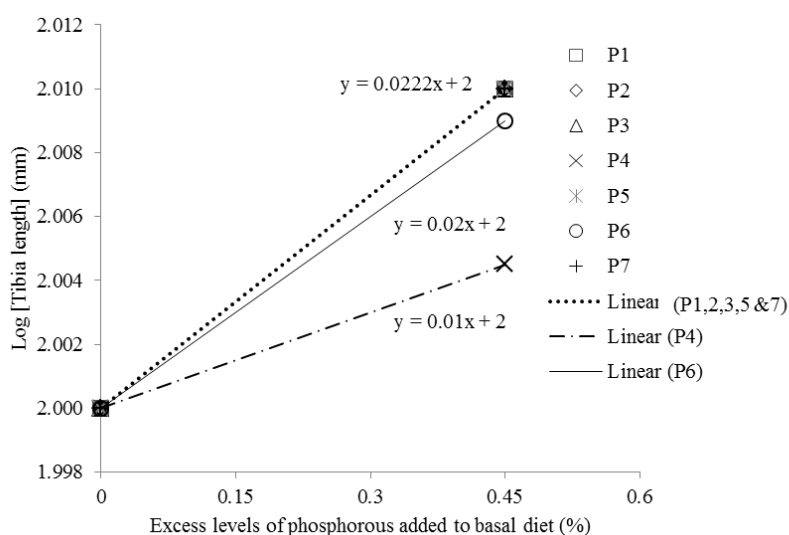


Figure 1 Comparison of bioavailability of 7 different commercial sources of dicalcium phosphate (DCP2: 17.75% P; DCP3: 17% P; DCP4: 17% P; DCP5: 18.5% P; DCP6: 17% P; DCP7: 18.06% P) relative to standard source (P1=Phosphoric acid 85%, contains 26.87% P) for broiler chickens

Conclusion Phosphorous originated from sources no. 2, 3, 5 and 7 were 20% and 40% more available to broilers than sources no. 4 and 6, respectively, in enhancing tibia length (Figure 1).

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Assessing the genetic diversity of indigenous chicken genotypes in South-western Nigeria using microsatellite markers

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Application Understanding of Nigerian indigenous chicken genetic diversity is required in contributing to meeting the production needs, allow sustainable genetic improvement, and to facilitate rapid adaptation to changing breeding objectives

Introduction Despite the importance of indigenous chickens, little is known about their genetic diversity leading to increasing research work on their genetic variation to characterise the genetic structure of locally available populations which is an important step towards revealing the uniqueness of these populations and identify their valuable genetic resources for conservation since they are largely free of systematic selection (Ajibike, 2015). Hence, this study was carried out to assess the genetic variation between the available indigenous chicken genotypes in South-western Nigerian.

Material and methods 2ml of blood was collected from the wing vein of 69 individual chickens: *Normal feather* (*N*, n=38), *Naked neck* (*Na*, n=4), *Fulani* (*Fu*, n=3), *Frizzle* (*Fr*, n=13) and “*Opipi*” (*Op*, n=11) using a new needle and syringe for each animal to avoid cross contamination. Deoxyribonucleic acid (DNA) was extracted from Fast Technology for Analysis (FTA) paper. The extracted DNA was amplified with 15 predefined microsatellite primer sets. Multiplexing was carried out following the recommendation of the ISAG/FAO panel. Allele size calling was carried out after separation using a ABI 3730XL automated capillary sequencer (Applied Biosystems, USA) with Liz 500-350 internal size marker. Genotypic data were analyzed using POPGENE (Yeh *et al.*, 1999) and GenAlex (Peakall and Smouse, 2012) to calculate the observed number of alleles, effective number of alleles, observed (H_o) and expected (H_e) heterozygosity, and test for Hardy–Weinberg equilibrium (HWE). The extent of inbreeding was further studied using GENEPOP software (Raymond and Rousset, 1995) by estimating the F_{IS} values and their significance level within each of the populations.

Results The mean number of alleles (MNA) per population range from 2.93±0.25 alleles for *Na* and *Fu* chicken to 5.93±0.62 for *N* chicken, while the effective number of allele (NEA) contributing to the population range from 2.36±0.21 for *Op* to 2.92±0.26 for *N* chicken. The lowest observed heterozygosity (H_o) was observed in *Fr* chicken (0.50±0.06) while *Na* chicken has the higher values (0.62±0.08). The highest expected heterozygosity (H_e) was observed in *N* chicken (0.61±0.04) while *Na* and *Fu* chickens has the lowest value (0.52±0.06). The inbreeding coefficients (F_{IS}) for all the sampled indigenous chicken genotypes were observed to be positive, except for *Na* chickens which shows a negative value of -0.05 at $P > 0.01$.

Table 1 Genetic diversity indicators for the sampled indigenous chicken genotypes

Pop	MNA	NEA	I	Ho	He	F_{IS}
<i>N</i>	5.93±0.62	2.92±0.26	1.25±0.10	0.53±0.05	0.61±0.04	0.15
<i>Na</i>	2.93±0.25	2.40±0.22	0.89±0.10	0.62±0.08	0.52±0.06	-0.05
<i>Fr</i>	4.33±0.27	2.70±0.22	1.12±0.08	0.50±0.06	0.57±0.04	0.18
<i>Fu</i>	2.93±0.25	2.39±0.23	0.90±0.09	0.57±0.07	0.52±0.05	0.14
<i>Op</i>	4.13±0.45	2.36±0.21	1.00±0.10	0.54±0.07	0.53±0.05	0.03
Mean	4.05±0.22	2.56±0.10	1.03±0.04	0.55±0.03	0.61±0.02	0.13

Conclusion The used microsatellite markers indicated that there was a high genetic diversity and moderate differentiation, despite the observed high degree of inbreeding and genetic admixture between the sampled chicken genotypes.

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The effects of forage type and molybdenum and sulfur supplementation on copper status in growing lambs

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Application Copper retention in the liver has been shown to be influenced by forage type. This may be due to the effect of rumen pH on thiomolybdate production in the rumen.

Introduction Copper (Cu) is an essential trace element involved in numerous enzyme and protein systems that have a range of functions in animal systems from antioxidants to hormone releasing proteins (Suttle, 2010). However, Cu interacts with molybdenum (Mo) and sulfur (S) in the rumen to produce Cu thiomolybdates which are unavailable to animals. It has been observed that the effects of Mo and S on Cu retention in dairy cows varies with forage type (Sinclair *et al.*, 2013). However, the mechanism for this effect is unclear. The objective of this experiment was to investigate the effects of forage type and Mo and S supplementation on Cu status of growing lambs.

Material and methods Forty-eight, Swaledale weaned lambs with a mean live weight of 27.1 kg were used in experiments, with eight representative animals slaughtered as an initial slaughter group. The remaining 40 lambs were then individually penned, blocked by live-weight (LW) and allocated to one of four treatments. Lambs were offered either grass haylage (GH) or maize silage (MS), together with one of two concentrates formulated to supply Cu 10 mg/kg DM in the overall diet, and either no antagonists (-) or antagonists (+). Antagonists (S 4 g/kg DM and Mo 5 mg/kg DM) were included with the intention of reducing the efficiency of Cu absorption by 0.5 (Suttle and Price, 1976). Forages and concentrates were fed in a 60:40 ratio on DM basis at a restricted level calculated to support a LW gain of 200 g/day (AFRC, 1993) over a period of 10 weeks. Throughout the experiment lambs were fed twice daily with water being available *ad libitum*. Dry matter intake (DMI) and LW gain were monitored weekly and weekly blood samples collected by venepuncture. At the end of the experiment all lambs were slaughtered at a commercial abattoir. Rumen fluid samples were collected and stored in ice prior to determination of pH within 1 hour of slaughter. Liver samples were also collected and stored at -20 °C. Following processing, blood plasma and liver samples were analysed for mineral content by ICP-MS. The experiment was analysed by ANOVA as a 2x2 design using Genstat 17.

Results The DMI of lambs offered diet GH was significantly higher ($P < 0.05$) and live weight gain significantly lower ($P < 0.001$) than those offered diet MS. However, there was no effect of antagonists on lamb performance. Lambs offered diet GH had a significantly higher ($P < 0.001$) rumen pH than those offered diet MS, but there were no significant effect of forage type on final liver or plasma Cu concentration, However, total liver Cu store was significantly higher ($P < 0.05$) in lambs fed diet MS compared to GH. Inclusion of antagonists (+) significantly ($P < 0.05$) reduced final liver Cu concentration, total liver Cu store and plasma Cu concentration.

Table 1 Effect of basal forage grass haylage (GH) and maize silage (MS) fed with (+) or without (-) supplemented Mo and S on the performance and Cu status in growing lamb

	Treatments				P Value			
	GH-	GH+	MS-	MS+	s.e.d	F	A	Int.
Intake, kg DM/d	0.82	0.85	0.75	0.78	0.041	0.029	0.398	0.992
Live weight gain, kg/d	0.07	0.08	0.13	0.13	0.017	<.001	0.947	0.768
Rumen pH	6.19	6.25	6.00	5.84	0.094	<.001	0.47	0.113
Liver Cu concentration, µg/g DM	192	125	255	138	52.2	0.308	0.019	0.499
Total liver Cu storage, mg/liver	19.50	11.50	32.90	17.00	5.76	0.028	0.007	0.337
Plasma Cu, µmol/L	15.14	13.49	15.10	12.84	1.075	0.651	0.016	0.687

Conclusion The higher Cu status of lambs offered MS compared to GH was associated with a lower rumen pH. The inclusion of antagonists reduced lamb Cu status.

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Effect of dietary iron with and without sulphur on copper metabolism in sheep

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Application High dietary iron levels but not high sulphur levels reduce the copper status of sheep.

Introduction Copper (Cu) availability is reduced by other mineral antagonists such as molybdenum (Mo), sulphur (S), and iron (Fe) when present in high concentration in sheep diets (Suttle, 1991). To date, research has concentrated on the effects of Mo and S on Cu metabolism (Ryssen, 1979). No work has been conducted to investigate the possible interaction between Fe and S in sheep on Cu metabolism. Therefore, an experiment was conducted to investigate the effect of dietary supplementation with Fe with or without supplemental S on liver Cu concentration and performance of growing lambs.

Material and methods Fifty-four Texel crossbred weaned lambs with average live weight (LW) of 29.06 ± 3.67 kg, were used in a 2×3 factorial experiment. Lambs were blocked by LW and sex, and then randomly allocated to one of six treatments. Dietary treatments were: 1) Low Fe: Low S (L:L), 2) Low Fe: Medium S (L:M), 3) Low Fe: High S (L:H), 4) High Fe: Low S (H:L), 5) High Fe: Medium S, and 6) High Fe: High S (H:H). Low Fe diets were not supplemented with Fe (302 mg/kg DM) and high Fe diets were supplemented with 800 mg Fe/kg DM (as FeSO_4), while low, medium and high S diets were supplemented with 0, 1.5, and 3.0 g S/kg DM (as $(\text{NH}_4)_2\text{SO}_4$); the analysed S in the basal diet was 2.89 g/kg DM. Lambs were housed individually throughout the trial period, and fed iso-nitrogenous, iso-energetic diets at a restricted level to gain 200 g/day (AFRC, 1993) over a 12 week trial period. Live weight was recorded weekly and jugular blood samples were taken fortnightly to monitor plasma Cu levels and once every 4 weeks for haematology analysis. At the termination of the trial, all lambs were slaughtered in a commercial abattoir and liver samples collected and analysed for Cu by ICP-MS. The data was analysed by ANOVA as a 2×3 factorial design.

Results There was no significant effect of dietary treatment on LW (41.95 ± 1.36 kg) or daily gain (0.15 ± 0.01 kg/d). Liver Cu concentration was significantly lower in lambs received high Fe diets compared to lambs fed low Fe diets, i.e. 304 vs. 439 mg/kg DM respectively (s.e.d. = 44.1; $P = 0.004$). Iron supplemented lambs had a significantly high liver Fe levels compared with un-supplemented lambs ($P < 0.001$). At week 12, the lambs that received high Fe supplemented diet had a significantly lower plasma Cu concentration ($P < 0.05$) than lambs given low Fe diets (10.88 vs. 11.65 $\mu\text{mol/l}$, respectively). Similarly in week 12 blood haemoglobin (Hb) concentration was higher ($P < 0.05$) in groups received Fe added diet in week 12 of the trial.

Table 1 Effect of iron and/or sulphur on liver Cu and Fe concentration (mg/kg DM), and on plasma Cu ($\mu\text{mol/l}$) and Hb (g/dl) of growing lambs

Item	Treatments						s.e.d	P			
	H:L	H:M	H:H	L:L	L:M	L:H		Fe	S	Fe.S	
Liver	Cu	274	332	306	545	344	427	76.5	0.004	0.415	0.064
	Fe	889	817	928	355	320	355	83.7	0.001	0.447	0.812
Plasma Cu		11.49	10.78	10.38	12.11	11.67	11.16	0.60	0.030	0.060	0.950
Blood Hb		13.22	13.86	13.21	12.32	13.01	11.96	0.77	0.031	0.275	0.921

Conclusion Dietary Fe supplementation significantly increased liver Fe concentration but decreased liver Cu concentration. Additional levels of supplemental S did not affect liver minerals stores and there was no significant Fe \times S interaction to decrease liver Cu content. Liver mineral levels are mainly influenced by high dietary Fe rather than S.

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Effects of growth promoters on expression of genes associated with lipid metabolism in ovine skeletal muscle, subcutaneous adipose tissue and liver

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Application The understanding of lipid metabolism in response to growth promoters will enable the identification of potential changes in nutrient utilisation and deposition, which may be targets for improving feed efficiency.

Introduction The growth promoters, growth hormone (GH) and beta-adrenergic agonist (BA), alter nutrient mobilisation and utilisation, which results in muscle hypertrophy and leaner carcasses (Bell *et al.*, 1998). The aim of this study was to determine the effects of these agents on the expression of genes associated with lipid synthesis (fatty acid synthase, FAS) and degradation (adipose triglyceride lipase, ATGL) in various tissues of lambs.

Material and methods Male lambs (120 days old) were all fed a high protein/energy diet *ad libitum*, with the GH group (n=10) receiving a single subcutaneous injection of bovine GH (3.75mg/kg body weight, POSILAC, Monsanto) on day 1; the BA group (n=10) receiving BA (cimaterol) at 10mg/kg in the feed, whereas the control group (CO), n=11 only had the *ad libitum* feed. After 6 days all lambs were slaughtered and samples of the *longissimus dorsi* (LD) and *supraspinatus* (SS) muscles, subcutaneous adipose tissue (SC) and liver (Li), were snap frozen in liquid nitrogen and stored at -80°C. Total RNA was extracted (Trizol) and first strand cDNA generated using random primers. Gene expression was determined using quantitative RT-PCR analysis (Roche) relative to beta-actin gene expression (not significantly affected by treatment). Metabolomic analysis of plasma fatty acid concentration taken at slaughter was carried out by Metabolon Inc (USA) using GC/MS and LC/MS/MS platforms. Treatment groups were compared using one-way ANOVA (Genstat) and *Post hoc* Dunnett's test, error bars are standard error of the mean. Significance was accepted at P<0.05.

Results In both muscles, the expression of ATGL mRNA was decreased in BA treated relative to control, whilst FAS mRNA was reduced in response to both BA and GH in LD and there was a trend in SS (P<0.1, ANOVA). There were no effects of either treatment on gene expression in SC. In Li, there were no effects on ATGL mRNA expression, whilst FAS gene expression was decreased in GH treated lambs relative to control (Figure 1). There was a significant increase in the plasma concentrations of the free fatty acids, palmitate, stearate and oleate, in GH treated lambs (Figure 2).

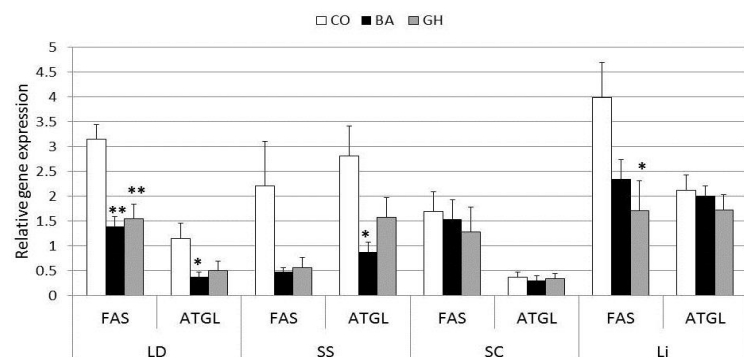


Figure 1 Effects of BA and GH on FAS and ATGL mRNA expression in *longissimus dorsi* (LD) and *supraspinatus* (SS) muscles, subcutaneous (SC) adipose tissue and liver (Li). (*P<0.05, ** P<0.01)

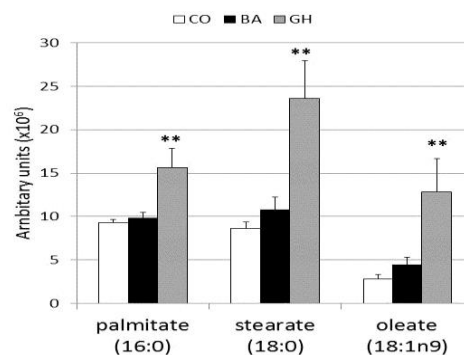


Figure 2 Effects of BA and GH on plasma fatty acid concentrations. (*P<0.05, ** P<0.01)

Conclusion Following a relatively short term treatment (6 days) with GH, but not BA, there was an increase in plasma fatty acids but this was not associated with changes in the expression of genes associated with either lipogenesis or lipolysis in SC. Changes in FAS suggests growth promoters may reduce fatty acid synthetic capacity of muscles and Li, whilst lipolysis in muscle is also decreased, particularly with BA treatment, suggesting a reduced turnover of lipids in muscle.

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Effects of age at first joining and ewe genotype on performance of ewes lambing at 2 years of age

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Application Regardless of genetic value for prolificacy joining replacement ewes to lamb as one-year olds has no negative effect on ewe performance when lambing at 2 years of age.

Introduction Keady (2014) concluded that the mean cost nationally of rearing a replacement ewe to first joining at ~19 months equated to approximately 25% of the value of the lamb carcass output produced during her lifetime. Joining ewe replacements to produce their first lambs at 1 year, with the objective of increasing the number of litters produced during their lifetime, may provide an opportunity to reduce replacement costs. Previously, Keady and Hanrahan (2014) reported that Belclare (B), B×Suffolk (B×S) and >75% S replacements joined at ~7 months reared 1.34, 1.03 and 0.93 lambs per ewe lambing. The aim of the current study was to evaluate the effects of age at joining (~7 or 19 months) and ewe genotype on the performance of ewes lambing at 2 years and the performance of their lambs.

Material and methods A total of 424 ewes [157 B, 114 B×S, 153 >75% S] were joined with Charollais rams to lamb in March at 2 years of age. A random 50% of each genetic group had been joined at ~7 months while the remainder were joined for the first time at ~19 months. Ewes were shorn at housing (early December) and offered grass silage *ad libitum* until lambing. During the last 7 weeks pre lambing a total of 15, 23 and 28 kg concentrate was offered to ewes carrying (scan data) singles, twins and triplets, respectively. Ewes rearing singles or twins received no concentrate supplementation post lambing while those rearing triplets received concentrate (0.5 kg/d) for 5 weeks post lambing, and their lambs had access to concentrate (up to 300 g/head daily) until weaning. All lambs were managed as one flock between weaning and slaughter, and offered grazed grass as the sole diet. The data were analysed using Procs GENMOD or MIXED of SAS, as appropriate.

Results The grass silage had DM and metabolizable energy concentrations of 213 g/kg and 11.5 MJ/kg DM, respectively. The effects of age at first joining and ewe genotype on animal performance are presented in Table 1. There were no age at first joining by ewe genotype interactions ($P>0.05$). Ewes that were joined for the first time to lamb at 2 years were heavier at joining and lambing, tended to have a lower condition score ($P=0.07$) at joining and produced lighter lambs at birth ($P<0.001$). Age at first joining had no effect ($P>0.05$) on ewe weight at weaning, litter size, or lamb gain from birth to weaning. Belclare ewes were lighter at joining and lambing relative to the B×S and >75% S ewes. Ewe genotype had no effect ($P>0.05$) on weight at weaning or body condition score at joining or weaning. Relative to B and B×S ewes, 75% S ewes produced smaller litters and reared fewer lambs per ewe joined. Belclare ewes tended to produce ($P=0.09$) lighter lambs at birth. Lambs born to the B×S ewes grew faster ($P<0.01$) from birth to weaning than those born to <75% S ewes.

Table 1 Effect of age at first joining and ewe genotype on ewe and lamb performance

	First mating (FM)			Ewe genotype ¹ (G)				Sig. ²	
	7 months	19 months	s.e.	B	B×S	>75% S	s.e.	FM	G
Ewe weight (kg)-									
joining	59.6	61.8	0.46	59.3 ^a	61.2 ^b	61.6 ^b	0.56	***	**
lambing	64.5	62.3	0.54	61.4 ^a	64.3 ^b	64.5 ^b	0.67	**	***
weaning	65.2	64.7	0.61	64.5	65.1	65.4	0.75	NS	NS
Condition score -									
joining	3.11	3.03	0.031	3.03	3.08	3.11	0.038	0.07	NS
weaning	3.31	3.24	0.034	3.26	3.25	3.31	0.042	NS	NS
Litter size	1.78	1.79	0.046	1.92 ^b	1.85 ^b	1.60 ^a	0.057	NS	***
Lambs reared per ewe joined	1.41	1.38	0.053	1.51 ^b	1.49 ^b	1.18 ^a	0.065	NS	***
Lamb weight -									
birth	4.83	4.53	0.066	4.55	4.74	4.74	0.079	***	0.09
weaning	29.8	29.3	0.52	29.6	30.2	28.9	0.57	0.10	NS
Lamb gain 0-14 weeks (g/day)	257	255	5.0	256 ^{ab}	263 ^b	248 ^a	5.4	NS	*
Ewes joined at 30 months ² (%)	78	77	-	77	76	80	-	NS	NS

¹B = Belclare, S = Suffolk; ²Relative to initial number assigned to the study. ^{2 ab}Means with a superscript in common are not significantly different

Conclusion Age at first joining (~7 or 19 months) had no effect on the performance of ewes lambing at 2 years of age. Belclare and B×S ewes reared 0.33 and 0.31 extra lambs per ewe joined than >75% S ewes.

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Effect of stocking rate and prolificacy potential on lamb performance and carcass output from a grass based production system

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Application Increasing stocking rate and ewe prolificacy can increase carcass output from grass based production systems

Introduction Increasing output (kg carcass) is vital to maintaining sustainable lamb production systems. Grazed grass is the most economic source of nutrition available to grazing ruminants (Finneran, *et al.*, 2010) and has the potential to supply 90 to 95% of the energy requirements of sheep (Davies and Penning, 1996). Stocking rate (SR; ewe ha⁻¹) and ewe prolificacy potential (PP; predicted number of lambs born ewe⁻¹ year⁻¹) are two important factors influencing output in grass-based production systems. The objective of this study therefore was to investigate the effect of SR, ewe PP and their interaction on lamb performance and carcass output from a grass based production system.

Material and methods A 2 x 3 factorial study comprising two production years was completed between October 2012 to October 2014. The study consisted of 180 medium prolificacy potential (MP) - Suffolk crossbred ewes (target weaning rate of 1.5 lambs ewe⁻¹), and 180 high prolificacy potential (HP) - Belclare crossbred ewes (target weaning rate of 1.8 lambs ewe⁻¹; Hanrahan, 1994) Within PP, ewes were blocked by live weight (kg) and body condition score (BCS) on a scale of 1 to 5, before being randomly assigned to one of three stocking rate (SR) pasture-based systems: a low SR (LSR; 10 ewes ha⁻¹), medium SR (MSR; 12 ewes ha⁻¹), and high SR (HSR; 14 ewes ha⁻¹) in October 2012. Ewes were mated in October each year to Charollais rams over a six week period. Post-lambing, ewes and their progeny were rotationally grazed. Lamb average daily gain (ADG; g day⁻¹) was recorded from birth to weaning and from weaning to slaughter, with lambs weighed at two week intervals from six weeks of age. Lambs were weaned at 14 weeks of age and drafted for slaughter once pre-defined live weight targets of 42, 43, 44, 45 and 46 kg were reached in the months June, July, August, September and October, respectively to produce a carcass of 19 to 20 kg. Post-weaning a leader follower grazing system was operated with lambs offered pasture access in advance of ewes. The effect of SR, PP and their interaction on lamb ADG, days to slaughter (DTS), carcass weight and carcass output (kg ha⁻¹) were modelled using linear mixed models in PROC HP MIXED (SAS Inst. Inc., Cary, NC, USA) with ewe included as a random effect.

Results

A significant PP by SR interaction was observed for pre-weaning lamb ADG, with MP lambs having a lower ADG at the MSR (-18.9 g day⁻¹, SEM = 6.4; P<0.01, Table 1) compared to HP, however at the LSR and HSR lambs from HP ewes had greater ADGs. Post-weaning; LSR lambs achieved a greater ADG (P<0.001) compared to the MSR and HSR lambs which did not differ from each other. Also post-weaning the HP lambs achieved a greater ADG. Lifetime lamb ADG was highest at the LSR, intermediate at the MSR and lowest at the HSR (P<0.001). A second PP by SR interaction was observed for DTS, with MP lambs at the MSR requiring a greater number of days to reach slaughter (+14.9 days; SEM = 6.6; P<0.01) compared to lambs finished off the LSR and HSR. High prolificacy lambs had a greater carcass weight (P<0.001). Carcass output increased as both SR (P<0.001) and PP (P<0.01) increased.

Table 1 The effect of stocking rate and prolificacy level on lamb performance and carcass output

Parameter	SR			SEM	PP			P-value		
	LSR	MSR	HSR		MP	HP	SEM	SR	PP	PP x SR
Pre-weaning ADG g day ⁻¹	272 ^a	263 ^b	242 ^c	7.9	263	255	4.3	<0.001	<0.01	<0.01
Post-weaning ADG g day ⁻¹	154 ^a	137 ^b	135 ^b	1.3	136	149	7.4	<0.001	<0.01	NS
Lifetime ADG g day ⁻¹	195 ^a	168 ^b	155 ^c	1.2	169	177	7.0	<0.001	NS	NS
Days to slaughter (days)	209 ^a	225 ^b	241 ^c	7.1	226	225	6.9	<0.001	NS	<0.01
Carcass weight (kg)	19.8	19.7	19.6	0.21	19.4	19.9	0.12	NS	<0.001	NS
Carcass output (kg ha ⁻¹)	320 ^a	379 ^b	419 ^c	24.9	344	401	13.6	<0.001	<0.01	NS

a,b,c Within rows superscripts indicates significant difference (P<0.01) in SR, ADG: Average daily gain (g day⁻¹)

Conclusion Increased carcass output from grass based production systems is achievable through increased stocking rate and prolificacy potential levels despite lower individual performance.

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Techniques to advance oestrus in ewes – a comparative study between the use of progestagen sponges and the ‘ram effect’

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Application Sponge treated ewes (SE) lambed earlier than teaser ram ewes (TE). The lambing percentage and the lamb birth weights did not differ between the techniques. Lamb weights at 35 days were greater in SE lambs due to differences in lambing resource availability.

Introduction The onset of oestrus in sheep can be manipulated in the last few weeks of anoestrus via several techniques (Martin, 2004). Producers use these techniques to manage for an earlier lambing time frame and consequently allow lambs to be marketed earlier in the year. The aim of the study was to see if the sponge technique compromised the performance of the ewes and lambs compared to the use of teaser rams. The key performance indicators of lambing timings; number of lambs per ewe and individual lamb weights were assessed between the two different techniques.

Material and methods The study investigated lambing outcomes following administration of flugestone acetate in combination with Pregnant Mare Serum Gonadotrophin (PMSG) or the utilisation of the ram effect on ewes during the non-breeding season. In the study, 138 Suffolk x Mule ewes were separated into two groups. The treatment group (n=60) was given an intravaginal progestagen (20mg flugestone acetate). The sponges were left in for 14 day and immediately after sponge removal an intramuscular injection of PMSG (300IU) was administered. Thirty-six hours after termination of the progesterone and PMSG the ewes were introduced to the ram. For the teaser group (n=78) vasectomised rams were introduced for two weeks on the same day the first treatment group were sponged. Once the teaser rams were removed, entire rams were introduced and left to run with the ‘teaser’ group. Subsequent weights of live lambs at birth and 35 days later were recorded. Data were analysed using the t-test to compare key performance indicator diaferences between the two techniques.

Results Lambing was significantly earlier ($p<0.001$) by 17 days in SE than the TE group. There was no significant difference in the numbers of singles, double and triplets per ewe and in average weights at birth between groups. However, a significant difference was identified in average lamb weights at 35 days old with the SE lambs being 1.4kg heavier ($p<0.01$).

Table 1 Early oestrus technique key performance indicators comparison

	Technique		s.e.d.	p
	SE	TE		
Ram introduction to lambing interval (days)	147.4	164.4	1.2	<0.001
Lambs per ewe	1.76	1.8	0.05	n.s.
Lamb birth weight (kg)	6.0	6.2	0.14	n.s.
35 day lamb weight (kg)	13.6	12.2	0.36	0.01

Conclusion Progestagen sponged ewes is an early oestrus technique which offers producers the advantage of a significantly shorter ram introduction to lambing interval compared to teaser rams, without the lambing percentages and lamb birth weights being compromised. The better growth rates observed in SE lambs because of less demand placed on lambing resources warrants further investigation, such as lambing shed stocking density and disease build up as lambing progresses.

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Relationship between body weight and milk production in dairy sheep

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Application Monitoring body live weight in dairy sheep in each stage of their productive cycle allows the implementation of feed management strategies that promote adequate nutrient availability for milk synthesis.

Introduction Ewe nutrition is vital for successful dairy performance. Although all nutrients are ultimately derived from food intake, the nutrients stored in body tissues are fundamental to specific production stage e.g. late gestation and early lactation. Nutrient demand is increased to achieve an adequate development and performance of the mammary gland. In dairy animals there is a high priority in physiological functions i.e. pregnancy and milk production (Bauman and Currie, 1980).

Material and methods The aim of the present study was to analyse the effect of body weight at mating, late pregnancy (day 120 of pregnancy), lambing, early lactation and late lactation on milk yield and patterns of milk production. Furthermore to investigate the effects of environmental factors such as: number of lambing (NL) and type of lambing (TL) on live weights and milk production. Monthly milk yields (MMY) were recorded throughout a period of 157±8.6 days from 52 multiparous East Friesian ewes from an experimental flock in Puerto Natales, University of Magallanes, Chile. The flock was managed under a strip grazing system on mixed swards of rye grass (*Lolium perenne*), orchard grass (*Dactylis glomerata*) and white clover (*Trifolium repens*) and supplemented at milking time with Lucerne hay (*Medicago sativa*) (400g / ewe / d) and commercial concentrate (15% CP; 400g / ewe / d). The animals were mechanically milked once daily, with the first MMY record at day 34±5.6; total milk yield (TMY) was computed using the Fleischmann's method (Ruiz *et al.* 2000). The ewes were weighed at mating (WM), late pregnancy (WLP), lambing (WL), early lactation (WEL), and late lactation (WLL). Higher-order polynomials were used (PROC REG, SAS Institute 2002) to analyse the relationship between total milk yield with measured body weights. The General Linear Model (PROC GLM, SAS Institute 2002) was used to analyse the effect of TL (1, 2 and 3 lambs) and NL (1st, 2nd, 3er, 4th and 5th) on milk production. To analyse the effect of the analysed variables (NL, TL, WM, WLP, WL, WEL and WLL) on lactation curves the mathematical model proposed by Wood gamma model was utilised. The parameters of the Wood model (a, b and c) were estimated individually for each lactation curve through an iterative procedure of nonlinear regression analysis (PROC NLIN, SAS Institute 2002).

Results There was a significant effect ($P<0.05$) of NL on WM, WLP, WL, WEL and WLL. All weights were lower in younger animals compared with older ones; ewes of first and second lambing (55.62 and 56.48 kg; $P=0.007$; s.e.d.=9.67) were lighter than ewes of third to fifth lambing (63.12, 65.17 and 65.17 kg, respectively). Ewes at their first and second lambing had significantly lower TMY (86.88 kg and 97.87; $P<0.001$; s.e.d.=2.23) compared to those at their third to fifth lambing (123.77, 123.91 and 127.02 l, respectively). There was a trend of effect of TL on TMY ($P=0.08$; s.e.d.=10.2), ewes of triple lambing had higher TMY in comparison with simple and double lambing (137.23>103.59 and 110.74 l, respectively). There was a positive quadratic relationship between WM and TMY ($\beta_1=9.6$, $\beta_2=-0.04$; $P<0.001$; $R^2=0.51$). TMY increased as WLP (quadratic effect, $\beta_1=20.0$, $\beta_2=-0.13$; $P=0.003$; $R^2=0.58$), WL (linear effect, $\beta_1=3.13$; $P<0.001$; $R^2=0.44$) and WEL (quadratic effect, $\beta_1=14.9$, $\beta_2=-0.09$; $P=0.04$; $R^2=0.48$) increased. There was no effect of WM, WLP, WL, WEL and WLL on their different parameters in the Wood model ($P>0.05$). The live weight of ewes at mating, late pregnancy, lambing, early and late lactation had a positive effect on milk production ($P<0.05$). Higher milk yields were obtained when ewes had weights from 59 to 66 kg at late pregnancy and 57 to 64 kg at lambing.

Conclusion To achieve appropriate production performance farmers must ensure adequate body weight that promotes suitable mammary development in pregnancy and body reserves that will favours galactopoyesis.

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Association of milking parlours and milking procedures with subclinical mastitis in dairy sheep

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Application Results from this study will enhance our knowledge regarding the role of machine milking and relative procedures on the prevalence of subclinical mastitis in dairy sheep.

Introduction Although most farmers have adopted automatic milking, udder health has not improved as expected (Hovinen *et al.*, 2011). The standard operating procedures concerning machine milking are usually underestimated or ignored by the majority of dairy farmers. As a result, udder health problems and large variation in milk quality dominate the routine of dairy flocks. The objective here was to assess the role of milking parlours on udder health and particularly the prevalence of subclinical mastitis in dairy sheep.

Material and methods A random number of twenty-eight dairy sheep farms, located in Northern Greece, were used. Each farm was visited by a team of two veterinarians that assessed the structure and function of the milking parlour. A designated recording form was used to assess the following: i) type and structure of the milking parlour, including dimensions and holding area, numbers of stalls and milking units, diameters of pipelines, number of milkings per day, frequency of teat cup liners replacement and general maintenance. Moreover, a specific mobile device (Exendis Milking System Analyser PTV) was used to measure the vacuum level in the clusters (in order to compare it with the vacuum of the pump), the pulsation rate and the duration of pulsation phases, in three milking units of each line in every milking parlour. A random sample of milking ewes from each flock, representing about 20% of the total population in the flock, was selected for milk sampling in order to perform California Mastitis Test (CMT; scores 0-4) as an indicator of subclinical mastitis. If the percentage of ewes with milk assigned CMT score 3 and 4 was over 20%, then the flock was considered as positive for subclinical mastitis. Data were analysed using univariate analysis of variance with SPSS 22 statistical software. Vacuum level, difference of vacuum level between clusters and vacuum pump, pulsation rate, pulsation ratio and cluster removal after vacuum cessation were transformed into categorical variables and used as fixed effects.

Results A general evaluation of the milking procedure and performance is shown in Figure 1 as a spider diagram, where 0 is the lowest and 10 the highest evaluation score. In 71% of the flocks, the percentage of animals with CMT 3 and 4 was over 20%. The average percentage of ewes with CMT 3 and 4 was 7% and 22%, respectively. The holding area was small compared to the number of milking ewes in the majority of the flocks. Also, the length of milking stalls was < 90cm in 36% of the flocks and the width was < 35cm in 25% of the flocks. Additionally, in 14% of farms, the diameter of pipes in the milk line was smaller than normal, which resulted in a decrease in vacuum level. Furthermore, only one farmer replaced the liners after 2500 milkings; all others were well above this number. The effect of ewes per milking unit was statistically significant, with the flocks that had more than 11 ewes per unit showing a higher percentage of subclinical mastitis (24.5% to 31.4%, $P=0.012$). The vacuum level measured in clusters differed significantly from that in the vacuum pump (≥ 2 kPa) at two or more measurements in 50% of flocks and at one measurement in 21% of flocks. In case such difference existed at two or more measurements, subclinical mastitis was observed in a significantly higher percentage (33.5%) than in the other cases (24.7%, $P=0.047$).

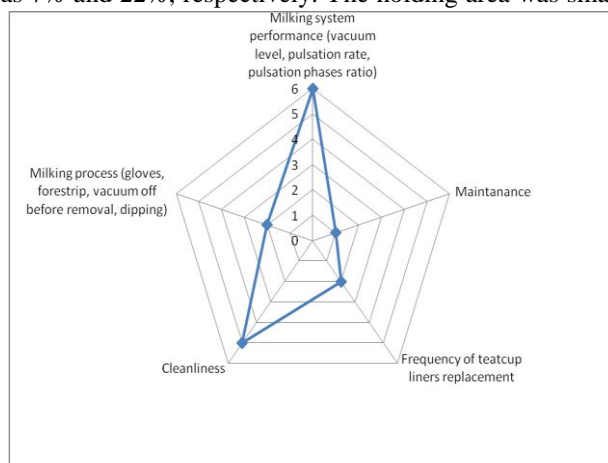


Figure 1 Evaluation of milking procedures and performance

Conclusion Errors in milking process and lack of proper maintenance of the milking parlour increased the prevalence of subclinical mastitis in dairy sheep. Farmers should have the milking parlours serviced by specialists in order to limit any issues that have negative consequences on animal health and welfare.

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Zinc concentration changes in the tissues with supplementation of different zinc sources in growing Baluchi lambs

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Application Supplemental zinc (Zn) is usually added to animal diets in the form of inorganic and organically bound Zn supplements. Feeding growing Baluchi lambs, with higher Zn concentration (100 mg Zn/kg of diet) of zinc supplements resulted in higher accumulation of Zn in the tissues of liver, kidney, muscle, spleen, and testis in lambs.

Introduction Little information is available in the literature regarding the optimum productivity effects of Zn organic and inorganic different sources (NRC, 2007). The objectives of this experiment were to examine the effects of inorganic and organic Zn supplementation on accumulation of Zn in the tissues of growing lambs.

Material and methods Thirty-six male growing lambs with an average weight 27 ± 0.3 kg were used to determine the effects of level of the zinc (Zn) supplementation in diet from inorganic and organic sources on performance of Baluchi lambs, an indigenous sheep breed in Iran. Animals were allocated to six groups, with the control group (C) receiving the basal diet (no zinc supplementation); the other five groups were offered the basal diet supplemented with 50 or 100 ppm Zn from zinc sulfate monohydrate (Zn-S) and Zinc proteinate (Zn-P) and added Zn from Zn-P+Zn-S (50+50 mg/kg, respectively). Ingredients of the basal diet contained (% DM) Alfalfa hay 55%, Barley 20%, corn 15.75%, Soybean meal 1.8%, Cottonseed meal 1.8%, Wheat bran 4.5%, Urea 0.2%, Calcium carbonate 0.45%, Salt 0.2%, and Minerals and vitamins mix 0.5%. Upon termination of experiments, lambs were killed by exsanguination and liver, heart, kidney, lung, muscle, spleen and testis were quantitatively excised. Zinc determinations were performed by a modified method of Parker *et al.* (1967) using a Perkin-Elmer Model 603 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT). The data was analyzed using the mixed procedure of SAS (9.1) for a completely randomized design.

Results (Table 1) showed that the Zn concentration of liver, kidney, muscle, spleen, and testis in lambs fed diets supplemented 100 mg/kg Zn as zinc proteinate, 100 mg/kg Zn as zinc sulfate and the mixture of inorganic and organic Zn were greater ($P < 0.05$) than other groups. The Zn concentrations of heart and lung in lambs on treatments 100 Zn-P and ZnP+Zn-S were higher ($P < 0.05$) than other groups.

Conclusion This experiment concluded that if supplementing inorganic or organic Zn sources with zinc levels lower than 100 mg Zn/kg of the diet then in lambs tissue did not accumulate zinc.

Acknowledgements

The authors wish to acknowledge the financial support received from the Islamic Azad University, Mashhad branch.

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Table 1 Effect of zinc sources on zinc concentration of tissues in lambs (mg/kg)

Tissue	Treatments*						SEM
	C	50 Zn-P	100 Zn-P	50 Zn-S	100 Zn-S	Zn-P+ Zn-S	
Heart	44.3 ^b	49.0 ^b	50.2 ^b	46.1 ^b	53.0 ^a	55.3 ^a	0.05
Kidney	52.1 ^c	54.4 ^b	56.2 ^a	51.9 ^b	55.5 ^a	57.7 ^a	0.03
Liver	42.1 ^b	43.7 ^{ab}	54.8 ^a	50.1 ^{ab}	57.3 ^a	59.2 ^a	0.04
Lung	22.3 ^b	26.0 ^b	48.2 ^a	31.1 ^b	45.0 ^a	55.3 ^a	0.02
Muscle	62.1 ^c	66.2 ^b	71.2 ^a	65.9 ^b	73.5 ^a	77.7 ^a	0.03
Testis	34.3 ^a	39.0 ^b	46.2 ^a	38.1 ^b	43.0 ^a	45.3 ^a	0.05
Spleen	62.1 ^b	65.7 ^{ab}	74.8 ^a	66.1 ^{ab}	77.3 ^a	79.2 ^a	0.04

C, control, Zn-S: zinc sulfate monohydrate, Zn-P: zinc proteinate.

a,b,c Means in the same row lacking a common superscript differ ($P < 0.05$).

Effect of *Acacia erioloba* and *Dichrostachys cinerea* supplementation on doe and kid growth of indigenous Namibian goats

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Application Feed shortage and expensive commercial feeds is a challenge in goat farming. Indigenous woody plants are adapted to harsh environment conditions. Their pods and fruits can be easily collected and used as protein supplements in dry season.

Introduction Indigenous woody plants form a major source of the diet of livestock in arid, semi-arid zones in Africa (Salem and Smith, 2008; Yayneshet *et al.*, 2008). Tree pods and fruits have protein contents that range from 100-190 g/kg and can provide significant protein supplements especially in the dry season but contain anti-nutritional factors such as tannins that could be detrimental to ruminants. Feeding limited quantities of these pods or fruits could increase weight gain and milk production. However, there is inadequate knowledge on the types of pods and their inclusion levels in the diet of lactating does. The aim of the present study was to assess the effect of *Acacia erioloba* (AE) and *Dichrostachys cinerea* (DC) pods on intake and growth rates of Namibian Caprivi and Ovambo does ecotypes.

Material and methods The study was carried out at John Pandeni Research Station under the Ministry of Agriculture, Water and Forestry (MAWF), Grootfontein, Namibia. Forty eight (48) indigenous does aged between 18 - 30 months were used in a 2x3 factorial arrangements of treatments. The main factors were pod type (2; AE and DC) and level of supplementation (3; 20% 40% and 60%). The pods of AE, DC were milled and mixed with Lucerne, and grass hay to obtain protein target between 130-140 g/kg in the ration using try and error method. Seventy nine (79) goat kids from single and twin birth types were used to determine the pre-weaning growth. Does were supplemented on individual units each fitted with a feeding trough. Animals were supplemented with 200 g once a day in the morning 7:00AM before they were allowed out for grazing. Phosphate lick and clean water points were available *ad libitum*. Kids were allowed to overnight with their mothers except the night before weighing. Does and kids weights were recorded once a week. Before the onset of the experiment, does were dipped in Alphamethrin solution to control external parasites and injected with 1% of Dectomax against internal worms. The feeding trial lasted for 91 days and all kids were weaned. Data for live weight and intake of supplement were analysed as a completely randomized design in a 2x3 factorial arrangements of treatments.

Results The results showed that type of supplement significantly ($P < 0.05$) improved intake with DC does having a higher intake. Level of supplementation did not affect intake ($P > 0.05$). Level of supplementation significantly ($P < 0.05$) affected final live-weight of does and weaning weights of kids. Does offered supplement at 40% had higher final weights than does of the other two levels, but there was no difference in weaning weights of kids due to supplement level ($P > 0.05$).

Table 1 Effect of supplementation at different levels on intake and growth of doe and kid

Item	POD TYPE			LEVEL			s.e.d.	Significance		
	AE	DC	s.e.d	20%	40%	60%		S	L	S x L
Supplement intake g DM/d	289.3	307.6	12.99	307.4	338.0	249.8	35.90	*	ns	ns
Supplement intake g/M ^{0.75} /d	69.3	72.9	2.55	73.0	78.5	61.9	6.30	*	ns	ns
Crude protein consumed g/d	47.7	52.1	3.11	53.6	53.7	42.5	6.43	*	ns	ns
Doe final weight, kg	35.6	33.4	0.72	34.8	35.7	31.2	0.86	*	ns	ns
Kid weaning weight, kg	14.3	14.9	0.40	15.0	15.2	13.8	0.48	ns	ns	ns
Kid ADG, g	98.6	102.5	3.45	101.1	105.2	94.5	4.06	ns	ns	ns

AE=*Acacia erioloba*; DC= *Dichrostachys cinerea*; S= supplement; L= level; ADG=average daily gain; *=sig; ns=not sig.

Conclusion Does supplementation with DC at 40% had highest total intake and better kid weights at weaning.

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Effects of high dietary zinc concentration and zinc sources on serum biochemical parameters in growing lambs

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Application Feeding growing lambs, with organic and mixed of inorganic and organic Zn sources, improved some biochemical parameters and concentration of minerals in the serum of lambs. However, insignificant differences of GLU and ALB, TC, TG, and BUN levels between treatments may be due to the transfer of insufficient zinc on synthesis of these metabolites.

Introduction According to NRC Nutrient Requirements of Small Ruminants (2007), the recommended level of zinc for optimum productivity is 20-33 mg Zn/kg in sheep. In practice, feed manufacturers tend to use higher concentrations than those specified by the small ruminant NRC (2007) to achieve optimum productivity performance. The objectives of this experiment were to investigate the response of feeding high concentrations of either organic, inorganic or mixture of inorganic and organic Zn supplements on the biochemical parameters of the blood of growing lambs.

Material and methods Thirty-six male growing Baluchi lambs, an indigenous sheep breed in Iran, with an average weight 26.5 ± 0.3 kg were randomly allocated to one of the dietary treatments. The treatments were: basal diet (no zinc supplementation, BD), other five groups were offered BD supplemented with 50 or 100 ppm Zn from feed-grade ZnSO₄. H₂O (Zn-S) and Zinc Proteinate (Zn-P) and added Zn from Zn-P+Zn-S (50+50 mg/kg), respectively. Ingredients of the basal diet contained (% DM) Alfalfa hay 55%, Barley 20%, corn 15.75%, Soybean meal 1.8%, Cottonseed meal 1.8%, Wheat bran 4.5%, Urea 0.2%, Calcium carbonate 0.45%, Salt 0.2%, and Minerals and vitamins mix 0.5%. Serum samples were analyzed for total protein (TP), glucose (GLU), blood urea nitrogen (BUN), total cholesterol (TC), triglyceride (TG), and albumin (ALB), Concentration of calcium (Ca) and phosphorus (P) by a Selectra-auto analyzer (Vital Scientific NV, DIERN, Netherland). The data was analyzed using the mixed procedure of SAS (9.1) for a completely randomized design.

Results (Table 1) showed that there was a significant difference between groups for TP, Zn, Ca, and P concentrations, although there were no significant differences between treatments in concentrations of GLU and ALB, TC, TG, and BUN levels.

Table 1 Effects of Zn sources on biochemical parameters in lambs

Parameter	Treatments*						SEM
	C	50 Zn-P	100 Zn-P	50 Zn-S	100 Zn-S	Zn-P+ Zn-S	
Glucose (mg/dL)	71.68	77.58	72.75	75.58	73.15	72.08	2.19
Albumin (mg/dL)	3.01	3.41	3.33	3.05	3.35	3.11	1.16
Total protein (g/L)	9.06 ^a	8.55 ^{ab}	8.70 ^{ab}	7.94 ^b	8.59 ^{ab}	8.46 ^{ab}	0.02
Triglyceride (mg/dL)	17.54	15.87	18.55	13.22	14.83	17.41	2.86
Total cholesterol (mg/dL)	47.20	44.85	52.31	44.21	42.52	44.38	2.88
Phosphorus (mg/dL)	4.79 ^{bc}	4.67 ^{bc}	5.20 ^{ab}	4.50 ^c	4.85 ^{abc}	5.45 ^a	0.01
Calcium (mg/dL)	9.82 ^{ab}	9.72 ^{ab}	9.75 ^{ab}	9.44 ^b	10.20 ^a	10.27 ^a	0.04
Zinc (mg/dL)	0.96 ^c	1.09 ^b	1.15 ^a	1.03 ^c	1.09 ^b	1.20 ^a	0.03
Blood Urea Nitrogen (mg/dL)	52.68	53.25	50.53	51.75	50.06	50.34	2.67

C, control, Zn-S: zinc sulphate monohydrate, Zn-P: zinc proteinate.

a,b,c Means in the same row lacking a common superscript differ ($P < 0.05$).

Conclusion In conclusion, feeding Baluchi lambs, with organic and mixed of inorganic and organic Zn, improved some biochemical parameters and minerals in blood serum of lambs. However, in the present study, insignificant differences of GLU and ALB, TC, TG, and BUN levels between treatments may be due to the transfer of insufficient zinc on synthesis of these metabolites.

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Effect of dietary inclusion of cashew nut shell liquid on performance and rumen fermentation in West African dwarf goats

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Application West African dwarf goats had reduced dry matter intake, increased weight, low rumen ammonia nitrogen and no change in rumen total volatile fatty acids with 10-15 ml/kg DM inclusion of cashew nut shell liquid (CNSL) in the diet.

Introduction Rumen fermentation processes have nutritional losses as methane and ammonia which limit efficient nutrient utilization in ruminants. Plant extracts have increasingly been proven as alternatives to feed antibiotics to improve nutritional efficiency but most studies are laboratory based with little information available on animal performance. The aim of the present study was to assess the effects of dietary CNSL inclusion on performance and rumen fermentation in West African dwarf goats.

Material and methods Cashew nut shell liquid was included in a complete concentrate pellet at four dietary treatment levels of 0 (control diet), 5, 10 and 15 ml/kg DM. The concentrate pellets were fed as supplement alongside a basal diet of *Panicum maximum* grass to twenty four West African dwarf goats. The animals (n=24) were balanced for weight and divided into four groups. Each group of animals was allocated to one of the four dietary treatments with six animals per treatment group in a completely randomized design. The grass and concentrate pellets were fed at a ratio of 60: 40, respectively of the animals' daily dry matter requirement. Clean drinking water was supplied to the animals' *ad libitum*. The animals' feed intake were measured on a daily basis while weight changes were taken weekly during a 90-day feeding trial. At day 89 and 90 of the feeding trial, rumen fluid samples (50 ml) were collected from each animal using stomach tubes. The rumen fluid was strained through four layers of cheese cloth, pooled over the collection days and stored frozen in sample bottles for determination of rumen ammonia nitrogen, NH₃-N (AOAC, 2000) and total volatile fatty acids, TVFAs (Barnett and Reid, 1957). 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, dry matter intake was lower in animals fed diets containing 10 – 15 ml CNSL /kg DM than in those fed on the control diet. Dietary CNSL increased weight gain in animals at inclusion rate of 10 - 15ml/kg DM. Percentage ammonia nitrogen concentration in the rumen reduced with inclusion of 10 – 15 ml/kg CNSL while the total volatile fatty acid was not significantly altered with CNSL inclusion in the diet.

Table 1 Effect of dietary inclusion of CNSL on animal performance and rumen fermentation

	Level of CNSL inclusion in concentrate pellets				s.e.m	P
	0 ml/kg DM	5 ml/kg DM	10 ml/kg DM	15 ml/kg DM		
Average dry matter intake (g/d)	600 ^a	580 ^{ab}	525 ^{bc}	510 ^c	13.43	0.017
Average final live weight (kg)	9.6 ^b	10.2 ^b	11.5 ^a	12.2 ^a	0.35	0.004
Average daily weight gain (g)	20.0 ^b	33.3 ^{ab}	38.9 ^a	42.2 ^a	3.23	0.039
Rumen NH ₃ -N (mg/100 ml)	12.0 ^a	10.8 ^{ab}	9.6 ^{bc}	8.5 ^c	0.45	0.006
Total VFAs (mM/100 ml)	5.8	6.2	6.5	7.0	0.24	n.s.

Conclusion The reduced dry matter intake and increased weight gain in animals fed 10 – 15 ml/kg DM of dietary CNSL indicates the potential of CNSL to improve animal's efficiency to convert feed to appreciable weight gain. Reduction in level of ammonia nitrogen in the rumen at 10 – 15 ml/kg DM CNSL inclusion suggests a reduction in dietary protein degradation in the rumen which signifies better utilization of protein at the lower tract.

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Influence of feed restriction on the dairy goat mammary gland proteome: a study in two breeds with different levels of tolerance to weight loss

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Application The use of high-throughput proteomics is of high importance to the establishment of weight loss tolerance biomarkers. These are of use in the establishment of adequate dairy goat selection strategies in the tropics. Seasonal weight loss tolerance in mammary goats was found to be related to the expression of immunity system proteins.

Introduction Seasonal weight loss (SWL) is one of the many constraints to animal production in tropical regions. In this work, we used two different goat breeds from the Canary Islands (Spain) with common ancestors and contrasting levels of adaptation to SWL: Majorera (adapted to SWL) and Palmera breed (not adapted to SWL) as described (Lerías *et al.*, 2013). In pasture conditions, Majorera goats are known to maintain their productive levels whereas Palmeras tend to experience rapid production decreases. Accordingly, Majorera animals have been exported to the Sahel regions of Mauritania to increase production levels of local goats (Lerías *et al.*, 2015). The ultimate objective of this work was to study the effect of SWL on the mammary gland secretory tissue proteome in these two goat breeds.

Material and methods Within each breed, goats with the same age and stage of lactation were divided into two groups: control (constant weight) and underfed (15% liveweight reduction): PC (Palmera Control), MC (Majorera Control), PE (Palmera Experimental) and ME (Majorera Experimental). Five animals were used in the Majorera groups and 4 animals were used in the Palmera groups. At day 22, mammary gland biopsy samples were extracted. Samples (100 µg) were added to 500 µl of ammonium bicarbonate 50 mM, urea 8M, thiourea 2M buffer and homogenized, centrifuged and supernatant recovered. Per sample, 15 µg of proteins were trypsin digested (FASP protocol) and desalted. Peptides were loaded onto reverse-phase C18 columns and analysed on an LTQ-Orbitrap Velos mass spectrometer. Protein identification and label free quantification were performed using Mascot (Matrixscience) and Progenesis software (Nonlinear Dynamics).

Results A total of 1010 proteins were identified, from which 96 proteins were considered statistically different (fold change > 1.98 and P<0.05) between two of the possible comparisons between the four experimental groups: PC vs PE: 63 proteins; MC vs ME: 57 proteins; MC vs PC: 13 proteins and ME vs PE: 19 proteins. After SWL, there was an increase of proteins related to apoptosis and stress processes in both breeds. Moreover, both breeds showed a decrease in the number of proteins related to protein, carbohydrates and fat biosynthesis. When both breeds were compared after SWL, the Majorera breed showed higher expression of immune system related proteins compared to Palmera breed. In contrast, Palmera breed showed higher expression of proteins related to apoptosis, ketone bodies formation (Fat liver) and protein metabolic processes compared to Majorera breed.

Conclusion The two goat breeds have a different metabolism reaction to SWL, highlighting differences particularly related to the immune system (higher expression in the tolerant breed) and apoptosis (higher expression in the susceptible breed).

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Comparison of composite and traditional sheep breeds using a range of key performance indicators to identify areas of economic impact

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Application The use of composite genetics as a way of introducing increased efficiency is an option available to lowland sheep farmers however the economic impact of their use has not been fully investigated.

Introduction There is a wide variety of breeds and crosses available for use within lowland prime lamb production, with increasing emphasis being placed on the value of maternal genetics. There is great reliance on the mule, a cross breed that combines the fertility of an upland breed with the hardiness and mothering characteristics seen in a variety of hill breeds. New alternatives exist in the form of composite breeds that enable them to select for desirable maternal characteristics such as smaller ewes with lower maintenance requirements without compromising on performance, and sires that have a greater output when compared to traditional breeds. The aim of this study was to compare KPIs such as live births, lamb mortality, daily liveweight gain (DLWG) and carcass information for lambs born to Highlander (H) ewes or mule (M) ewes when crossed with either Primera (P), Suffolk (S) or Texel (T) tups.

Material and methods 271 Ewes were randomly allocated into tup groups having been balanced for condition score, age and breed. Tup groups were as follows: PxH, SxH, TxH, PxM, SxM and Tx M. The resulting 556 lamb crosses were recorded for birth weight, weaning weight, live slaughter weight, deadweight, carcass grade and carcass price. Still births and lamb mortality were also recorded. All lambs were reared on forage alone and subjected to the same management strategy for gut parasite control, lameness and trace element supplementation. On assessment as being fit for slaughter individual carcass reports were obtained. Statistical analysis using ANOVA was carried out to establish effects of dam, sire and dam x sire effects on lamb performance. KPI data was compared to Eblex benchmark data and where appropriate raw data was converted to 'per 100 ewes put to tup' to aid comparison.

Results H had significantly more live lambs than M, but also experienced significantly higher mortality rates in the first 48 hours. Despite this significantly more lambs born to H were sold finished. DLWG to weaning for H born lambs was not compromised despite more lambs, and DLWGs post weaning for H born lambs were significantly higher than for those born to M. H produced more kg of lamb and returned more money per kg of ewe than M born lambs for all sires except S. P and S sired lambs produced significantly more kg lamb/kg ewe than other sires. T sired lamb had generally better carcass grades (data not shown) however gave some of the poorest returns. Key results are presented in Table 1. Data obtained from subsequent years of this project (2014 and 2015) show similar results however are not yet complete (data not shown).

Table 1 Comparison of breed crosses for a variety of KPIs and where appropriate Eblex industry benchmarks (2013)

* Per 100 ewes to tup	Lambs born alive*	Lambs dead in 1 st 48 hrs*	Lambs dead in >48 hrs*	Lambs sold*	DLWG birth – weaning	DLWG weaning - slaughter	£ lamb (DW)/kg ewe	kg lamb (DW) per kg ewe
BM ⁺	165	5	4	157				
PxH	228 ^b	4 ^a	16 ^b	208 ^b	0.32 ^a	0.12 ^b	2.24 ^c	0.66 ^b
PxM	203 ^a	7 ^a	15 ^b	178 ^b	0.33 ^a	0.06 ^a	1.03 ^b	0.56 ^a
SxH	244 ^c	33 ^c	0 ^a	211 ^b	0.38 ^b	0.15 ^b	2.62 ^c	0.67 ^b
SxM	194 ^a	7 ^a	13 ^b	173 ^a	0.35 ^a	0.06 ^a	2.45 ^c	0.55 ^a
TxH	224 ^b	38 ^c	17 ^b	166 ^a	0.33 ^a	0.09 ^a	2.14 ^c	0.52 ^a
TxM	204 ^a	16 ^b	20 ^b	169 ^a	0.34 ^a	0.06 ^a	0.77 ^a	0.54 ^a
SEM	0.09	0.06	0.05	0.10	0.01	0.01	0.08	0.04

^{abc} Indicates a significant difference (P<0.05) within column ⁺ Top third benchmark taken from Eblex Stocktake 2013 for lowland lamb production

Conclusion H ewes out-performed M in production KPIs however lamb mortality was significantly higher. There was insufficient detail to adequately explain mortality rates and is the subject of continuing investigation. PxM produced crosses that grew too big before finishing (data not shown). Fluctuations in market price have inevitably influenced returns and are subject to further analysis incorporating days on farm data. Initial analysis shows that H ewes appear to offer a more cost effective and harder working ewe compared to M under this management regime. Results for P tups would suggest a need for careful use to prevent against over-sized finished lambs.

Genomic regions underlying bovine tuberculosis resistance in Holstein Friesian dairy cattle

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Application Genomic regions harbouring genes associated with resistance to bovine tuberculosis were identified. This information may be used to develop breeding strategies to enhance disease resistance.

Introduction Bovine tuberculosis (bTB) has resulted in high social and economic losses in the UK despite longstanding eradication schemes. However, within animal variation in cattle susceptibility to the disease has led to the exploration of alternative control methods, one of which is selecting and breeding cattle for bTB resistance to augment existing control measures. Although many genome-wide association studies (GWAS) aiming at identifying genes underlying cattle resistance to bTB have been undertaken in different populations, none to date has identified any major common quantitative trait locus. The objective of the present study was to conduct a genomic analysis for bTB resistance in Holstein Friesian dairy cattle in Great Britain.

Material and methods Data consisted of genetic evaluation results for bTB resistance that had been based on skin test and *post mortem* examination records; the latter were provided by the Animal and Plant Health Agency. De-regressed estimated breeding values (EBVs) of Holstein Friesian bulls were calculated as per the procedure of Garrick *et al.* (2009) and 804 of these bulls were genotyped with the 50K Illumina Bovine chip. A total of 56,134 SNPs were available per animal. After quality control, where SNPs with MAF <0.02, SNP or animal call rate <0.95 and deviation from HWE <10⁻⁶ were excluded, 36,643 SNPs and 799 individual bulls were kept. De-regressed EBVs of these bulls and the genomic relationship matrix were included in GWAS using the GenABEL software (Aulchenko *et al.* 2007). In addition, Regional Heritability Mapping was conducted considering genomic regions comprising 100 SNPs per 'window', overlapping by 50 SNPs (Nagamine *et al.* 2012). Gene annotation was based on the *Bos_taurus_UMD_3.1.1* (<http://www.ncbi.nlm.nih.gov/genome/82>).

Results Two SNPs on chromosomes 2 and 11 were significantly associated with bTB at chromosome-wide significance level (Figure 1). The estimated polygenic heritability for resistance to bTB was 0.39 ± 0.07, while the proportion of variance explained by the two SNPs was 0.02 and 0.11, respectively. A genomic region consisting of 100 SNPs, also found on chromosome 2, had suggestive association with bTB resistance. This region accounted for 0.04 of the total variance. The most plausible candidate gene falling within this region, integrin alpha 4, is a protein coding gene previously associated with monocyte count in white blood cells, which play an important role in response to inflammation.

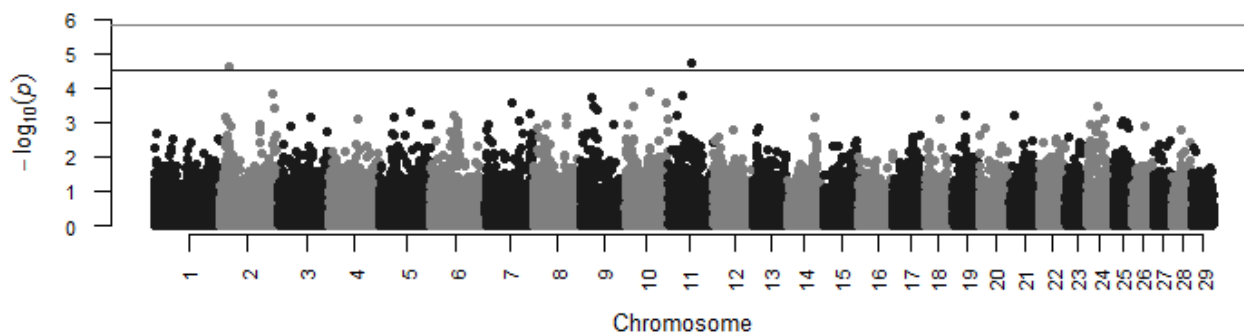


Figure 1 Manhattan plot displaying $-\log_{10}$ of P-values of association of each SNP to the phenotype.

Conclusion Genomic regions associated with bTB resistance and a plausible candidate gene were identified, offering the potential to assist in understanding the pathways critical to cattle resistance to bTB. Validation of these regions and associated genes with independent data is warranted.

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Polymorphisms in genes regulating aspects of innate immunity are associated with life expectancy in a population of UK Holstein dairy cows

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Application Life expectancy was correlated with coding differences in genes with key roles in innate immunity. Selective breeding of cows for a “better” innate immune response is a promising way to reduce disease incidence in dairy populations.

Introduction Mastitis, infertility and lameness are the three major reasons for involuntary culling of dairy cows. These conditions all reduce life expectancy in the herd, which is in turn a major determinant of profitability (Kossaibati and Esslemont, 2000). We initially undertook a review of the literature and databases to identify key genes involved in the regulation of innate immune responses which are associated with the risk of a cow having a raised somatic cell count (SCC) or developing clinical mastitis (Chen *et al.* 2015). This highlighted a number of genes relating to granulocyte adhesion and diapedesis signaling and the ephrin receptor signaling pathway. These genes are important regulators of the early innate immune response by controlling granulocyte recruitment and migration to infected tissues and subsequently an inflammatory response. The candidate gene list included *SELE*, *SELP*, *SELL* (all adhesion molecules involved in granulocyte diapedesis), *TREMI* (encoding an Ig superfamily receptor expressed on myeloid cells such as granulocytes and important for amplifying inflammatory responses) and *RelA* (a member of the NF-kappaB family of transcription factors which co-ordinate regulation of host defense). The SNP rs41579632 was also studied as it had previously been associated with clinical mastitis in Norwegian Red cattle (Sodeland *et al.*, 2011).

Material and methods DNA from a well characterised population of 337 Holstein cows from 19 UK herds (Brickell and Wathes, 2011) was used to sequence a number of candidate genes with a known role in innate immunity and to determine SNP incidence. Associations between genotype, SCC, diseases recorded in the first lactation and survival time in the herd were calculated. SCC was taken as either a quantitative (mean lactation SCC) or binary trait (affected v unaffected; SCCbin). All analyses were performed using a 3 x 2 (genotype by status) chi-squared model except for the mean SCC analysis which was carried out on environmental residuals (Aulchenko *et al.*, 2007) with an allele substitution model in PLINK. Fisher’s exact test was used for binary traits. Differences between genotypes in the life expectancy of animals with each SNP were measured by Kaplan-Meier survival analysis. Censored animals were those that survived until the end of their second lactation. The proportions of cows censored were compared using the Cox proportional hazards regression model.

Results The initial sequencing identified 8 SNP in *SELP*, 3 in *SELL*, 1 in *SELE*, 2 in *TREMI* and 1 in *RelA*. From these, 5 SNP in *SELP*, 2 in *SELL*, 1 in *SELE*, 2 in *TREMI* and 1 in *RelA* in addition to rs41579632 were taken forward for analysis, giving a total of 12 SNP. None of the SNP tested showed significant associations with SCC by any of the analytical methods used ($P > 0.1$), apart from *RelA*-intron5 with SCCbin ($P = 0.08$). Two SNP in *SELP* in exons 4 and 8 demonstrated significant differences between genotypes with a variety of health traits including mastitis, uterine disease and lameness ($P = 0.01-0.05$). *SELL*-EX3 was associated with uterine disease ($P = 0.02$). As results on health traits indicated that the same SNP were associated with several disparate diseases, a survival analysis was next performed. Three SNP (*SELP*-EXON4, *TREMI*-UTR and *RelA*-intron5) all showed significant associations with survival to the third lactation ($P = 0.02, 0.04$ and 0.003 respectively). In all cases the differences in life expectancy according to genotype became more pronounced from about 4 years of age, when most cows would be in their second lactation.

Conclusion Our results support the concept that coding differences in *SELP*, *SELL*, *TREMI* and *RelA* can impact on the life expectancy of Holstein dairy cows, potentially through altered functions of granulocytes, the first line of cells involved in the defence to invading pathogens. Functional differences in this cell type may impact on the susceptibility of the host to a variety of diseases which lead to earlier culling, particularly from the second lactation onwards.

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Phenotypic and genetic correlations between age at first kidding and 305 day milk yield in dairy goats

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Application This study estimated the heritability (h^2) of age at first kidding in mixed breed UK dairy goats, as well as the phenotypic and genetic correlations between this trait and 305 day milk yield for the first and second lactation. The results suggest that age at first kidding has a low h^2 and a low positive phenotypic correlation with 305 day milk yield in the first lactation, however this relationship is not observed at a genetic level.

Introduction Recently, much progress has been made in UK dairy goat breeding programs but much of the focus has been on producing breeding values for milk yield. In dairy cattle, unfavourable genetic correlations have been found between milk yield, and other traits such as fertility and mastitis. In order to develop a selection index suitably weighted for a variety of traits that will lead to optimum improvement, it is important to understand the genetic relationships between production, health, and fertility traits. Estimation of h^2 and correlations with other traits of interest is an essential first step towards incorporating multiple traits into a breeding index. This study estimated the h^2 of age at first kidding (AFK), as well as genetic and phenotypic correlations between AFK and 305 day milk yield (MY) in lactations 1 (L1) and 2 (L2).

Material and methods Records from 2,434 dairy goats on 1 UK dairy farm on 2 sites born between 2007 and 2012 were utilised. Goats were a composite of 3 breeds: Alpine, Saanen, and Toggenburg, and were highly related on the 2 sites. Heritabilities, as well as phenotypic, residual, and genetic correlations were estimated between AFK, and MY (L1 and L2). Milk yields were calculated as per Gantner *et al.* (2008). Animals with a minimum of 3 records covering at least 90 days in milk were included in the analyses. Daily yield records below 0.2 and above 12 kg were assumed to be anomalies and were excluded from the dataset. All animals included in the analyses had milk records from both lactations. For AFK and MY, records above or below 3 SD of the mean were excluded. Univariate analyses were used to estimate breeding values for all traits using the following animal model: $y = Xb + Za + e$ where the incidence matrices X and Z relate phenotypic records contained in vector y to fixed (b), additive genetic (a) effects, and vector e represents residual error. Farm, year of birth, and season of birth were included as fixed effects, and the animal was fitted as a random effect for both AFK and MY, while age of dam at birth was fitted as a covariate for AFK. All fixed effects were found to be significant ($P < 0.05$) and were therefore retained in the model. A multivariate analysis was used to calculate correlations between AFK and MY (L1 and L2). Fixed and random effects were fitted for each trait based on the univariate analyses as described previously.

Results Following quality control, mean AFK was 464 ± 91.4 days, while mean MY for L1 and L2 were $1,168 \pm 318.5$ and $1,247 \pm 427$ kg, respectively. Heritabilities for AFK, MY L1, and MY L2 for were 0.11 ± 0.04 , 0.32 ± 0.05 , and 0.18 ± 0.05 , respectively. The estimated h^2 of AFK was lower than those calculated in US (0.23; García-Peniche *et al.* 2012), Polish (0.13; Bagnicka *et al.* 2007), and Mexican dairy goat populations (0.31; Torres-Vázquez *et al.* 2009). In turn, the phenotypic, residual, and genetic correlations between AFK and MY were 0.15 ± 0.02 , 0.21 ± 0.04 , and -0.08 ± 0.19 for L1, and -0.07 ± 0.02 , -0.07 ± 0.04 , and -0.07 ± 0.22 for L2. A genetic correlation of -0.18 between AFK and MY has previously been reported, however this estimate was also associated with a high SE (0.23). The genetic correlation between lactations for MY was 0.60 ± 0.11 . The results presented here suggest a low, positive phenotypic correlation between AFK and L1 MY, but not L2. As the genetic correlation did not significantly differ from zero, this association must be due to the environmental correlation observed. It may be that more mature does are physically able to produce more milk, or can allocate more resources to milk production, due to reduced growth rate at this time. This would explain the lack of phenotypic correlation at L2. Goats that readily breed at a younger age are desirable, as it offers the opportunity for more rapid genetic improvement, as well as reducing the amount of time and resources required to raise the animals to producing age. However, the phenotypic correlations presented here suggest there may be a trade-off between age at first kidding and productivity in L1. The disadvantage of the present study is that it utilised 305 day MY, a standard based on dairy cattle, which typically have shorter lactation periods than dairy goats. It would be of interest to further explore how age at first kidding relates to milk yield across the duration of L1, as well as lifetime productivity.

Conclusion Heritabilities suggest that age at first kidding would respond to selection. The current results suggest that this could be achieved without adversely affecting 305 day MY. Positive phenotypic correlations suggest that later lactation is associated with improved performance, however more work is required to determine whether this is related to lifetime performance, and to calculate a marginal economic value to apply to this trait.

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Genomic selection for conformation traits in UK dairy goats

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Application This paper presents the first implementation of genomic selection for conformation in UK dairy goats. This is to ensure that improvements in productivity are accompanied by improvements in functional conformation traits relating to locomotion and milking.

Introduction Conformation traits are of interest to dairy goat breeders not only as descriptive traits, but also because of their influence on production, longevity and profitability. As these traits often have low heritability (McLaren *et al.*, 2016) and are expensive to score, the application of genomic technology could potentially offer significant benefits for breeders. The objective of this study was to estimate genomic breeding values for 9 conformation traits (from groups of udder, teat, legs, and feet).

Material and methods The research was based on data provided by two sites of one commercial goat farm in the UK comprising scores for udder (udder furrow, udder depth, udder attachment), teat (teat shape, teat angle, teat placement), back legs and feet (front feet, and back feet) conformation on 6,723 goats. The pedigree file contained 37,462 individuals. In total 4518 animals with phenotypes were genotyped with the Illumina 50K caprine chip. The training population had between 76 and 498 animals (trait dependent), whereas validation populations had between 22 and 896 animals. Genomic breeding values were estimated using single-step BLUP (Legarra *et al.*, 2009; Misztal *et al.*, 2009). The software package BLUPf90 (Misztal *et al.*, 2002) was used to fit the following model: $\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e}$

where: \mathbf{y} = vector of observations for the analysed conformation score; \mathbf{b} = vector of fixed effects: farm, lactation stage, birth year, scoring year and scorer; \mathbf{a} = vector of random additive animal effects; \mathbf{e} = vector of random residuals. \mathbf{X} and \mathbf{Z} = incidence matrices. Random effects were assumed to be normally distributed with zero means and the following covariance

$$\text{structure: } \text{Var} \begin{bmatrix} \mathbf{a} \\ \mathbf{e} \end{bmatrix} = \begin{bmatrix} \mathbf{H}\sigma_a^2 & 0 \\ 0 & \mathbf{I}\sigma_e^2 \end{bmatrix}$$

where \mathbf{I} is an identity matrix, and \mathbf{H} is a relationship matrix calculated by blending Van Raden's (2008) genomic relationship matrix \mathbf{G} and pedigree relationship matrix \mathbf{A} . The model was parameterised with (co) variance components estimated in a previous study (McLaren *et al.*, 2016). Accuracy of genomic predictions was calculated as a correlation between the GEBVs and de-regressed EBVs for a given conformation trait.

Results Accuracy of genomic breeding values was between 0.23-0.39 for udder, 0.18-0.48 for teat, 0.04-0.55 for legs and feet conformation. The respective regression coefficients were between 0.13-0.35 for udder, 0.15-0.52 for teat, and 0.05-0.61 for legs and feet conformation. Accuracy obtained for the analysed conformation traits was lower than the accuracy of GEBVs for milk yield of 0.61 (Mucha *et al.*, 2015). This is mainly due to small size of the reference population and lower heritability of the traits in the current study.

Conclusion This is the first estimation of genomic breeding values for conformation traits in UK dairy goats. Due to the small size of the reference population for the conformation traits, these preliminary results should be treated with caution and will be re-estimated as the reference population expands towards 12,000 goats and more phenotypes become available. Current results indicate that the single-step methodology maximises accuracy, which is important particularly for traits with lower heritability and with a small reference population.

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Detection and utility of ancestral haplotypes in cattle breeds

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Application The study of ancestral haplotypes identifies regional variants of potential interest in selection programmes to improve productivity and fitness. It also provides information regarding population history to be used in management.

Introduction In cattle, intense selection has led to the development of strong linkage disequilibrium with different patterns across breeds. The analysis of ancestral haplotypes is expected to provide information about the evolutionary history of diverse populations and identify regions of common interest for both conservation and breeding programmes. The main objective of the present study was to develop a simple procedure to detect ancestral haplotypes in populations with genetic links. The procedure was applied to Fleckvieh, Simmental and Brown-Swiss population data. Regions of common interest were identified and patterns of the population history and dynamics were assessed.

Material and methods

Fleckvieh/Simmental dataset: 473 bulls genotyped for 714,759 Single Nucleotide Polymorphisms (SNPs) with Fleckvieh (315 bulls) and Simmental (158 bulls) being treated as different populations with up to 7 generations.

Brown-Swiss dataset: 412 bulls genotyped for 714,759 SNPs and with each of the five involved countries considered as a different population: Austria (21 bulls, 6 generations), Germany (54 bulls, 6 generations), Italy (77 bulls, 7 generations), Switzerland (184 bulls, 7 generations) and USA (77 bulls, 7 generations).

Analysis: Genotypes were phased using clusters of 100 SNPs. Within each population, haplotypes in the base (oldest) generation were identified in each cluster and considered as ancestral. In subsequent generations, ancestral haplotype frequency and average similarity (molecular co-ancestry) was estimated and averaged across all clusters. Linear regression was used to assess trends in frequency and co-ancestry across time. Haplotype variability was computed as the average number of haplotypes identified per cluster, and ancestral haplotypes identified in a given population were also traced in others to assess the shared percentage.

Results A higher haplotype variability (71.55) and reduced co-ancestry trend ($b=7.43 \times 10^{-5}$) was observed for Fleckvieh than for Simmental (44.98 and $b=4.18 \times 10^{-4}$, respectively). This is contrary to what might have been expected from the origin of Fleckvieh (imported nucleus of Simmental cattle into Germany and Austria). Possible explanations are the enrichment of Simmental haplotypes with local breed haplotypes during the formation of the Fleckvieh breed and an unbalanced genetic flow between the two populations. Both explanations are concordant with the history of Fleckvieh.

The Brown-Swiss breed originated in Switzerland and was exported to USA in 1869, where it was intensively selected and exported back into Italy, Germany and Austria. In concordance, the Swiss population showed the highest variability (37.22) and a low proportion of shared ancestral haplotypes with other European populations ($5.8\% \pm 1.09$), but also a high shared proportion with the USA population (33.19%). Furthermore, the non-Swiss European populations and the USA population had similar proportions of shared ancestral haplotypes ($9.33\% \pm 1.76$ and $11.58\% \pm 1.98$, respectively).

The analysis of the most common ancestral haplotypes (Table 1) also revealed selection signatures in the populations, in agreement with previous studies (Mészáros *et al.*, 2015; Stella *et al.*, 2010) and showing interesting genes closer than 0.3 Mb of the most frequent haplotypes.

Table 1 Analysis of the top 5 common ancestral haplotypes in Fleckvieh (Fleck), Simmental (Sim) and Brown-Swiss (BS)

Chr	Breeds (number of haplotypes)	Related genes (function)
5	Sim (2), BS (2)	SYT10 (longevity/maturity), PMEL and ERBB5 (coat colour)
6	Fleck (3), Sim (3), BS (5)	MEP6, ISBP, LAP3, MED28 (milk protein/fat) and KIT (coat colour)
7	Fleck (2)	Previous QTL detected for milk production
21	BS (1)	MEF2A (milk production)

Conclusion Detection of ancestral haplotypes has led to the identification of signatures of selection in three common cattle breeds, mirroring also their population dynamics.

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Random regression analysis to determine herd profiles for carcass weight in UK beef cattle

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Application Knowledge of the variability in herd performance across years, by drawing inferences from carcass weight at slaughter, could be used as a management tool to monitor herd performance.

Introduction Random regression models can provide information of the genetic variability in carcass weight across a given time trajectory defined by age at slaughter. Random regression models also allow for modelling of herd-specific deviations to describe differences in growth curves across herds (De Roos *et al.*, 2004). The objective of the present study was to determine growth curve and herd curve parameters, using a random regression model, for carcass weight measured on young bulls across multiple breeds.

Material and methods Carcass trait information was available on 58,655 bulls from 2,387 UK herds slaughtered between 2002 and 2012. Records from bulls slaughtered <12 months or >24 months of age were discarded as were bulls with no known sire or dam. Records outside ± 4 standard deviations from the mean carcass weight or age at slaughter were discarded. Dam records were restricted to parity 1 to 10, and parity was categorised as 1, 2, 3, 4, and ≥ 5 . Only records from sires with at least 5 progeny records were retained. Two contemporary groups were defined 1) abattoir-date of slaughter, and 2) finishing herd-year of slaughter; only contemporary groups with five or more records were retained. Following edits, 22,971 young bulls from 2,239 sires remained. A random regression model was fitted in ASReml (Gilmour *et al.*, 2009). A fixed effect of age at slaughter was modelled with a Legendre polynomial and reflected the fixed curve. Other fixed effects included breed of sire and breed of dam, parity of the dam, contemporary group of abattoir-date of slaughter as well as heterosis and recombination coefficients of the animal. Random effects included the sire of the animal and the contemporary group of finishing herd-year of slaughter; both were modelled across age at slaughter with Legendre polynomials. Random effects reflected individual deviations from the fixed curve. The random residual term was modelled as heterogeneous across age at slaughter classes. Covariance function coefficients were estimated using the variance covariance matrix and the matrix of Legendre polynomials: $G = \Phi K \Phi$ where G is the variance covariance matrix for slaughter ages, Φ is the matrix of Legendre polynomial age regression coefficients, and K is the estimated variance covariance matrix of the random polynomial coefficients K_a or K_{hy} ; K_{hy} is the estimated finishing herd-year of slaughter variance covariance matrix; K_a is the estimated genetic variance covariance matrix multiplied by four to transform from a sire variance to a genetic variance.

Results The average (standard deviation in parentheses) carcass weight and age at slaughter across the data was 354.3 (52.2) kg and 454.8 (51.4) days, respectively. A random quadratic Legendre polynomial on sire and a random linear Legendre polynomial on finishing herd-year of slaughter fitted the data best. The heritability of carcass weight (standard error in parentheses) was least (i.e., 0.19; se=0.04) at 673 days of age and greatest (0.25; se=0.02) at 536 days of age indicating that sufficient genetic variation exists for selection on this trait (Fig. 1). Finishing herd curve variance (standard error in parentheses) ranged from 802.63 (70.98) units² to 1594.90 (172.19) units² for carcass weight (Fig. 2). Analysis of eigenvalues of finishing herd-year covariance across age at slaughter, revealed considerable variation between herds in their growth curve parameters for carcass weight.

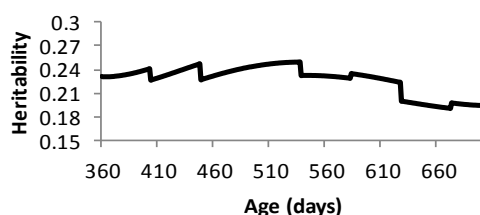


Figure 1 Estimated animal genetic variances for carcass weight across age at slaughter. Standard errors ranged from 0.02 to 0.06.

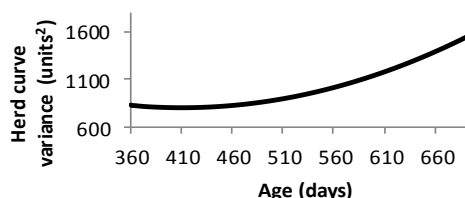


Figure 2 Herd curve variance estimates for carcass weight across age at slaughter. Standard errors ranged from 68.93 to 172.19.

Conclusion Random regression models can be used to model herd variance in carcass weight across an age trajectory. Considerable variation exists between herds in their growth curve parameters for carcass weight, reflecting different management practices.

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Genetic parameters of lamb survival for Dorset, Lleyn and Texel sheep

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Application The proportion of Dorset, Lleyn and Texel lambs unaccounted for, and therefore deemed not to have survived up to 8 weeks of age, ranged from 10%–13%. Lamb survival is a heritable trait in all three breeds indicating that genetic improvement for this trait is possible.

Introduction A key factor affecting the profitability of sheep farms is lamb survival. In order to investigate the potential for including this trait in future breeding programmes, the heritability of lamb survival was investigated using data collected from Dorset, Lleyn and Texel lambs.

Material and methods Lamb survival was considered as a binary trait, measured as a trait of the lamb, not as a trait of the ewe. Lambs were coded 0 if they did not have an 8- or 21-week weight recorded or 1 otherwise. The data used were from performance recording flocks throughout the UK and contained survival records for 15,433 (from 20 flocks), 51,174 (from 47 flocks) and 48,995 (from 108 flocks) Dorset, Lleyn and Texel lambs, born between 2003–2013, respectively. Inconsistencies in the level of recording associated with this trait were observed therefore data from flocks with less than 5% of their lambs coded 0 were removed. Univariate analyses, using ASReml (Gilmour et. al, 2009), were carried out using the following models to determine the genetic and permanent environmental maternal effects:

$$\text{i) } \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}; \quad \text{ii) } \mathbf{y} = \mathbf{Xb} + \mathbf{Z}_1\mathbf{a} + \mathbf{Z}_2\mathbf{m} + \mathbf{e}; \quad \text{iii) } \mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Zp} + \mathbf{e}$$

where: \mathbf{y} - vector of observations for lamb survival; \mathbf{b} - vector of fixed effects: litter size at birth, sex, farm, birth year, dam age, lamb birth weight (treated as a covariate), month of birth and the interaction between month and birth year; \mathbf{a} - vector of random direct genetic effects; \mathbf{m} - vector of random maternal genetic effects; \mathbf{p} - vector of random permanent environment effects of the dam; \mathbf{e} - vector of random residuals. \mathbf{X} and \mathbf{Z} - incidence matrices.

Due to the binary nature of the trait, the initial heritabilities estimated were transformed to an assumed underlying continuous and normally distributed scale, using the method described by Dempster and Lerner, (1950).

Results The overall proportion of lambs unaccounted for, and therefore assigned to code 0, were 13.0%, 10.6% and 13.4% for the Dorsets, Lleyns and Texels respectively. Direct heritability estimates, once transformed, were low for all three breeds ranging from 0.04 – 0.08. Maternal heritability and permanent environment estimates ranged from 0.004 – 0.02 and 0.01 – 0.02 respectively (Table 1).

Table 1 Direct (h^2), maternal (h^2_m) and permanent environment (h^2_{pe}) heritability estimates for Dorset, Lleyn and Texel lamb survival

Breed	Model	h^2	Transformed h^2	h^2_m	Transformed h^2_m	h^2_{pe}	Transformed h^2_{pe}
Dorset	i)	0.21 (0.02)	0.08				
	ii)	0.20 (0.03)	0.07	0.01 (0.02)	0.004		
	iii)	0.18 (0.03)	0.07			0.03 (0.02)	0.01
Lleyn	i)	0.16 (0.01)	0.05				
	ii)	0.12 (0.01)	0.04	0.05 (0.01)	0.02		
	iii)	0.12 (0.01)	0.04			0.06 (0.01)	0.02
Texel	i)	0.21 (0.01)	0.08				
	ii)	0.18 (0.01)	0.07	0.06 (0.01)	0.02		
	iii)	0.17 (0.01)	0.06			0.04 (0.01)	0.02

Conclusion Lamb survival has a low genetic basis for all three breeds estimated. Nonetheless, it would still be important to include lamb survival as a breeding goal to improve animal resilience and identify families with good survival. Improvements in the level of recording of this trait would also be beneficial.

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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Comparative maternal performance of high and low replacement index beef cows

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Application Availability of maternal genetic evaluations should enable breeders to improve maternal efficiency of the beef suckler cow herd by selecting superior cows for maternal traits.

Introduction The ideal suckler cow is one that has a good milk yield, maintains a 365 day calving interval and produces a live calf each year. Weanling output is a key indicator of a cow's maternal performance as her milking capability is the driving force of calf weaning weight (Boggs *et al.*, 1980). Animals identified as high genetic merit for maternal traits through the replacement index should be superior for milk production and fertility traits, therefore driving the overall system performance and ultimately profitability. Consequently, the objective of this study, carried out at Teagasc Grange, Dunsany, Co. Meath, Ireland, was to compare the performance of high and low replacement index cows during their first and second lactation.

Material and methods Data were available from 179 cows and their progeny over two years; 101 and 78 cows of high and low index, respectively. Animals were selected from high reliability (>70%) Angus and Limousin sires on the basis of their replacement index. The replacement index for high and low index cows were €119 and €50, respectively. Cows calved for the first time at 24 months of age. Mean calving date was March 22. Animals were managed under a rotational grazing system with an average pre- and post- grazing height of 11.6 (s.d. 1.9) cm and 4.1 (s.d. 0.8) cm, respectively. Cow and calf live weight and cow body condition score (BCS; 0 to 5) were recorded every three weeks. The weigh-suckle-weigh technique was used at 120 and 156 days in milk in 2014 and 50, 117 and 184 days in milk in 2015 to establish cow milk yield during the grazing season (McGee *et al.*, 2005). Gradual weaning was completed over a five day period in both years. Calf weight was recorded at weaning and calves were valued by three experienced assessors. Calf quality was determined on a 1 to 5 scale as outlined in the DAFM Suckler Cow Welfare Scheme (ICBF, 2008). The effect of cow genetic merit on cow and calf performance was analysed using a mixed model in PROC HPMIXED (SAS Inst. Inc., Cary, NC). Fixed effects included for all traits were genetic merit of the cow, year and parity. Depending on the trait under investigation (i.e. cow or calf trait) the heterosis coefficient and recombination loss coefficient of the cow or calf were included as fixed effects. For all calf traits, sex and sire were included as fixed effects. For calf live weight, age and the interaction between age and sex were included as fixed effects. Within all cow and calf traits the sire of the cow was included as a random effect.

Results Live weight ($P<0.01$) and BCS ($P<0.05$) were 12 kg and 0.08 greater for low index cows, respectively (Table 1). High genetic merit cows had a 1.1 kg/d greater ($P<0.001$) milk yield than low merit cows. Genetic merit did not influence calf birth weight, calf average daily gain, weaning weight, calf quality or calf value.

Table 1 Effect of genetic merit on cow live weight, body condition score and progeny performance

	High Genetic Merit	Low Genetic Merit	s.e. ¹	P-value
Cow live weight (kg)	585	597	9.3	<0.01
Body condition score ²	2.81	2.89	0.030	<0.05
Milk yield (kg/d)	7.8	6.7	0.19	<0.001
Calf birth weight (kg)	42	43	0.9	0.4188
Weaning weight (kg)	286	279	5.1	0.5258
Calf ADG ³ (kg/d)	1.09	1.04	0.03	0.5380
Calf quality (1 to 5)	3.23	3.24	0.070	0.8629
Calf Value (€)	760	752	16.7	0.8770

¹Weighted standard error of the mean, ²Body condition score = 0 emaciated to 5 extremely fat, ³ADG = average daily gain

Conclusion Results from the current study showed that high genetic merit cows were lighter, had a lower BCS and a greater milk yield than low merit cow but cow genotype did not affect calf performance.

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Joint genetic analysis of Jersey dairy cows performing in two countries in Sub Saharan Africa

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Application Currently, there are a few countries that carry out genetic evaluation for dairy cattle in Sub Saharan Africa. Where this is done, it is at individual country level. Results from a joint genetic evaluation may provide robust and accurate genetic parameters.

Introduction Genetic improvement of farmed livestock has had a major impact on productivity and its effects being permanent, cumulative and usually highly cost effective. However, genetic improvement has not been carried out systematically in most Sub Saharan Africa countries because of lack of performance recording and pedigree information. However, some data has been collected in some countries which are currently used for national evaluation. A joint across-country analysis may result in more accurate evaluations in cases where common foreign sires have been used. Using a case study approach with data from Jersey cattle performing in Kenya and South Africa, the hypothesis was that joint genetic evaluation would result in robust and accurate genetic parameters and hence, improve genetic progress.

Material and methods Test interval method (ICAR 2003) was used to determine 305-day milk yield from test day records for Jersey cattle (n=46,242) obtained from the Kenya Livestock Breeders Organisation. In South Africa, 305-day milk yield records (n=1,858,021) were obtained from Agricultural Research Council. Data were from cows between first and fifth lactation from 1988 to 2012. A total of 400 sires with an average of 14 daughters per sire from Kenya and 9,962 sires with an average of 34 daughters per sire from South Africa were used. There were 31 sires that had daughters in both Kenya and South Africa from several foreign countries. The common sires were from USA (18), New Zealand (7), Denmark (1), Canada (1), Great Britain (1) and Australia (1). Variance component estimation was performed fitting a bivariate mixed linear model using ASReml (Gilmour *et al.*, 2009). The model was $y_{ijkl} = lac_j + HYS_k + animal_l + pe_m + e_{ijkl}$, where lac_j was the effect of the j^{th} lactation, HYS_k was the herd-year-season, $animal_l$ and pe_m were the random l^{th} animal and m^{th} pe effect. e_{ijkl} was the error term. In the joint evaluation, country of performance was included in the model to account for the production system.

Results Descriptive statistics for production and fertility traits for Jersey cattle performing in Kenya and South Africa are presented in Table 1.

Table 1 Means and standard deviations of Jersey cow performance traits in Kenya and South Africa between 1988 and 2012.

Trait	Kenya	South Africa	Joint data
Age at first calving (months)	31.0 ±7.21	28.0±4.10	29.0±4.50
Calving interval (days)	493.0±152.01	404.0±89.30	405.2± 91.30
305-day milk yield (litres)	4181.0±1428.1	5563.0 ±1417.3	5520.4± 1437.5

Cows in Kenya calved for the first time at a slightly older age than in South Africa. However, cows in Kenya had relatively lower milk yield than cows in South Africa with more variation in milk yield in Kenya (CV% = 34%) than in South Africa (CV% = 25%). Genetic parameters for individual and joint evaluations are presented in Table 2.

Table 2 Estimation of genetic parameters and standard errors for Kenyan and South African Jersey cattle.

Genetic parameters	Kenya	South Africa	Joint genetic evaluation
Heritability of 305-day milk yield (MY)	0.13 (0.10)	0.18 (0.01)	0.21 (0.01)
Heritability of age at first calving (AFC)	0.15 (0.05)	0.44 (0.05)	0.58 (0.05)
Heritability of calving interval (CI)	Non estimable	0.04 (0.01)	0.05 (0.01)
Repeatability of 305-day MY	0.13 (0.10)	0.43 (0.03)	0.43 (0.01)
Genetic correlation of 305-day MY and AFC	-0.53 (0.24)	-0.12 (0.10)	-0.20 (0.10)
Genetic correlation of 305-day MY and CI	Non estimable	0.60 (0.05)	0.58 (0.04)

Joint genetic evaluation increased the value of the genetic parameter estimates and accuracy as reflected in low standard errors associated with the estimates.

Conclusion A joint genetic evaluation between Jersey cattle from Kenya and South Africa is feasible and more appropriate than individual country evaluation. This would generally increase the value of genetic parameter estimates and accuracy of selection especially where there are insufficient data available in individual countries for a robust analysis.

Acknowledgement Thanks to the Agricultural Research Council, South Africa, Kenya Livestock Breeder's Organisation and International Livestock Research Institute for data and the SRUC International Engagement Strategy for funding.

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Investigating genomic selection in crossbred cattle using data from the Dairy Genetics Project for East Africa

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Application Successful application of genomic selection in crossbred dairy cattle population from and for small holder systems in some African countries would allow for the development of a selection programme to sustainably improve production and fitness traits.

Introduction In developing countries, a large proportion of dairy production is carried out by small holders whose stock comprises of fewer than ten cows. The majority of these cows are crosses between indigenous and exotic breeds. There is little phenotypic recording, and no reliable pedigree records exist for these animals. Traditional methods of breeding value estimation (which rely on phenotype and pedigree information) cannot, therefore, be used to select animals for breeding. Genomic selection may be a suitable tool for estimating breeding values for these animals, as it allows the inference of relationships between animals using genotypes as opposed to the pedigree. This study investigates the potential for genomic evaluation in a small, crossbred, dairy cattle population, using three methods.

Material and methods The dataset comprised of milk yield deviations (YD) for 1,013 crossbred cows from the Kenyan component of the Dairy Genetics East Africa Project, all of which had genotype data available from the Illumina BovineHD SNP chip. Individuals were of varying crosses between indigenous African breeds (various Small East African Zebu ecotypes), and exotic dairy breeds (Ayrshire, Friesian, Guernsey, Holstein and Jersey), and were split into five groups according to proportion of dairy ancestry. Three groups were identified to be used as separate validation populations based on PCA analysis. Group 1 contained all animals where a Zebu breed contributed to the cross (n=178), group 2 contained all animals where 60-87.5% of the animal was contributed by exotic dairy breeds (n=448) and group 3 contained all animals where >87.5% of the animal was contributed to by exotic dairy breeds. Genomic breeding values (GEBVs) were calculated using 3 methods, SNP-BLUP, GBLUP, and BayesC, using the following model: $\mathbf{y} = \boldsymbol{\mu} + \mathbf{Z}\mathbf{a} + \mathbf{e}$; where \mathbf{y} is a vector of YDs, $\boldsymbol{\mu}$ is the fixed effect of the overall mean, \mathbf{e} is a vector of residual effects with an assumed variance of $N(0, \sigma_e^2 \mathbf{I})$, and \mathbf{X} and \mathbf{Z} are respective incidence matrices. For the SNP-BLUP model, \mathbf{a} represents a vector of random unknown marker effects where all SNPs are assumed to affect the trait, and effect distribution is assumed $N(0, \sigma_a^2 \mathbf{I})$, for the GBLUP model, \mathbf{a} represents a vector of unknown animal effects with an assumed distribution of $N(0, \sigma_a^2 \mathbf{G})$, where \mathbf{G} is the genomic relationship matrix, and for BayesC \mathbf{a} represents a vector of SNP effects, where some SNPs have zero effect with probability of π , which was set 0.3 in this study.

Results Accuracies of evaluation for the three validation groups for all three methods are shown in Table 1. In all cases, the highest accuracy was obtained when predicting animals >87.5% dairy (group 3) from animals with lower dairy contributions, with observed accuracies of approximately 0.4. Accuracies for group 2 were slightly lower but not significantly different from group 3. Accuracies obtained for group 1 were slightly lower again, which is to be expected as these animals are crosses between *Bos Taurus* and *Bos Indicus* species.

Table 1 Accuracy of GEBVs for three validation groups based on SNP-BLUP, GBLUP and BayesC analysis

Validation group	N	Accuracy (standard error)		
		SNP-BLUP	GBLUP	BayesC
1	178	0.28 (0.06)	0.32 (0.06)	0.28 (0.06)
2	448	0.36 (0.04)	0.35 (0.04)	0.35 (0.04)
3	297	0.42 (0.04)	0.41 (0.04)	0.39 (0.05)

Conclusion Though the study population is of limited size, the results suggest that there is the potential for estimation of GEBVs in a crossbred cattle population. SNP-BLUP or GBLUP are likely the preferred method for estimation as they produce similar accuracies to BayesC but are less computationally intensive. Innovative methods of data collection should to be developed in order to increase the accuracy of GEBV estimation in the small holder system.

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Is a cross-validation approach useful to overcome stringent multiple testing correction procedures in small GWAS studies?

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Application Identifying allelic differences in genes which influence production and health traits may help improve the rate of genetic progress in selection schemes or pinpoint physiological pathways playing a key role in trait phenotypes.

Introduction Using the Bonferroni correction in genomewide association studies (GWAS) often results in few single nucleotide polymorphisms (SNP) achieving significance due to the large number of tests applied when using SNP chip data. Here an approach is explored to overcome this limitation using a type of cross-validation. This method is tested on data from the UK and China derived from a joint Holstein dairy cow study of somatic cell count (SCC) data.

Material and methods In this approach a GWAS was carried out on the data from Chinese cows (estimation set). The SNP were ranked on their probability. A second GWAS was carried using the UK data (validation set) but only for SNP identified in the UK dataset achieving significance at the 5% level. The second set of results was ranked on their probabilities and those achieving significance at the 5% level were considered to be in LD with polymorphic genes affecting the trait. This was repeated at more stringent cut-off points in order to see if the trade-off between stringency and number of significant result differed. The cows used in this study have previously been described by Brickell *et al.* (2010) and Wu *et al.* (2012). All cows were Holsteins maintained on commercial dairy farms. Wuhan dairy farm, in China, has ~3000 cattle housed in open-sided sheds with access to outside yards, providing shelter in winter while remaining ventilated in summer. Cows are milked 3 times a day producing an average 305-day milk yield of 6,616 kg. The UK data was from 20 dairy herds kept in a range of systems in the south-east of England averaging 7,218 kg of milk in a 305-day lactation. They were genotyped with the 50K Illumina SNP chip. After quality control 171 UK and 214 Chinese cows, and 36,092 SNP remained in the analyses. Cow SCC status was characterised as a binary trait after Yoshida *et al.* (2012). The GWAS were carried out with allelic, genotypic and dominance models in PLINK (Purcell *et al.*, 2007). In addition SCC analysis which was carried out on environmental residuals (Aulechenko *et al.*, 2007) with an allele substitution model using mean lactation SCC.

Results Running analyses on all 385 cows together resulted in no SNP achieving significance after Bonferroni correction for multiple testing when using any of the 4 models (allelic, dominance, genotypic or allele substitution). When using the allelic model on the Chinese data 117 SNP demonstrated an effect on SCC ($P < 0.05$). None of these SNP achieved significance in the UK dataset when using a Bonferroni correction at 0.05. However, when considering only those SNP which achieved significance at the more stringent $P < 0.01$ in the Chinese set then 4 SNP were found to be significant in the UK population. Repeating these analyses but using the genotypic and dominance models resulted in 3 and 6 significant SNP at the $P < 0.01$ level being found. Reversing the analysis by using the UK dataset as the estimation set resulted in 3 SNP with the allelic test, 3 SNP with the dominance test and 2 SNP with the genotypic test being significant at the $P < 0.01$ level in the Chinese set. Results from analysing the mean SCC data also found significant SNP using this method ($P < 0.01$).

Conclusion Discovering genes affecting traits from small GWAS studies is often limited by the stringent requirements of correction for multiple testing. This study reports a cross-validation approach which appears to be promising since significant SNP were found in the validation set when it was analysed just for SNP achieving significance in the estimation set. In this case no SNP were found to be significant in the combined-set analysis. Whereas here the approach has been used on two distinct populations, from the UK and China, it should be possible to extend this method by randomly splitting a dataset into two halves and analysing the resultant datasets as described above. Potentially this could be repeated many times and a meta-analysis used on the set of results produced.

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16S rRNA gene sequencing reveals temporal shifts in the porcine faecal microbiota post-weaning, independent of enterotoxigenic *Escherichia coli* challenge

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Application A 16S rRNA gene metabarcoding pipeline has been developed and was used to study microbial shifts in the porcine gut, which may inform the development of novel therapeutic strategies to improve enteric health.

Introduction The weaning transition period in pigs is linked with increased vulnerability to enteric disorders, such as post-weaning colibacillosis, primarily caused by enterotoxigenic *Escherichia coli* (ETEC). The gut microbiota has been subject to intense research for several decades, due to associations with enteric health. In recent years, the emergence of next-generation sequencing has allowed study of complex microbial communities with greater resolution. Here, a previously developed ETEC infection model (Athanasiadou *et al.*, 2011) was used to study the effect of ETEC challenge on the faecal microbiota, as well as the temporal dynamics of faecal microbial communities over the post-weaning transition.

Materials and methods Fifty-nine pigs (Large White x Landrace) were weaned at 26.7±0.7 days of age and weighed 8.65±1.77kg and housed in groups of four where possible (2 males and 2 females). On days 4, 6, 8, 11 and 13 post-weaning, pigs were either trickle challenged in-feed with 10⁸ cfu ETEC in PBS or sham-challenged with PBS only. Rectal faecal samples were taken on days 4 (pre-challenge), 8, 12, 15 and 19. Pigs from each treatment were selected for the sequencing study, balancing for weaning weight, sex, pen and experimental round (n=16). Following DNA extraction, the V3 hypervariable region of the 16S rRNA gene was amplified before sequencing using the Illumina MiSeq. Quality control and analysis of sequences was carried out using mothur (Schloss *et al.*, 2009). Changes in operational taxonomic unit (OTU) relative abundances were assessed using Metastats (Paulson *et al.*, 2011). Shifts in microbiota structure and stability were assessed using analysis of molecular variance and homogeneity of molecular variance, respectively.

Results ETEC challenge had no effect on faecal microbiota structure, stability or OTU relative abundances ($P > 0.05$) at any time point. However, changes in faecal microbiota structure and stability were observed over time (Figure 1) and shifts in particular OTU relative abundances were evident ($P < 0.05$). Specifically, three OTUs identified as *Prevotella* species increased in relative abundance over time in both ETEC- and sham-inoculated pigs from baseline (Table 1).

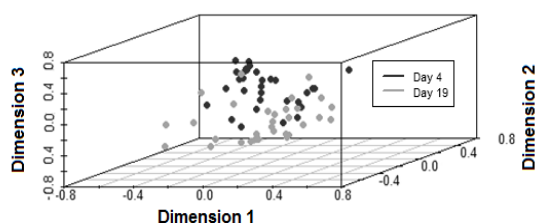


Figure 1 3D-NMDS ordination of faecal microbial communities pre-inoculation and 15 days post-inoculation showing changes in community structure over time

Table 1 Metastats comparison of Day 4 and Day 19 relative abundances (± SEM), only showing OTUs with significant shifts in both ETEC and sham groups ($P < 0.05$).

OTU phylotype	ETEC		SHAM	
	Day 4	Day 19	Day 4	Day 19
<i>Prevotella copri</i>	0.047 ± 0.015	0.216 ± 0.028	0.062 ± 0.020	0.200 ± 0.032
<i>Prevotella</i>	0.017 ± 0.003	0.061 ± 0.017	0.018 ± 0.005	0.057 ± 0.009
S24-7 (Bacteroidetes)	0.035 ± 0.008	0.014 ± 0.003	0.047 ± 0.013	0.018 ± 0.004
<i>Prevotella stercorea</i>	0.012 ± 0.004	0.033 ± 0.005	0.009 ± 0.002	0.029 ± 0.003
<i>Faecalibacterium prausnitzii</i>	0.004 ± 0.003	0.022 ± 0.006	0.003 ± 0.001	0.029 ± 0.007
Erysipelotrichaceae	0.000 ± 0.000	0.035 ± 0.007	0.001 ± 0.001	0.036 ± 0.006

Conclusion ETEC challenge had no significant effect on the faecal microbiota. However, the site of colonisation is the terminal small intestine, so any effects on the microbiota may be localised. Significant changes in microbiota structure and stability were observed over a short time frame post-weaning, highlighting the importance of this dynamic phase in shaping the porcine microbiota. Manipulation of the gut microbiota is viewed as a potential future therapeutic option for enteric disorders, and a better understanding of microbial shifts during this dynamic phase may lead to the development of dietary and probiotic management strategies.

Acknowledgements The authors greatly acknowledge BBSRC and Zoetis for sponsorship of this PhD project. Many thanks to Dave Anderson and Lesley Smith for technical assistance during this trial.

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The effects of xylanase on weaner-grower pig performance and peptide YY concentration in portal and peripheral blood

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Application The addition of xylanase to weaner-grower pig diets may be less effective when fed high quality wheat-barley based diets.

Introduction Xylanase, a glycosidase enzyme has been shown to improve pig performance through improvements in digestibility and reduced diet viscosity. Studies in broiler chickens have shown that the addition of xylanase increases peptide YY concentrations (Singh *et al*, 2012) which results in delayed gastric emptying, improving nutrient digestibility and growth performance. The aim of this study was to determine the effects of increasing xylanase levels on pig performance and the concentration of peptide YY in portal and peripheral blood.

Material and methods Five hundred and twelve weaner-grower pigs were allocated to pens (32 replicates) at 7 weeks of age on the basis of litter origin, sex and liveweight. Pigs had *ad libitum* access to one of four treatment diets over a 35 day period. Dietary treatments comprised 4 inclusion levels of xylanase (0, 8,000, 16,000 or 32,000 BTU/kg). Diets were wheat-barley based and formulated to meet or exceed nutrient requirements. Pigs were individually weighed at the start of the trial (7 weeks of age) and at 10 and 12 weeks of age. Pen feed intake and feed conversion ratios were calculated. Pig health was assessed and recorded daily. At the end of the trial 15 pigs per treatment were euthanised and blood was collected from portal and peripheral circulations into heparinised tubes. Mean pen data for liveweight, feed intake, daily gain and FCR were analysed using an ANOVA according to a general linear model (GLM) procedure (SPSS, version 22). Orthogonal polynomial contrasts were applied to test for linear and quadratic responses to the inclusion of xylanase (SPSS, version 22). The number of pigs treated with antibiotics and faecal and health scores were analysed by the non-parametric Kruskal–Wallis one-way ANOVA. An ANOVA was run to compare treatment effects on portal and peripheral levels of peptide YY, whereas a Repeated-Measures ANOVA was performed to compare portal and peripheral concentrations.

Results Pigs performed well across all treatments with an average growth rate of 0.781 ± 0.005 kg/day. The addition of xylanase to the diet had no effect on performance over the 35 days (Figure 1). Peptide YY levels in portal and peripheral plasma were not significantly different between treatments (Figure 2). There was no difference in peptide YY concentrations between portal and peripheral plasma (Figure 2). Health status was similar between treatments.

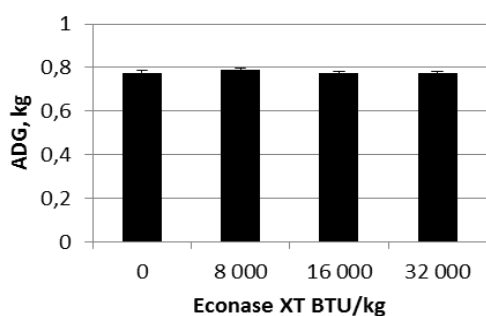


Figure 1 Average daily gain of weaner-grower pigs fed four levels of xylanase over a 35 day period

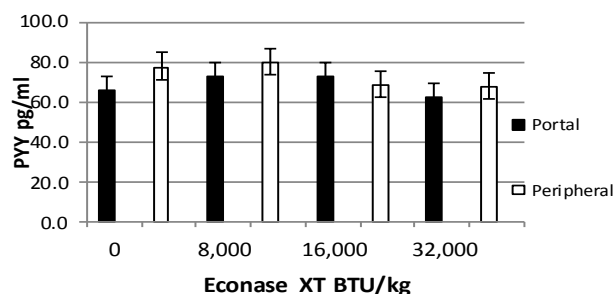


Figure 2 PYY concentrations in portal and peripheral plasma of weaner-grower pigs fed four levels of xylanase (d 35)

Conclusion The addition of xylanase to weaner-grower pigs fed a wheat-barley based diet had no effect on performance or portal and peripheral peptide YY concentrations. Pigs grew well when fed the non-supplemented diet which may suggest that diet quality was sufficient and thus there were no beneficial effects of adding xylanase with regards to growth performance. Another reason for the lack of response may be due to the trial duration as response to xylanase may develop when diets are fed over an extended period of time.

Acknowledgements This work was funded by AB Vista.

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Increasing *Buttiauxella* phytase dose up to 2000 FTU/kg increased nutrient retention and improved performance in weaned piglets fed wheat or corn based P deficient diets

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Application Additional benefits in performance and nutrient retention are seen with higher doses of phytase above 500 FTU/kg in weaned piglets fed wheat or corn based diets.

Introduction Phytase was traditionally used at 500 FTU/kg in piglet feed to improve phytate phosphorus (P) digestibility and to reduce P excreted into the environment. However, research has shown that a high phytase dose can degrade phytate more thoroughly and further reduce the anti-nutritional effect of phytate. This improves the utilization of nutrients beyond P and the growth performance of piglets (Zeng *et al.*, 2015). The aim of the present study was to determine the effect of increasing phytase dose on nutrient utilization and growth performance in piglets fed wheat or corn based diets.

Material and methods Two studies with similar designs, the main difference being grain type, were carried out to determine the effect of increased phytase levels on apparent faecal nutrient digestibility, retention and growth performance in weaned piglets during a 14 day period. A wheat/soybean meal based diet was used in study 1 and corn/soybean meal in study 2. A complete randomised block design was used in each experiment, with two runs in study 1 and four runs in study 2 in individual metabolic crates. Five treatments were tested: 1) positive control (PC) meeting piglet nutrient requirements; 2) negative control (NC) with reduced digestible P and Ca; and 3-5) NC supplemented with *Buttiauxella* sp. phytase (Danisco Animal Nutrition, DuPont Industrial Biosciences) at 500, 1000 or 2000 FTU/kg feed respectively (phytase activity was measured using the harmonised standard method ISO 30024:2009, at 37°C and pH 5.5). NC diets were formulated with a reduction of 0.15% Ca, 0.14 and 0.15% digestible P in experiment 1 and 2 compared to PC diets (the analysed total P content was 0.17% and 0.24% lower in experiments 1 and 2 respectively). Each treatment consisted of 8 replications (1 piglet per replicate) in study 1 and 12 replications in study 2. Piglets (11 kg BW) were provided pelleted diets and water at *ad libitum*. From day 10 to 14 urinary and faecal production was collected. TiO₂ was used as an indirect marker to measure apparent total tract digestibility (ATTD), and retention of P, N and energy. Data from both studies were analysed together investigating phytase effect, grain effect and their interaction. The experimental unit was each piglet. Treatment means were compared by Tukey's HSD using JMP 11 (SAS Institute Inc., USA); trial was used as a random effect. Linear and quadratic responses were analysed with increased phytase dose from 0 (NC) to 2000 FTU/kg.

Results Increasing phytase level up to 2000 FTU/kg significantly ($P < 0.05$) improved the final BW, average daily gain and feed conversion efficiency in a linear/quadratic manner, regardless of grain source. No significant differences were found for grain source and phytase interaction, except for P retention, where a higher increase was found in corn based diet than in wheat based diet. In general, phosphorus retention significantly ($P < 0.0001$) improved with increasing phytase dose. Similarly, increased phytase dose resulted in a linear increase in nitrogen retention, while gross energy retention increased quadratically (Table 1).

Table 1 Effect of increasing phytase dose on performance and nutrient retention in weaned piglets (pooled data from two trials)*

Dietary treatments	PC	NC	<i>Buttiauxella</i> phytase, FTU/kg			Phytase effect		
			500	1000	2000	<i>P</i>	<i>P</i> linear	<i>P</i> quadratic
ADG, g/d	537.0 ^a	445.4 ^b	510.3 ^{ab}	520.9 ^{ab}	557.6 ^a	0.003	<0.001	<0.001
ADFI, g/d	681.7	627.3	668.6	660.0	692.2	0.414	0.129	0.132
FCE	0.790 ^a	0.714 ^b	0.773 ^{ab}	0.795 ^a	0.811 ^a	0.001	0.001	<0.001
P retention, %	69.7 ^b	59.3 ^c	77.9 ^{ab}	76.5 ^{ab}	82.7 ^a	<.0001	<.0001	<.0001
N retention, %	69.7	67.0	69.6	67.5	74.3	0.330	0.048	0.161
GE retention, %	89.1	87.9	90.0	90.0	90.5	0.293	0.060	0.034

* PC: positive control; NC: negative control; FCE: feed conversion efficiency (gain: feed); P: phosphorus; N: nitrogen; GE: gross energy. SEM standard error of the mean; *P*: significance value.

Conclusion Increasing *Buttiauxella* phytase dose up to 2000 FTU/kg improved nutrient utilization and performance, providing environmental and production benefits in weaned piglets fed wheat or corn based P deficient diets.

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Impact of zinc oxide on the immediate post weaning colonisation of enterobacteria in pigs

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Application Supplemented at pharmacological dosage in piglet feed, zinc oxide (ZnO) shows a positive impact on gut health of weaned piglets. A new source of ZnO is evaluated at lower dosage.

Introduction Zinc oxide (ZnO) has been used for a long time with beneficial effects in animal production (Pettigrew, 2006) and is commonly supplemented at 3000 ppm in Asia and the Americas. The European legislation has limited the use of ZnO to a maximum level of 150 ppm in pig feed, because of suspected environmental pollution (Jondreville *et al.*, 2003).

It is generally believed that a direct effect of ZnO exists on enterobacteria induced post-weaning diarrhoea, but it is unknown exactly when its beneficial effect starts and for how long it proceeds. Thus, the aim of this study was to monitor the impact of ZnO after weaning on the presence of *E. coli* pathogenic genes.

Material and methods 48 weaned piglets were fed diets with 150 or 3000 ppm Zn from standard ZnO sources and 150 or 300 ppm Zn from a new original ZnO preparation (HiZox[®], Animine, France). They were housed in 24 stainless steel pens (2 piglets per pen); 5 piglets per treatment (20 piglets) were randomly chosen for the fecal analyses.

Daily fecal samples were taken 4h after feeding, after rectal simulation, on day 0, 1, 2, 3, 4, 6, 8, 10 and 14 after weaning. Faecal consistency was scored, from 1 (liquid diarrhoea) to 5 (hard and dry feces). DNA was extracted from samples using a commercial extraction kit (Qiagen Stool kit, Qiagen, Hilden, Germany). The development of *Escherichia* spp. were analysed in 180 samples, by qPCR (16S rRNA gene copy number). A multiplex-PCR targeting important *E. coli* toxins and fimbriae was used in samples from day 0 and day 1 of the trial. Prominent gene occurrences were further analysed by qPCR (*eltIIa estIb, estII, fedA, fae, fan*).

Total Zn was analysed in fecal samples via AAS.

Data were analysed by ANOVA (total fecal Zn). Non parametric tests (Kruskal-Wallis, Mann-Whitney U) were performed for non-normally distributed data (fecal scoring, quantitative results for *Escherichia* spp. and toxins). The Chi-Square test was conducted for qualitative data of the presence of pathogenic *E. coli* factors. Differences among least squares means with a probability of $P < 0.05$ were considered as statistically significant.

Results Fecal scoring showed significant differences between the trial groups. While the control group showed the lowest scoring values (3.38 ± 0.17 for the entire period), the group fed with the highest Zn dose had the best scoring (3.90 ± 0.16). In the groups with HiZox, intermediate values were observed (3.70 ± 0.19 with 150 ppm, 3.73 ± 0.23 with 300 ppm); the control group and the other groups were significantly different ($P < 0.01$).

Starting on the third day of the trial, animals fed the highest Zn dose showed increasing fecal Zn level, which seemed to plateau (14.28 ± 0.8 g/kg Zn wet weight) after day 10. Fecal Zn levels between both 150 ppm Zn trial groups and the 300 ppm HiZox trial group started to differ numerically on day 4, then significantly ($P < 0.05$) until the end of the trial; they remained below 2g/kg Zn wet weight: 0.86 ± 0.15 for the control group, 1.04 ± 0.09 with the 150 ppm HiZox group, 1.83 ± 0.14 with the 300 ppm HiZox group at day 14.

During the first three days of the trial, an increase of the development of *Escherichia* spp. was observed, followed by a decline starting on day 4. Significant differences ($P < 0.05$) were observed between both 150 ppm Zn groups (6.85 ± 0.46 and $6.81 \pm 0.55 \log \cdot g^{-1}$ wet weight for the copy numbers at day 3) and the 300 ppm HiZox ($6.46 \pm 0.25 \log \cdot g^{-1}$) or 3000 ppm ZnO groups ($6.34 \pm 0.26 \log \cdot g^{-1}$). This trend continued on day 6, however without significance. No differences were observed after ten days, where all trial groups showed similar concentrations ($4.5 \log \cdot g^{-1}$ wet weight on average).

Several *E. coli* associated pathogenic factors were located in samples from day 0 and day 1. The most prominent pathogenic factors were the heat stable toxins *estIb* and *estII*. Although no significant differences were observed, based on the sum of all detected *E. coli* pathogenic factors, positive samples are more numerous in the control group (51%), compared to other groups (less than 34%). Quantitative results for *estIb* and *estII* showed that both toxin genes displayed the highest concentration in the 150 ppm ZnO trial group. For *estII*, the 3000 ppm ZnO trial group showed the lowest level over the whole trial period ($2.72 \pm 0.28 \log \cdot g^{-1}$ wet weight at day 10), followed by the 300 ppm HiZox group ($3.01 \pm 1.21 \log \cdot g^{-1}$). Differences between the 3000 ppm ZnO group and the 150 ppm Zn groups were significant ($P < 0.05$) from day 2 to day 10.

Conclusion This trial has shown an effect of ZnO on pathogenic bacteria until day 8 to day 10. The 3000 ppm ZnO level and the 300 ppm HiZox trial group were superior in reducing bacterial count as well as *E. coli* pathogenic factors.

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The interactive effects of two different low dose dietary copper sources with phytase on weaned pig performance

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Application Chelating dietary copper with hydroxyl-methylthio-butanoic acid (HMTBa), in combination with phytase, mediates a superior feed efficiency in weaned piglets provided with lower mineral diets to reduce environmental impact.

Introduction Weaning is associated with many stressors known to adversely affect piglets, resulting in post-weaning growth depression. Copper (Cu), often in the form of inorganic CuSO₄, is commonly utilised as a multifunctional in-feed growth promoter at concentrations <175ppm until 8-10 weeks of age in the EU (Council Directive 70/524/EEC) (Zhoa *et al.*, 2014). Microbial phytase is commonly included in feed to improve the degradation of phytate phosphorus, the main phosphorus (P) storage compound in cereals, increasing the bioavailability of P in cereal grains. Thus, phytase reduces P excretion and can decrease other anti-nutritive effects of phytate. Inorganic sources of Cu are known to dissociate in the stomach, releasing Cu²⁺ ions which have a very high affinity to phytic acid, resulting in a phytase resistant Cu-phytate complex which is excreted (Banks *et al.*, 2004). Chelating Cu with HMTBa prevents the dissociation of Cu²⁺ ions and the subsequent antagonism with phytase. Consequently, the bioavailability of Cu is increased, reducing the concentrations required to promote equivalent growth promotion compared to CuSO₄ (Zhao *et al.*, 2014), enabling lower mineral diets to be utilised for environmental benefit. This study assessed whether supplementation of chelated Cu (Mintrex Copper, Novus Europe, Belgium) in combination with a standard dose of phytase in weaned pig diets would additively improve performance, as a result of the increased Cu and P bioavailability and reduced anti-nutritive effects of phytate.

Material and methods Ninety-six crossbred pigs ([Large White x Landrace] x Hylean Maxgro) were weaned onto trial at 28 days of age. Pigs were allocated between four dietary treatments, fed over two post-weaning phases for a period of five weeks (Phase one (weeks 1-2): 21.5% CP, 2.5% CF, 8% Fats, 5.5% Ash, 0.75% Ca, 0.55% P. Phase two (weeks 3-5): 21% CP, 3% CF, 6.5% Fats, 5.5% Ash, 0.72% Ca, 0.5% P). The basal diet contained only nutritional levels of zinc and no added fish meal. The treatments were: 1) 80ppm/kg Mintrex copper (MC), 2) 80ppm/kg MC + 500FTU/kg phytase (manufacturer's declaration, Phyzyme XP, DuPont) (MC+Phy), 3) 80ppm/kg CuSO₄ (CS) and 4) 80ppm/kg CuSO₄ + 500FTU phytase (CS+Phy). Pigs were housed in pens of four, balanced for weight, sex ratio and litter origin, to provide six replicates per treatment, blocked over two time batches. Feed and water were available *ad libitum*, with each pen having access to one nipple drinker. Mean daily live weight gain (DLWG), daily voluntary feed intake (DVFI) and feed conversion ratio (FCR) were measured from weekly weighing. The data obtained were analysed by the GLM function in Minitab version 16 statistical software; pen served as the experimental unit for all analyses. Factors included in the model were copper treatment, phytase treatment and copper/phytase interaction; start weight was included as a covariate and replicate as a blocking factor.

Results Pigs receiving Mintrex copper or phytase were significantly heavier at the end of the study (+7.2 and +6.0% respectively), attributed to their superior DLWG and FCR, demonstrating independent treatment effects. There was also an interactive effect of copper source and phytase, with pigs receiving the MC+Phy diet recording a lower FCR.

Table 1 The interactive effects of copper source and phytase on pig performance during the 5 week period after weaning

	MC+ Phy	MC	CS+ Phy	CS	S.E.M	Cu	Significance Phy	Cu x Phy
Start weight (kg)	7.91	7.97	7.99	7.96	0.053	n.s.	n.s.	n.s.
End weight (kg)	22.39	20.54	20.30	19.75	0.343	<0.001	0.002	n.s.
Overall DLWG (kg)	0.414	0.359	0.352	0.339	0.010	0.001	0.002	n.s.
Overall DVFI (kg)	0.612	0.588	0.584	0.553	0.017	n.s.	n.s.	n.s.
Overall FCR (feed/gain)	1.48 ^b	1.64 ^a	1.66 ^a	1.66 ^a	0.037	0.005	0.013	0.015

Conclusion Dietary supplementation with 80ppm/kg of MC, particularly when in combination with phytase, can enhance weaned pig performance when utilised in lower mineral diets designed to reduce environmental impact.

Acknowledgements The authors gratefully acknowledge funding from Newcastle University and Novus International.

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Ileal digestibility of crude protein and amino acid in rapeseed co-products fed to pigs

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Application Modification of rapeseed de-oiling processes may be beneficial to the feed and livestock industry, as it might produce products with better nutritional values in terms of crude protein (CP) and amino acid (AA) digestibility.

Introduction Rapeseed cultivars, harvest years and processing conditions might influence digestibility of rapeseed co-products in animal diets (Zhou *et al.*, 2013). The objective of the current experiment was to study the effects of these variables on the standardised ileal digestibility (SID) of CP and AA in eight rapeseed co-products fed to growing pigs.

Material and methods From 2013 Harvest, DK Cabernet, PR46W21 and V2750L rapeseed varieties were processed by soft hexane extraction, producing soft rapeseed meal (SRSM), with the DK Cabernet also being cold-pressed obtaining rapeseed cake (RSC). DK Cabernet and PR46W21 varieties harvested in 2014 were processed by both soft and standard commercial hexane extraction producing SRSM and rapeseed meal (RSM) respectively. The soft hexane extraction was specifically designed to exclude the cooking step and heat supply whilst crushing the seed during the standard de-oiling method. Forty eight white Duroc × Landrace males pigs (initial weight: 41 ± 2.7 kg) were allotted to 8 diets over 6 time periods in a randomised design, testing each diet across six pigs. The experiment was approved by Ethical Review Committee, University of Nottingham. At the end of experiment (body weight: 47 ± 3.6 kg) the pigs were culled via barbiturate (not electrical stunning). Digesta were collected from the ileum, measured as 1 m from the ileal-caecal-colonic junction towards the jejunum. Digestibility was assessed using titanium dioxide as an inert marker and evaluated by one-way ANOVA without blocking (GenStat, 15th edition). Data were considered statistically significant at $p < 0.05$.

Results The cold pressing method produced the greatest SID of CP and essential AA in the DK Cabernet RSC across all samples tested. Within Harvest 2014 samples and compared to their RSM counterparts, the soft hexane extraction significantly ($P < 0.05$) improved the SID of CP, Arg, His, Leu, Lys, Met+Cys, Thr and Val in DK Cabernet, and significantly ($P < 0.05$) increased the SID only of Lys in PR46W21. However, the SID of Ile, Leu, Lys, Met+Cys, Phe, Thr, Val and Trp did not significantly vary between the SRSM of DK Cabernet and PR46W21 varieties harvested in 2013 and 2014.

Table 1 Standardised ileal digestibility of crude protein and amino acids.

Sample	CP	Arg	His	Ile	Leu	Lys	MetCys	Phe	Thr	Val	Trp
2013 RSC DK Cabernet	0.80 ^{ab}	0.91 ^a	0.90 ^a	0.86 ^a	0.83 ^a	0.85 ^a	0.84 ^a	0.84	0.77 ^a	0.77 ^a	0.78 ^a
2013 SRSM DK Cabernet	0.72 ^{abc}	0.82 ^c	0.81 ^{cd}	0.79 ^{ab}	0.78 ^{ab}	0.69 ^{bc}	0.71 ^b	0.80	0.69 ^{ab}	0.73 ^a	0.77 ^a
2013 SRSM V2750L	0.75 ^{abc}	0.89 ^{ab}	0.88 ^{ab}	0.84 ^a	0.83 ^a	0.77 ^{ab}	0.79 ^{ab}	0.83	0.75 ^a	0.79 ^a	0.79 ^a
2013 SRSM PR46W21	0.81 ^a	0.87 ^{abc}	0.86 ^{abc}	0.83 ^a	0.81 ^a	0.76 ^{ab}	0.72 ^b	0.83	0.74 ^a	0.75 ^a	0.79 ^a
2014 SRSM DK Cabernet	0.69 ^c	0.86 ^{abc}	0.83 ^{bcd}	0.79 ^{ab}	0.79 ^a	0.72 ^b	0.72 ^b	0.79	0.71 ^a	0.73 ^a	0.75 ^{ab}
2014 RSM DK Cabernet	0.55 ^d	0.73 ^d	0.70 ^e	0.72 ^b	0.70 ^b	0.54 ^d	0.60 ^c	0.71	0.59 ^b	0.63 ^b	0.65 ^b
2014 SRSM PR46W21	0.71 ^{bc}	0.85 ^{bc}	0.84 ^{abcd}	0.80 ^{ab}	0.80 ^a	0.73 ^b	0.70 ^{bc}	0.78	0.73 ^a	0.73 ^a	0.76 ^{ab}
2014 RSM PR46W21	0.67 ^c	0.84 ^{bc}	0.78 ^d	0.78 ^{ab}	0.78 ^{ab}	0.62 ^{cd}	0.69 ^{bc}	0.78	0.68 ^{ab}	0.72 ^{ab}	0.45 ^c
Mean	0.71	0.85	0.83	0.80	0.79	0.71	0.72	0.80	0.71	0.73	0.72
CV, %	11.5	6.4	7.7	5.4	5.2	13.4	9.8	5.3	8.0	6.5	16.3
p value	<0.001	<0.001	<0.001	0.043	0.059	<0.001	0.001	0.091	0.029	0.035	<0.001

Conclusion Nutritional value of rapeseed co-products, in terms of their CP and AA digestibility, is sensitive to oilseed rape variety and oil extraction processing.

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Rapeseed meal as home grown alternative to soya bean meal for fattening pigs

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Application Rapeseed meal may be used at greater levels than the typical 150 g/kg upper limit in nutritionally balanced finisher feeds under UK conditions, and has the potential to replace soya bean meal with improved feed conversion ratio.

Introduction Rapeseed meal (RSM) is a protein source for fattening pig diets, with typical upper inclusion limit in the UK of ~15%. Recent Canadian studies indicate that greater levels of RSM may be used in weaned pigs without detrimental impact on performance (Landero *et al.*, 2011). Here, we hypothesized that RSM prepared from modern varieties of UK oilseed rape may be used at relatively large inclusion levels to replace soya bean meal (SBM) in fattening pig diets.

Material and methods RSM from two contrasting oilseed rape varieties (DK Cabernet and PR46W21, with glucosinolates of 2.72 and 4.61 $\mu\text{mole/g}$ dry matter, respectively) were used at 0, 50, 150 and 250 g/kg, gradually and completely replacing SBM in finisher pig diets. The RSM were prepared at the oilseed crushing pilot plant CREOL under commercial oil-extraction conditions, were analysed for amino acids, and using book values for standardized ileal digestibility (SID) and net energy (NE) supply, iso-energetic diets were formulated (NE 9.3 MJ/kg), at similar SID lysine (8.9 g/kg). Soya oil gradually increased from 13.7 to 45.4 g/kg, whilst wheat reduced from 265 to 165 g/kg. Pure amino acids and macro-minerals levels were modified to meet requirements of methionine, threonine, tryptophan, calcium and digestible phosphorus (BSAS, 2003). Barley, wheatfeed, salt and trace element / vitamin premix levels were kept constant. Each RSM diet was fed *ad libitum* to two groups of three male and two groups of three female terminal line finisher LW \times L pigs, for three weeks, after one week of adaptation period ($n=4$); the control diet was fed to four groups of males and females ($n=8$). Body weight at housing was 61.7 ± 0.9 and 61.9 ± 0.9 kg for male and female pigs, respectively. Weekly live weights for individual pigs, and pen feed intakes were taken to assess body weight gain (BWG, g/pig/day), average daily feed intake (ADFI, g/pig/day) and feed conversion ratio (FCR as ADFI/BWG). Data was analysed for diet and sex effects using a 7×2 factorial ANOVA, with pen as experimental unit, and housing body weight as covariate for ADFI and FCR, and adaptation period BWG as covariate for test diet BWG. Orthogonal contrast statements were used to locate feeding treatment effects as effects of RSM inclusion *per se*, RSM variety, RSM level, and their interaction.

Results There were no sex or diet effects on ADFI (Table 1). However, males grew faster than females (1207 vs 1130 g/day; s.e.d. 34 g/day; $P=0.022$), which tended to be more pronounced in the absence than in the presence of RSM (+175 vs +45 g/day; s.e.d. 86 g/day; $P=0.066$). Males also grew at better FCR than females (2.24 vs 2.43; s.e.d. 0.04; $P<0.001$), whilst FRC improved at greater levels of RSM, which tended to be stronger in DK Cabernet than in PR46W21 (Table 1).

Table 1 Effect of replacing soya bean meal (SBM) with rapeseed meal (RSM) on finisher pig performance.

RSM Variety	RSM level (g/kg)	SBM level (g/kg)	ADFI (g/day)	BWG (g/day)	FCR
None	0	180	2684	1150	2.33
DK Cabernet	50	120	2807	1112	2.46
	150	60	2647	1157	2.31
	250	0	2601	1168	2.22
PR46W21	50	120	2706	1197	2.31
	150	60	2921	1160	2.43
	250	0	2702	1254	2.29
		s.e.d.	120	60	0.07
P-values					
Diet effects	RSM		0.562	0.546	0.881
	Variety		0.269	0.228	0.800
	Level		0.299	0.321	0.040
	Interaction		0.317	0.963	0.070

Conclusion This data support the view that RSM from modern varieties of oilseed rape may be used up to 250 g/kg inclusion level, and as such completely replace SBM, in nutritionally complete finisher pig diets. This work is being extended with grower pigs, whose performance is expected to be more sensitive to RSM feeding.

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Finishing pig performance and diet digestibility when offering diets with up to 300 g/kg of wheat or maize Dried Distillers Grains with Solubles (DDGS)

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Application Although the dry matter, energy and nitrogen digestibility of the diet was reduced, pig performance was not affected when high levels of wheat or maize dried distillers grains with solubles (DDGS) were included in finisher pig diets. In this work the digestible energy of the maize and wheat DDGS was found to be 14.8 and 14.0 MJ/kg respectively.

Introduction Dried distillers grains with soluble (DDGS) have become a common feed ingredient in pig diets, especially in the US due to the large bio ethanol industry that has evolved there in recent years. Whilst maize is a common feed stock for the EU bio ethanol plants, as in the USA, wheat is also commonly used. As such both wheat and maize DDGS are available to European feed manufactures. However, knowledge on the nutritive value of wheat DDGS is scarce compared to maize DDGS and comparisons between the two are uncommon. This trial aimed to compare pig performance and diet digestibility using increasing levels of wheat and maize DDGS.

Material and methods Two trials were conducted. In trial 1 (performance study) 720 pigs (PIC 337 x (LR x LW)) were penned in groups of 10 from 12 weeks of age (44kg) to slaughter at 100kg over eight time periods creating (8 pen replicates per treatment). Pigs were assigned to group so that each group was balanced for weight and gender (gilt and boar) within each time period. Each group was then randomly assigned to a dietary treatment. Pigs were reared in commercial, fully slatted housing with Automatically Controlled Natural Ventilation. All diets were offered *ad libitum* through wet and dry single space feeders. In trial 2 (digestibility trial) 72 boars were used to determine diet digestibility over eight time periods (eight replicates/treatment). The average start weight of the pigs was 54.5kg and pigs were housed in metabolism crates, for a period of 14 days (7d prefeed + 7d balance collection). Samples of the diets, faeces and urine were analysed to determine digestibility of dry matter (DM), crude protein (CP), oil, neutral detergent fibre (NDF) and energy. Nine diets were offered in both trials. A control diet was formulated to contain (g/kg) 206 barley, 200 maize, 200 wheat, 232 soya, 123 pollard, 14 soya oil, 5 molaferm and 20 minerals, vitamins and additional amino acids. A diet containing 300 g/kg maize DDGS was formulated to contain (g/kg) 300 maize DDGS, 168 barley, 150 maize, 150 wheat, 98 soya, 83 pollard, 26 soya oil, 5 molaferm and 20 vitamins and additional amino acids. A diet containing 300 g/kg wheat DDGS was formulated to contain 300 wheat DDGS, 168 barley, 150 maize, 150 wheat, 89 soya, 92 pollard, 26 soya oil, 5 molaferm and 20 vitamins and additional amino acids. Diets containing 75, 150 and 225 g/kg of maize or wheat DDGS were respectively formulated. All diets contained 20 g/kg of Mycosorb. All results were analysed by ANOVA to determine differences. The DE of DDGS was calculated using a linear regression approach using the data from the digestibility trial and standard matrix DE values for other ingredients.

Results There were no significant interactions between DDGS type and level of inclusion on either pig performance or diet digestibility. There was no significant effect ($P>0.05$) of DDGS type or level of inclusion on pig average daily gain (average 851 g/day) or average daily feed intake (average 2136 g/day) between 44 and 100kg. Over the finishing period, although there was no effect of inclusion rate, FCR was improved ($P<0.05$) when DDGS was included compared with when it was not (FCR average for all diets with DDGS was 2.50 compared to the control which had an FCR of 2.59). With regard to diet digestibility, the type of DDGS had little effect except for NDF and oil B digestibility which were lower (both $P<0.05$) when maize DDGS was offered (0.585 and 0.798 respectively) compared with when wheat DDGS was offered (0.632 and 0.814 respectively). However there was a more pronounced effect of the inclusion level on diet digestibility (Table 1). As inclusion level increased energy, dry matter and nitrogen digestibility decreased whilst oil B digestibility increased. The DE of the wheat and maize DDGS raw material was calculated as 14.0 and 14.8MJ/kg respectively.

Table 1 Diet digestibility as inclusion level (g/kg) of wheat and maize DDGS increased

	Ctl	75	150	225	300	SEM	P Value
DE (MJ/kg)	15.81	15.82	15.88	16.05	16.06	0.105	NS
Energy	85.46	84.02	83.50	82.86	81.90	0.550	<0.05
Dry matter	85.91	84.51	84.15	83.27	82.00	0.511	<0.001
Nitrogen	86.63	84.80	84.35	82.88	83.01	0.646	<0.05
Ash	63.92	61.94	62.4	61.63	60.93	1.381	NS
NDF	61.33	61.86	61.01	61.19	59.34	5.929	NS
Oil B	70.17 ^a	77.02 ^b	79.61 ^c	82.35 ^d	83.26 ^d	0.739	<0.001

Conclusion The DE of the DDGS was found to be higher than what was used to formulate the diets. As a result more oil was used in the formulation than was required. Nonetheless this study, using these sources of DDGS (European wheat and US maize) and a mycotoxin binder found no significant impact on pig performance even though energy, dry matter and nitrogen digestibility were reduced as DDGS inclusion increased. In this study pig performance and diet digestibility was similar between the wheat and maize DDGS.

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The effect of high phytase doses on phytate degradation and *myo*-inositol release in the grower pig

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Application Phytase at levels above the current standard recommendation further improve phytate degradation and *myo*-inositol (MYO) release in pigs fed wheat-barley based diets.

Introduction Microbial phytases are commonly added to monogastric diets to degrade dietary phytate (*myo*-inositol hexaphosphate, InsP₆) and in doing so reduce the need for inorganic phosphate supplementation. Recent research has shown that phytase can yield further improvements in nutrient bioavailability and growth performance in pigs when included at levels above the standard recommendation (Santos *et al.*, 2014). The underlying mechanism for this favourable remains to be elucidated. It has been suggested that MYO, the end product of complete phytate hydrolysis, may be involved (Cowieson *et al.*, 2015). This study set out to determine the effect of high phytase levels on phytate degradation and MYO generation in the grower pig.

Material and methods A total of 288 crossbred ((Large White x Landrace) x MAXGRO) grower pigs (initial BW 36.7 kg) were used in this 28 day experiment. Pigs were blocked into pens of 4 (mixed sex) on the basis of body weight (BW), sex and litter. Within block, pens were randomly assigned to one of 6 wheat-barley based diets ($n = 12$). Dietary treatments included: (PC) a positive control formulated to meet or exceed the nutritional recommendations according to BSAS (2003); (NC) a negative control diet similar to the PC but with reductions in Ca (1.6 g/kg), P (1.24 g/kg), Lys (0.17 g/kg), Thr (0.33 g/kg) and ME (0.217 MJ/kg); and the NC diet supplemented with phytase at 500, 1,000, 2,000 or 8,000 FTU/kg. Feed was offered *ad libitum* throughout the experiment. At the end of the trial (d 29 and 30) 10 pigs per treatment, each from different pens, were euthanised for the collection of blood and ileal digesta. Portal and peripheral blood samples were collected into heparinised tubes from the portal and the jugular vein respectively prior to MYO analysis. Ileal digesta were obtained from the terminal ileum, freeze dried and analysed for phytate, phytate hydrolysis products *myo*-inositol penta- to di- phosphate (InsP₂₋₆) and MYO. Data were analysed as a randomised complete block design using the GLM procedure of SPSS. Orthogonal polynomial contrasts were used to test for linear and quadratic responses to phytase supplementation.

Results Ileal InsP₆ and InsP₅ concentrations were reduced in response to phytase treatment (linear $P < 0.01$, quadratic $P < 0.01$; Table 1). Concomitantly, there were linear increases in ileal InsP₄, InsP₃ and MYO concentrations ($P < 0.01$). In support of the ileal phytate degradation data are the plasma MYO data (Figure 1) which showed linear increases in portal and peripheral plasma MYO in response to phytase ($P < 0.001$). MYO levels in the portal blood were higher than in the peripheral blood ($P < 0.001$).

Table 1 Effect of dietary phytase level on ileal InsP₂₋₆ and MYO concentration (nmol/g DM).

Diet	MYO	InsP ₂	InsP ₃	InsP ₄	InsP ₅	InsP ₆
PC	4,422	5,330	917	1,711	3,052	21,616
NC	4,309	5,142	657	1,204	2,950	25,189
500	7,729	5,634	1,236	2,658	2,075	15,217
1000	9,964	4,663	1,195	1,972	1,271	12,505
2000	12,812	5,841	1,172	1,771	987	11,038
8000	14,086	5,149	413	304	574	7,868
SEM	1,744	821	177	400	311	2,817
<i>P</i> value						
Linear	0.001	0.907	0.002	<0.001	<0.001	0.001
Quadratic	0.008	0.449	0.012	0.162	<0.001	0.007

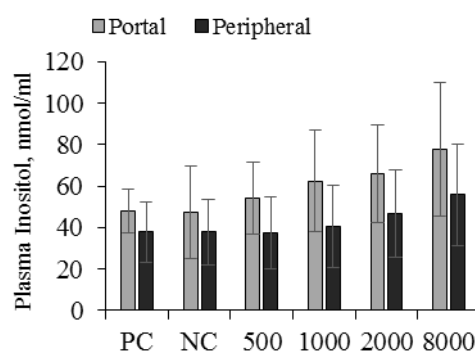


Figure 1 Effect of dietary phytase level on portal and peripheral plasma MYO concentration.

Conclusion Increasing phytase dose reduced ileal phytate concentration and increased MYO release, resulting in higher MYO blood levels. Lower levels of phytase increased InsP₄ and InsP₃ to a peak at 1000 FTU/kg with further increments reducing these below the control. These data support the current tenet that MYO is involved in the high phytase dosing response.

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Effects of single and double feeder space on the feed intake and growth performance of pigs post-weaning from four to eight weeks of age

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Application Doubling feeder space did not significantly improve pig live weight, average daily gain or feed intake in pigs from weaning at 28 days of age to eight weeks post-weaning.

Introduction For pigs, available feeder space standards are dependent on weight, potentially limiting growth performance of faster growing genotypes by reducing simultaneous feeder access and feed intake. This may result in a post-weaning growth check and affect subsequent growth performance in weaner pigs. A previous study showed restricted feeder space increased competition for trough access, leading to adaptation of feeding behaviour (Hyun and Ellis, 2001). This study aimed to determine if doubling feeder space results in increased weight gain and feed intake post-weaning.

Material and methods A randomised control block design, carried out in four repeated trials, was used to compare single (4.82 cm/pig) and double (9.64 cm/pig) feeder space, adapted from methodology previously described by Wolter *et al.*, (2002). A total of 272 pigs sourced from 37 litters, weaned at 28 days of age ($8.8 \text{ kg} \pm 1.42$) were randomly allocated to mixed-litter pens (2 identical pens per treatment, per trial) of 17 pigs, balanced for sex and weight. Each pen provided the same floor space of $0.51 \text{ m}^2 \text{ pig}^{-1}$, and consisted of fully-slatted plastic flooring, two double-headed nipple drinkers and identical enrichment. Two four-space feeders (30 cm x 20 cm x 15 cm spaces) were placed on one side wall of each pen. All pens received the same amount of feed daily, with single-treatment pigs only provided food in one feeder, whilst double-treatment pigs had food distributed between two feeders. Feed intake was monitored for the first two weeks post-weaning and individual weight measurements were taken weekly over eight weeks. Data were analysed in Genstat v17.1. Live weight gain was analysed via an analysis of variance (repeated measures) and feed intake and average daily gain via T-tests. The four trials were treated as blocks and no trial effect was found.

Results Feeder space treatments had no impact on average pig live weights over the eight week period ($20.6 \text{ kg [S]} \text{ vs } 20.5 \text{ kg [D]} \pm 0.37$, $P = 0.760$) (Figure 1) or average pig feed intake ($26.1 \text{ kg. wk}^{-1} \text{ [S]} \text{ vs } 25.4 \text{ kg. wk}^{-1} \text{ [D]} \pm 3.13$, $P = 0.806$) between weaning and week eight post-weaning. There were no significant interactions between time and treatment on average pig live weight or feed intake over this period. In addition, feeder space treatments had no impact on average daily gain over the first two-weeks post-weaning ($0.20 \text{ kg. d}^{-1} \text{ [S]} \text{ vs } 0.20 \text{ kg. d}^{-1} \text{ [D]} \pm 0.01$, $P = 0.662$) or over the first eight weeks post-weaning ($0.6 \text{ kg. d}^{-1} \text{ [S]} \text{ vs } 0.5 \text{ kg. d}^{-1} \text{ [D]} \pm 0.01$, $P = 0.434$).

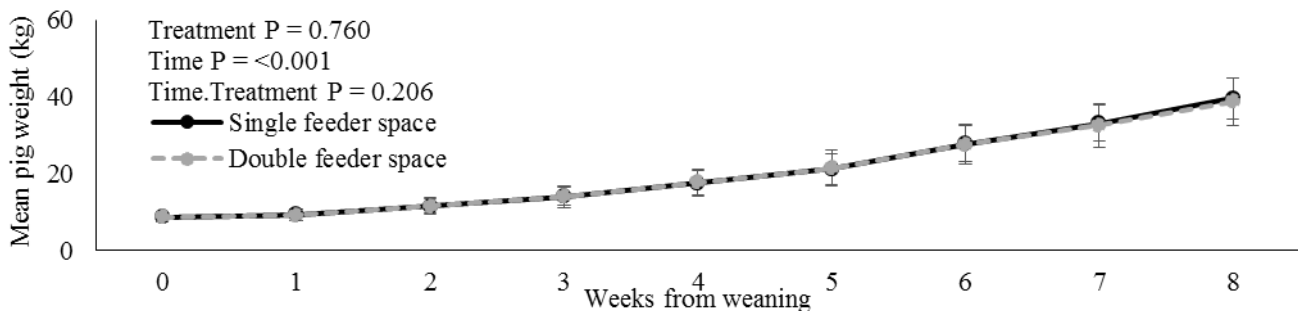


Figure 1 Pig weight from weaning through eight weeks post-weaning, for single and double feeder space treatments

Conclusion Doubling feeder space did not significantly affect pig growth or feed intake over the eight weeks post-weaning, which is supported by previous findings (Morrow *et al.*, 1994; Weber *et al.*, 2015). In the present study, no post-weaning growth check was observed and all pigs regardless of treatment gained more weight per day than the UK average of 480 g/d in the rearing phase ($546.4 \text{ g [S]} \text{ vs } 536.7 \text{ g [D]}$). This indicates current feeder space recommendations do not restrict growth of faster growing genotypes under the conditions of the trial conducted.

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Performance of individual piglets when reared on sows divergent in output potential

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Application Sows in the top quartile, in terms of litter performance, weaned an average of 123kg of litter weight, while sows in the bottom quartile weaned 94kg. This increase in total litter weaning weight had no effect on within litter variation.

Introduction It is well documented that larger birth weight (BW) pigs reach heavier weaning weights (WW) (Fix *et al.*, 2010). How individual pigs perform on sows that have divergent outputs, in terms of weaning weight, is yet to be discussed. Identifying how piglets respond within the litter could help producers improve performance across the whole herd. This report aims to identify how pigs with a range of birth weights perform when reared on sows with different levels of output.

Material and methods Using data from a trial reported by Craig and Magowan (2015), sows were assigned to top quartile (n=29), mid-range (n=57) or bottom quartile (n=29) according to litter weight weaned. Pigs were weighed at 0, 7, 14, 21 and 28 days. Body condition score (BCS), backfat depth (measured at P₂) and sow weight were recorded at farrowing and weaning. Sow feed intake was also recorded. Pig and litter average daily gain (ADG), birth and wean coefficient of variation (CoV) and daily digestible energy (DE), crude protein (CP), lysine and valine intakes were calculated. Individual pigs were categorised within each litter according to BW into small (<1.2kg), medium (1.2-1.8kg) or large (>1.8kg). Data was analysed using analysis of variance (ANOVA). Details of covariates are presented below.

Results Top quartile sows weaned an extra 28.2kg (P<0.001) of litter weight and an extra piglet (P<0.001) compared to the bottom quartile (Table 1). Top quartile sows also had a higher ADFI (P=0.002) and ate an average of 7.9kg per day (i.e. 116.14 MJ DE/day and 79.9g lysine/d). There was no significant effect of sow ranking on the birth (0.17) and wean (0.21) CoV, sow P₂ change (-3.6), BCS change (-0.4) or weight change (6.2kg) between farrowing and weaning. Top quartile sows had a higher proportion of large BW pigs at birth than bottom quartile sows (30% v. 11%). All pigs suckling top quartile sows grew significantly faster (P<0.001) compared to their counterparts reared on the bottom quartile sows (Table 2). The weaning weight of medium and large pigs increased as overall litter weight increased but for small pigs although weaning weight increased when pigs were reared on 'mid' sows compared with 'bottom' sows, the weaning weight of light birth weight piglets did not increase any further even when they were reared on 'top' quartile sows.

Table 1 Performance of sows according to percentile*

	Bottom 25%	Mid 50%	Top 25%	P. Value	SEM
Total Litter WW (kg)	94.1 ^a	109 ^b	122.7 ^c	<.001	1.19
No. Weaned	12.2 ^a	12.9 ^b	13.2 ^b	<.001	0.11
ADFI (kg/d)	7.1 ^a	7.6 ^b	7.9 ^b	0.002	0.16

*Covariates used: lactation length, no. of pigs after cross-fostering and litter weight after cross-fostering

Table 2 WW (kg) and ADG (kg) of individual piglets of different BW within each percentile*

	Small (<1.2kg)		Medium (1.2-1.8kg)		Large (>1.8kg)	
	Wean Wt.	ADG B-W	Wean Wt.	ADG B-W	Wean Wt.	ADG B-W
Bottom 25%	6.7 ^a	0.193 ^a	8.0 ^a	0.230 ^a	8.8 ^a	0.241 ^a
Mid 50%	7.6 ^b	0.230 ^b	8.5 ^b	0.251 ^b	9.4 ^b	0.263 ^a
Top 25%	7.6 ^b	0.231 ^b	9.2 ^c	0.272 ^c	10.5 ^c	0.297 ^b
P. Value	<.001	<.001	<.001	<.001	<.001	<.001
SEM	0.18	0.0072	0.093	0.0034	0.273	0.0104

*Covariates used: lactation length and number weaned

Conclusion The difference in sow potential during lactation within this data set equated to a difference of 28kg per litter weaned. Commercially, litter WW are commonly 100kg and this examination of data has demonstrated the future potential of sows. Furthermore, it appears that these high outputs can be achieved with no detrimental effect on body condition. Whilst the WW of medium and large BW pigs increased as sow overall output increased, the WW of light BW pigs did not. Although small BW pigs have been found to have WW of over 8kg at an individual pig level, this study would suggest that, at a population level, the growth potential of small pigs becomes limiting compared with medium and heavy BW piglets.

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Identifying the optimum average pig weight to change diet in the finisher stage

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Application Changing the diet from a grower to a finisher pig diet when pigs are on average 60kg optimises the performance of the group of animals and represents the lowest feed cost per kg gain during the finishing period.

Introduction Finishing feed accounts for approximately 70% of the feed used in a birth to bacon unit. Financially 60% of feed cost is in the finishing period and a 0.1 unit improvement in feed conversion ratio (FCR) can increase profitability by £18000 on a typical 500 sow unit. Pigs normally change from a grower to a finisher diet on entry to the finishing pig accommodation (approximately 40kg) even though weight within that group of pigs could range from 25 to 50kg. This trial investigated the impact of targeting a specific weight to change from the grower to the finisher diet on finishing pig performance.

Material and methods The study was carried out on a commercial farm. There were five dietary treatments with each being replicated eight times over four time periods. Dietary treatments represented a positive control where the grower diet was offered through the entire finisher period or the diet changed from a grower to a finisher diet when average pig weight in the pen was either 40, 50, 60 or 70kg. There were 15 pigs per pen and within time periods pens of pigs were balanced for weight and gender (entire boars and gilts). The grower diet was a commercial mix and contained CP 185 g/kg, total lysine 12.5 g/kg, DE 14.6 MJ/kg. The finisher diet was also a commercial mix and contained CP 170 g/kg, total lysine 10 g/kg, DE 13.8 MJ/kg. Diets were offered in dry pelleted form through single space wet and dry feeders with one feeder per 15 pigs. Pens of pigs were weighed at the start (approximately 13 weeks of age) and every two weeks until the first pigs went for slaughter after which they were weighed every week. Pigs were weighed individually when being sent for slaughter. Pen feed intake was recorded daily and any feed left at the end of the trial was weighed. Cold weight and back fat depth at P₂ was recorded at the factory before chill. Data was analysed using ANOVA with pen being the experimental unit and start weight was used as a covariate.

Results Average daily feed intake was not significantly different ($P>0.05$) over the finishing period (37 to 118kg) and averaged 2.48kg/day. There was a significant quadratic response ($P<0.05$) of diet on growth rate with growth rate increasing as transfer weight increased to 60kg but numerically decreasing again when transfer weight was 70kg (Table 1). FCR showed a similar quadratic response ($P<0.01$) with FCR improving as transfer weight increased to 60kg but no further improvement was noted when transfer weight was 70kg (Table 1). There was no difference in back fat depth at P₂ across treatments with the average being 13.8mm (sed 0.46). The growth rate and FCR of pigs offered the positive control was similar to those pigs whose diet changed at 60 or 70 kg. When offered the grower diet (within treatments 50, 60 and 70) the daily gain of pigs averaged 1099 g/d and their feed conversion ratio averaged 1.93. When these pigs moved to the finisher diet (within treatments 50, 60 and 70) their growth rate averaged 1017 g/d and FCR was 2.66. Using a differential of £30/tonne the cost per kg gain followed the same pattern as the performance figures with changing diet at 60 kg being optimum at 49.6p/kg while changing before or after 60 kg averaged 50.5p/kg.

Table 1 Pig performance when pigs changed from a grower diet to a finisher diet at 40, 50, 60 or 70kg or were offered the grower diet throughout finishing.

	Weight of diet change (kg)				P Values				
	40	50	60	70	+ve	SED	Treatment	Linear	Quadratic
FCR	2.51 ^b	2.48 ^b	2.41 ^{ab}	2.40 ^{ab}	2.33 ^a	0.055	0.017	0.874	0.005
ADG (g/day)	984 ^a	998 ^{ab}	1061 ^c	1031 ^{bc}	1055 ^{bc}	22.0	0.002	0.413	0.013
ADFI (g/day)	2467	2461	2554	1476	2459	36.7	0.150	0.256	0.611
Cost/kg gain (p)	50.4	50.0	49.6	50.9	54.0				

Conclusion Pigs offered the finisher diet from 40 kg had the same average daily feed intake as pigs offered the grower diet throughout the finishing period. However due to the different nutrient levels in these diets pigs which were offered grower diet throughout consumed 6% more energy and 20% more lysine and as a result increased growth rate by 73 g/d and reduced FCR by 0.17 units. This study suggests that offering the more nutrient dense grower diet to 60kg will optimise herd performance and economic return.

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An association analysis of sow live-weight and back-fat depth as indicators of reproductive performance

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Application Live-weight and back-fat depth at service may be less critical than previously thought to reproductive success in modern sow genotypes.

Introduction The modern sow can produce up to 29.6 pigs/sow/year (BPEX, 2015). High prolificacy increases the likelihood of stillborn and the proportion of low-birth weight, unviable piglets. For this reason there is a renewed focus on the nutrition of highly prolific sows during gestation. To this end it is important to determine appropriate targets for sow live-weight and back-fat depth during gestation. The objective of this study was to quantify the association of both sow live-weight and back-fat depth on litter size and numbers born alive by an association analysis using data collected during 8 feeding experiments.

Material and methods Data were available from feeding experiments conducted at Teagasc Pig Development Department, Moorepark, Co. Cork, Ireland, between 1999 and 2010. Sows and piglets originated from 8 studies which evaluated gestation and lactation diet composition, feed allowance and timing during gestation. A total of 898 sow gestation and lactation records were available for analysis; phenotypes available included total born (TB) and born alive (BA). Sow live-weight and back-fat depth, recorded using a back-fat scanner at the P2 site (65 mm from the edge of the dorsal mid-line, at the level of the last rib), were taken at service, day 25 (d25), 50 (d50), 80 (d80), 110 (d110) and weaning. The association of both total born and born alive (dependent variables) with both sow live-weight and back-fat depth (independent variables) was determined separately using mixed models; parity, month of farrowing and year of farrowing were included as fixed effects while sow was included as a repeated effect, using an appropriate covariance structure for records within sow. In a separate analysis the association of total born and born alive with sow live-weight was quantified, with back-fat depth also included as a fixed effect in the model.

Results The average total born and born alive across all sows in the data set was 12.6 and 11.5 respectively. Sow live-weight and back-fat depth at service were not associated ($P>0.05$) with total born or born alive (Table 1). This was also the case on day25, 50 and 80 for sow live-weight and day25 and 50 for back-fat depth. With regard to sow live-weight there was a highly significant association with litter size on day110, even when sow live-weight was adjusted for back-fat depth (Table 1). Sow back-fat depth was negatively associated with both total and live born during both late gestation and at weaning. At weaning there were also significant negative associations between sow live-weight and total born and born alive. This was not apparent for sow live-weight when it was adjusted for back-fat depth.

Conclusion The results reflect the expected association between the sow's litter size and her weight and back-fat depth in late gestation and at weaning. However, within the range of sows studied, neither live-weight nor back-fat at service was associated with subsequent litter size. The majority of sows in this work were representative of maternal genetics between 2005 and 2010 and this study would suggest that for these sows their metabolic state at service was not important in driving litter size at birth.

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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Table 1 Regression coefficients (standard errors) of the association of sow live-weight and back-fat depth on reproductive performance

Variable	Total Born	Born Alive
Sow weight (kg x10)		
Service	0.063(0.075)	0.025(0.074)
d25	0.095(0.104)	0.044(0.103)
d50	-0.002(0.079)	-0.030(0.076)
d80	0.071(0.081)	-0.039(0.080)
d110	0.162(0.044)***	0.117(0.042)**
Weaning	-0.101(0.050)*	-0.134(0.049)**
Sow weight adjusted for back-fat (kg x10)		
Service	0.010(0.125)	-0.077(0.123)
d25	0.101(0.124)	0.042(0.122)
d50	-0.044(0.098)	-0.087(0.094)
d80	0.229(0.103)*	0.120(0.102)
d110	0.338(0.055)***	0.256(0.053)***
Weaning	0.022(0.065)	-0.021(0.063)
Sow back-fat depth (mm)		
Service	-0.016(0.049)	-0.042(0.048)
d25	0.020(0.047)	0.012(0.047)
d50	0.010(0.043)	-0.004(0.043)
d80	-0.057(0.042)	-0.094(0.042)*
d110	-0.066(0.028)*	-0.060(0.027)*
Weaning	-0.126(0.036)***	-0.144(0.036)***

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

Investigating physiological measures of lifetime welfare in slaughter pigs

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Application Elevated levels of haptoglobin (Hp), C-Reactive Protein (CRP) and hair cortisol sampled from pigs at slaughter were associated with health and welfare problems identified throughout life and therefore may be useful measures to integrate into abattoir-based welfare inspections.

Introduction Physiological measures indicative of medium to long-term welfare could form part of abattoir-based welfare assessments of farm animals. Individual physiological measures vary in how they respond to compromised health and welfare and it may therefore be beneficial to assess a combination of measures to gain a holistic view of herd health status. The aim of this study was to determine the extent to which Hp and CRP, measured from exsanguinated blood, and hair cortisol levels, sampled one day prior to slaughter, reflected welfare problems identified throughout the pigs life. Welfare assessments were based on lesions and health status (e.g. lameness and coughing) and qualitative behavioural assessments (QBA; Wemelsfelder, 1997).

Material and methods A total of 65 pigs from an experimental farm were used in this study. Data were collected between June and December 2014. Welfare assessments were carried out on each pig at 7 and 9 weeks of age (in the weaning unit), and at 10, 15, and 20 weeks of age (in the finishing unit). On each observation week, skin lesions scores were recorded from [0] no injuries to [5] many very big, deep and red lesions in 12 body zones. Tail lesions were also recorded ([0] no evidence of tail biting to [4] partial or total loss of tail), as was presence or absence (P/A) of different health issues (lameness, bursitis, hernias, rectal prolapse, scouring, coughing, poor body condition and aural hematomas). QBA scores were also made by scoring each pig on 5 positive (e.g. 'happy') and 5 negative (e.g. 'frustrated') behavioural descriptors. One day prior to slaughter, hair was collected from the rump area of each pig using a surgical scissors. Samples were washed and treated using the methods described by Davenport *et al.* (2006) with the following adjustments; hair samples were washed in isopropanol several times. Each sample was dried and cut into 1-2 mm sections. 60 mg of the sample was weighed into a 4 ml tube. The samples were pulverized, 2 ml methanol added and the vial incubated overnight at room temperature with constant gentle agitation for steroid extraction. Following extraction, methanol was transferred to a fresh vial, evaporated in a scanvan vacuum centrifuge and stored at -80°C . Subsequently, Enzyme Immunoassay analysis was performed as per Davenport *et al.* (2006). On the day of slaughter, blood samples were taken from each pig at the point of exsanguination. Concentrations of Hp and CRP were determined in duplicate using commercial assay kits in accordance with the manufacturer's instructions. QBA scores were analysed using principal components analysis. A number of fixed effect models were used to examine the effects of control variables (live weight, average daily weight gain, sex, weaning pen enrichment, pen size), and the following predictor variables; skin lesion scores, tail lesion scores, the P/A of each individual health issue, the P/A of at least one health issue in the lifetime, and QBA scores, on levels of serum Hp and CRP, and hair cortisol. All statistical analyses were carried out using SPSS version 20.

Results Pigs with tail lesions at any stage on farm had greater hair cortisol levels than those with no tail lesions (tail lesions: 50.49 ± 2.55 pg/mg, no tail lesions: 38.88 ± 3.18 pg/mg, $F = 8.94$, $P < 0.05$). Pigs lame on at least one observation week on farm had greater cortisol levels than those with no lameness on farm (lame: 51.91 ± 2.88 pg/mg, not lame: 43.55 ± 2.41 pg/mg, $F = 4.67$, $P < 0.05$). Pigs with moderate or severe tail lesions on farm had greater Hp levels than those with no or mild tail lesions (moderate/severe: 1.22 ± 0.41 mg/ml, none/mild: 0.70 ± 0.08 mg/ml, $F = 4.48$, $P < 0.05$). As 'good welfare' QBA scores decreased, Hp levels tended to increase ($F = 3.721$, $P = 0.062$) while CRP levels clearly increased ($F = 15.97$, $P < 0.001$). No control variables contributed significantly to explaining Hp, CRP or hair cortisol levels.

Conclusion Physiological measures recorded at slaughter differed depending on whether or not the pig had been recorded with different welfare-related conditions during its life. This suggests that they may be useful indicators of lifetime welfare status, and that the use of both hair cortisol and acute phase proteins provides a more holistic picture.

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Life cycle assessment (LCA) applied to Irish commercial pig production

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Application The study was the first life cycle assessment (LCA) of Irish pig production, providing producers and policy makers with insights into alternative strategies for reducing the sector's environmental footprint.

Introduction Intensive pig production is widely regarded as having a negative impact on the environment. One method for assessing a livestock system's environmental performance is LCA, which models systems' inputs and outputs and calculates potential impacts on various categories such as global warming potential (GWP). This paper applies LCA to Irish pig production under current and hypothetical production scenarios and highlights potential improvement opportunities.

Material and methods Figure 1 displays the system boundary from the production of crops for animal feed, to the slaughterhouse gate. The functional unit was 1 kg of pig meat leaving the slaughterhouse. Data on feed production were supplied by two different Irish feed mills (one on-site mill and one off-site mill). To build an inventory of Irish pig production, a combination of herd performance data for 2012 from Teagasc, and environmental reports from the Irish Environmental Protection Agency (EPA) were utilised. Four scenarios representing typical pig production in Ireland were created, depending on the source (and hence ingredients) of feed and herd feed efficiency (feed required for weight-gain). For comparative analysis, four

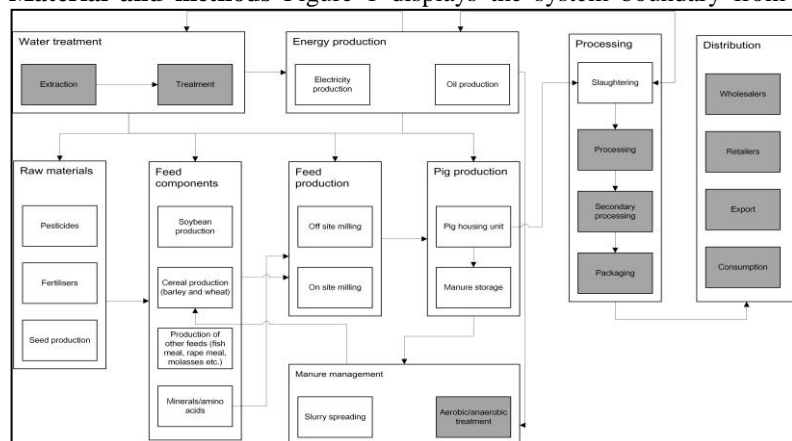


Figure 2 System boundary of the LCA model. Excluded processes are in grey.

replicate scenarios were also created to examine the effects of replacing internationally imported wheat and barley (the typical source in Ireland) with domestically produced wheat and barley. Data for feed ingredients were sourced from Blonk *et al.* (2014) and Weidema *et al.* (2013). The three impact categories addressed were global warming potential (GWP), acidification potential (AP), and eutrophication potential (EP).

Results The results suggest that animal feed consumption efficiency and the inclusion of Irish barley and wheat affect GWP, AP and EP considerably. For example, throughout the eight scenarios, GWP ranged from 2.87 to 3.36 kg CO₂-eq kg⁻¹ pig meat, AP ranged from 54.80 to 63.30 g SO₂-eq kg⁻¹ pig meat, and EP ranged from 29.10 to 34.70 g PO₄³⁻-eq kg⁻¹ pig meat. For each of the scenarios representative of current pig production practices (using imported wheat and barley), the alternative scenarios with Irish barley and wheat produced lower GWP, AP and EP. Table 1 provides results from the off-site mill with average feed efficiency. Overall, results were largely in agreement with previous European research (e.g. Nguyen *et al.*, 2011; Reckmann *et al.*, 2013).

Table 2 Results from the scenario of the off-site mill with average feed efficiency. This scenario excludes Irish wheat and barley. Numbers are expressed as kg⁻¹ pig meat leaving the slaughterhouse.

Impact Category	Total	Feed	Housing	Slaughtering
GWP (kg CO ₂ -eq)	3.36	2.02 (60%)	1.18 (35%)	0.16 (5%)
AP (g SO ₂ -eq)	63.30	27.87 (44%)	35.13 (55%)	0.30 (< 1%)
EP (g PO ₄ ³⁻ -eq)	34.70	22.79 (66%)	11.31 (32%)	0.60 (2%)

Conclusion LCA was applied to Irish pig production to determine the environmental performance of the sector. The results from the study largely fall within ranges from previous European LCA studies of swine production. Feed efficiency was the greatest factor determining environmental performance. In addition, the inclusion of Irish wheat and barley (results not shown) could potentially lower the environmental footprint of the sector significantly.

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The effects of dietary brown seaweed (*Ascophylum nodosum*) supplementation on alleviating heat stress in farrowing Landrace x Large White sows

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Application Sows supplemented with brown seaweed had lower core body temperatures, and produced piglets with higher growth rates, than sows fed basal feed, addressing increasing global heat stress concerns whilst maintaining profit.

Introduction Heat stress induces a number of adverse effects on afflicted animals, ranging from reduced fertility and milk production to growth retardation and immunosuppression, which have negative consequences for welfare and production (Kuczynski *et al.*, 2011). This is especially evident in pigs, which have low sweating capacities and thus find it more difficult to maintain thermal homeostasis. The vitamin niacin is known to induce vasodilation, increasing blood flow to the periphery of the body and thus promoting radiant heat loss via the skin (Zimbelman *et al.*, 2010). This remains untested in pigs, however, despite holding a number of advantages over traditional air and water cooling systems, which are costly and ineffective at reducing core temperatures. The aim of the present study was to examine the effects of dietary supplementation of niacin via fresh ground seaweed on farrowing sows under conditions of heat stress, and the subsequent effects on growth rate of piglets.

Material and methods Seventeen Landrace x Large White sows were used in this 60d trial. Ten sows (Batch 1) spread between three rooms were sampled during the initial 30d, whereas seven (Batch 2) spread between two rooms were sampled during the subsequent 30d. Of the seventeen sows, nine (five in Batch 1, four in Batch 2) were provided with unaltered basal feed (Normal Group), whilst eight (five in Batch 1, three in Batch 2) were provided with dried, ground brown seaweed (*Ascophylum nodosum*) at 50g kg⁻¹ basal feed (Seaweed Group). Ambient temperatures of farrowing house were recorded by temperature loggers. Sow skin and rectal temperatures were taken once per three days – at 16:30 to 17:00 – via thermal imaging camera and rectal thermometer, respectively. Piglet birth and weaning weights were recorded as per normal production processes. The data were tested for normality and homogeneity of variances. Rectal and skin temperatures were subsequently analysed by a Student's t-test, whilst piglet weights were analysed by a Mann-Whitney U test.

Results Average ambient temperature of pig housing was 20.67 °C, with an average maximum temperature of 27.72 °C and an average minimum temperature of 17.22 °C. No significant differences in temperatures were found between any of the five experimental rooms ($P > 0.05$). Seaweed Group sows exhibited a significantly lower average rectal temperature than the Normal Group sows ($P < 0.001$). Average skin temperatures for the Seaweed Group were significantly lower than in the Normal Group ($P < 0.01$). Piglet birth and weaning weights did not significantly differ, regardless of sow feed supplementation ($P > 0.05$). However, the average growth rates from birth to weaning of piglets from Seaweed Group sows was significantly higher than in piglets from Normal Group sows ($P < 0.05$).

Table 1 Average skin and rectal temperatures of sows fed basal (Normal) and supplemented (Seaweed) diets

Group	Rectal temperatures			Skin temperatures		
	T (°C)	SEM	P	T (°C)	SEM	P
Normal	38.60	0.057	< 0.001	33.47	0.238	< 0.01
Seaweed	37.93	0.054	< 0.001	34.40	0.216	< 0.01

Table 2 Average birth and weaning weights, and daily weight gain, of piglets from the Normal and Seaweed Groups

Group	Birth weights			Weaning weights			Growth rates		
	W (kg)	SEM	P	W (kg)	SEM	P	DWG (kg day ⁻¹)	SEM	P
Normal	1.63	0.021	> 0.05	7.97	0.086	> 0.05	0.22	0.003	< 0.05
Seaweed	1.50	0.025	> 0.05	8.63	0.104	> 0.05	0.25	0.004	< 0.05

Conclusion Results indicate that a 50g kg⁻¹ basal feed supplementation of brown seaweed reduces core temperature in farrowing sows during the late summer period. The increase in skin temperatures due to the natural vasodilatory effects of niacin was expected, and could benefit from additional water cooling to maximise efficiency. The increased growth rate of piglets from supplemented sows suggests improved milk quality and/or quantity. Further investigation into sow water/feed intake, breed differences, maternal behaviour, and financial feasibility will enhance opportunities for widespread use.

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Organic selenium and vitamin E supplementation improves feed conversion efficiency in heat stressed dairy cows

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Application Improving tolerance to heat stress (HS) in intensively farmed dairy cows by using supplemental anti-oxidants.

Introduction With increases in the frequency, intensity and duration of heat waves forecast plus increased participation in tropical agriculture, HS is both a current and emerging problem. To control body temperature during HS, cows reduce metabolic heat production by lowering feed intake and increase energy expenditure to dissipate heat. This results in a net loss in production efficiency that is characterised by increased oxidative stress. With milk production trending towards intensive production systems feeding TMR rather than pasture, the aim of this experiment was to investigate the effects of anti-oxidant supplementation on milk production and oxidative stress in TMR fed dairy cows.

Material and methods Sixteen Friesian x Holstein cows (ca. 120 DIM) were randomly allocated to control (0, $n=6$), “half” ($n=5$) or “full” ($n=5$) dose of mixed organic selenium (yeast) and vitamin E (15 mg and 2000 IU/d respectively at full dose). Cows were acclimatised to climate-controlled rooms and diets for one week before the following climates- 7d Thermoneutral (TN, 18°C), 7d Heat Treatment (HT, cyclic 8h 35°C/28°C) and 5 d recovery (TN conditions). Milk yield and feed intake were measured twice daily and FCE calculated as g milk/kg DMI per day. The oxidative status of the cows was assessed from plasma samples collected at d 1,5,8,10,12,14,16 and 18. Kits were used to assess reactive oxygen metabolites (ROMs, oxidant production), biological anti-oxidant potential (BAP, anti-oxidant capacity) and the oxidant stability index (OSI) was calculated as the ROMs/BAP. Furthermore glutathione content was quantified. Data were analysed for the effects of anti-oxidant dose (A, control, half or full), the day of experiment (D) and the interaction (A*D) using a REML with blocking on cow and replicate.

Results As expected the HS conditions in week two of the experiment reduced feed intake and milk yield compared to TN ($P<0.001$ for both). This resulted in reduced FCE, reflecting that the cows were diverting energy from production to heat dissipation ($P<0.001$). Overall anti-oxidant supplementation did not improve milk yield or feed intake during HS. However the half dose of anti-oxidants tended to have higher FCE during HS ($P=0.089$), which may reflect less energy being diverted to heat dissipation and improved tolerance to heat stress. HS reduced BAP ($P=0.020$) and there was a slight but not statistically significant increase in ROMs ($P=0.098$). The production of oxidants was unchanged with anti-oxidant diets (ROMs $P=0.56$), however the BAP was increased overall ($P=0.042$). Consequently the OSI increased sharply during HS ($P=0.012$) in control cows, with some buffering by anti-oxidants ($P=0.062$) and tendency to be lower overall during the experiment ($P=0.069$). The improvement in BAP was in part due to increase glutathione content, which was reduced in the control and to a lesser degree the half but not full dose during HS ($P=0.022$). As glutathione is synthesised by the selenoprotein glutathione peroxidase, this result indicates anti-oxidant activity mediated by the Se supplementation.

Conclusion Lactating dairy cows are prone to heat and oxidative stress, resulting in reduced productivity. These data show that supplementation of organic Se and vitamin E reduces oxidative stress. Anti-oxidants did not substantially improve milk production when lactating dairy cows were experiencing HS, however they appear to be diverting less energy from production as indicated by improved FCE which indicates improved tolerance to HS.

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Pre-treating the ration with a crude fermentation extract derived from *Trichoderma* improved the dry matter intake and milk yield of first lactation heifers

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Application Pre-treating the ration with *Trichoderma* extract led to an increase in D-value and ME of the ration as measured by NIR. This ration when fed to primiparous cows resulted in increased DMI, milk yield and better fertility.

Introduction Feeding rations which have been pre-treated by fermentation extracts derived from *Trichoderma* have been shown to improve the digestibility of the ration through the action of the extract on the surface of the fibre (McAllister *et al*, 2001). Lag time to digestion is reduced, and faster colonisation by the rumen microflora once the fibre has been ingested has been observed (Morgavi *et al*, 2000). This has led to improvements in animal performance and better feed efficiency (Holtshausen *et al*, 2011). The aim of the current study was to evaluate the effect of pre-treating a ration with a novel product, a crude fermentation extract derived from *Trichoderma*, on the performance of first lactation heifers during early lactation.

Material and methods Fifty first lactation heifers were assigned to two groups Control (CON) or treated (*Trichoderma* extract) on the basis of milk yield and dry matter intake measured over an initial 3 week co-variate period. The crude extract was applied to the ration daily (750 ml/ T DM), approximately 1 h before feeding. Samples of treated and untreated TMR were analysed by NIR to determine whether there was any effect on the ration. Individual milk yield and pen intake was measured daily for the duration of the trial, which lasted 84 days. Fertility records were also maintained. Data was analysed by the Mixed Procedure from SAS. Significance was declared at $P < 0.05$, and numerical tendencies $P < 0.1$.

Results Analysis of the treated and untreated TMR samples by NIR demonstrated that the predicted D-value was increased from 63% to 67% and the predicted ME value from 10.5 to 11.1 MJ/kg DM. Dry matter intake was increased on average by 2 kg/day over the duration of the trial in the treated group ($P < 0.05$). Milk yield was numerically increased in the treated group compared to the control over the duration of the trial (28.2 vs 27.6, +0.6 litres/ day, $P = 0.09$). This positive effect was further improved in the last three weeks of the trial when milk yield was significantly increased by 1.4 litres vs CON (27.7 vs 26.3 litres/day, $P < 0.05$). Fertility was also improved in the animals eating the ration pre-treated with *Trichoderma* extract, with 84% of animals confirmed pregnant compared with 64% in the control group 4 months after starting the trial. Insemination success rate was also higher, with the animals being fed feed pre-treated with *Trichoderma* extract requiring 2.3 inseminations versus 3.2 in the control group.

Conclusion Pre-treating the ration with a crude fermentation extract derived from *Trichoderma* led to an increase in the predicted digestibility and ME of the ration. The increase in digestibility significantly stimulated DMI. The extra intake and resulting available energy was used in a variety of ways by the treated group, resulting in increased milk yield and some positive effects on fertility.

Acknowledgements The authors would like to thank the staff at ADAS Drayton for monitoring the day to day running of the trial and AB Vista for providing financial support.

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Non-invasive indicators of rumen function and stress in dairy cows

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Application By understanding more about rumen function and the animal's response to changes, we can better understand true feed efficiency within a dairy herd.

Introduction Supporting correct rumen function enables a healthy cow to be feed efficient and produce more milk. Rumen function, efficiency of digestion and stress were investigated using non-invasive indicators: feed intake, milk yield, rumination rate, digestibility, methane emissions and faecal glucocorticoid concentration. Faecal glucocorticoids are metabolites of cortisol, so provide a non-invasive alternative to blood cortisol. The main objective of this work was to identify which animal and dietary factors, measured using non-invasive methods, are related to dietary change. The hypothesis was that a dietary change might cause a stress response in the cow that might be reflected by changes in faecal glucocorticoid concentration, rumination time, methane output, dry matter intake (DMI) and digestibility.

Material and methods An ELISA method (Mostl *et al.*, 2002) was used to measure concentrations of the faecal glucocorticoid metabolite 11-Oxoetiocholanolon. This assay is specific for cortisol metabolites. In the first study (n=31 cows) faecal samples were collected (via natural defecation) twice in a 24-hour period to investigate effect of time of day on faecal glucocorticoid concentration. In the second study, 26 cows were subjected to a mild stress event (routine blood sample). Faecal samples were collected before this event via natural defecation and again 10-12 hours post stress event via rectal grab sampling. In the third study (n=37 cows), responses to diet changes were recorded in four successive periods of three weeks. Dietary treatments were total mixed rations (TMR) containing distillers dried grains with solubles (DDGS) at four levels of inclusion (0, 75, 150, 225 g/kg diet DM) applied in a 4x4 Latin square design. Diets were balanced for energy and protein. Faecal samples were collected 24hr after diet change at the start of each period, and assayed for faecal glucocorticoid and acid-insoluble ash (AIA) concentrations. Rumination time, milk yield, DMI, and methane output were recorded for each individual cow throughout each period.

Results In Study 1, time of day had no effect on faecal glucocorticoid concentration. In Study 2, faecal glucocorticoid concentration increased 10-12 hours after a mild stress event ($P<0.001$). In Study 3, faecal glucocorticoid concentration increased with successive diet changes ($P<0.05$), methane was significantly lower in Period 1 than in Period 4, but rumination time, milk yield, DMI and faecal AIA were not different between periods (Table 1). There was no effect of DDGS inclusion level on faecal glucocorticoid concentration or any of the other putative indicators of rumen function.

Table 1 Effects of diet change on indicators of rumen function in four successive treatment periods

	Period				σ	P
	1	2	3	4		
Faecal Glucocorticoids (ng/g)	300	585	691	876	240.9	<0.05
Rumination time (min/d)	423	430	415	390	17.5	0.53
Milk yield (kg/d)	37.8	36.1	33.9	29.0	3.8	0.74
DMI (kg/d)	24.3	22.2	22.0	22.0	1.13	0.98
Faecal AIA (g/kg)	37.8	36.9	41.3	40.6	2.12	0.95
Methane (g/d)	373	448	425	471	42.0	<0.05

Conclusion The first two studies confirm that faecal glucocorticoid concentration is not affected by time of day, but does increase in response to mild stress. Faecal glucocorticoid concentration increased with each successive diet change in Study 3, which perhaps indicates a cumulative stress effect. There was no effect of dietary treatment, suggesting that diet change *per se*, rather than diet composition, was potentially responsible for the increase in faecal glucocorticoids. Although methane output differed between periods, other indicators of rumen function did not vary. This suggests that changes in faecal glucocorticoids were not associated with changes in rumen function. Further work will confirm if the cumulative effect of diet changes on faecal glucocorticoids is repeatable, or if the observation is an artefact of this particular experiment.

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Effects of concentrate nitrogen levels on production performance and nutrient digestibility of lactating dairy cows fed zero-grazed grass and concentrate diets

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Application Feeding dairy cows low nitrogen (N) diets on grass-based systems, without adverse effects on milk production and animal health, can increase N utilisation efficiency and reduce environment footprint for dairy production.

Introduction Nitrogen is an expensive dietary component, which when utilized inefficiently has severe implications on the environment, such as the increase of N excretion in faeces and urine, thus causing nitrate pollution to water resources and ammonia and nitrous oxide emissions to atmosphere. Optimizing N utilisation efficiency is both economically and environmentally beneficial for dairy production. The aim of the present study was to investigate the effect of concentrate N levels on feed intake, milk production and nutrient digestibility in dairy cows fed fresh grass.

Material and methods Twelve multiparous lactating dairy cows (6 Holstein and 6 Holstein Swedish Red cross) were used in a change-over design study with three dietary treatments and three 25-day periods. Cows within each breed were blocked into three groups based on live weight, milk yield and lactation stage, and then randomly assigned to treatments. Treatments were low, medium and high N concentrates (22.7, 26.1 and 29.4 g/kg DM respectively) fed at 33% dry matter (DM) intake (DMI), with 67.2% DMI being fresh perennial ryegrass. Half of the daily concentrate rations were offered at morning milking at 7am and half at afternoon milking at 3pm, while fresh-cut grass was offered at 10am each morning *ad libitum*. Herbage was harvested daily at regrowth intervals depending on the month (increasing from 22 to 30 d from June to September), thus offering grass of a similar quality that grazing animals would receive under routine management. Feed intake, milk yield and composition and nutrient digestibility were measured during the final 6 d of each period. The treatment effect was evaluated using one-way ANOVA with the effect of period being removed.

Results The ANOVA results are presented in Table 1. Concentrations of N in the total diet were 27.2, 28.0 and 28.8 g/kg DM (crude protein = 170, 174 and 180 g/kg DM) for low, medium and high N concentrate diets, respectively. Cows on medium N concentrate diets had a significantly lower body condition score than those given the low N concentrate diet ($P < 0.05$), while the former had a significantly higher live weight than those on high N concentrate diets ($P < 0.01$). However, the effect of diet was not significant on feed intake, milk yield or composition, and digestibility of energy or nutrients.

Table 1 Effects on feed intake, performance and nutrient digestibility

	Low concentrate	N concentrate	Medium concentrate	High concentrate	N SED	Sig.
Grass DM intake (kg/d)	13.8		14.0	14.1	0.23	NS
Concentrate DM intake (kg/d)	6.7		6.8	6.7	0.11	NS
Total DM intake (kg/d)	20.6		20.8	20.8	0.28	NS
Body condition score	2.37 ^b		2.29 ^a	2.33 ^{ab}	0.028	*
Live weight (kg)	580 ^{ab}		586 ^b	574 ^a	3.4	**
Energy corrected milk yield (kg/d)	27.4		27.7	28.0	0.62	NS
Milk fat content (g/kg)	42.2		41.5	41.9	0.59	NS
Milk protein content (g/kg)	36.1		36.4	36.5	0.30	NS
Milk lactose content (g/kg)	44.8		44.9	44.9	0.18	NS
DM digestibility	0.770		0.770	0.771	0.0071	NS
Digestible OM in total DM	0.718		0.718	0.718	0.0059	NS
Nitrogen digestibility	0.677		0.695	0.694	0.0132	NS
Energy digestibility	0.761		0.759	0.760	0.0076	NS
NDF digestibility	0.726		0.719	0.729	0.0093	NS

Conclusion Feeding zero-grazed lactating cows diets containing CP of 170 g/kg DM had no adverse effect on live weight or milk production within the constraints of the present study. However the results need to be evaluated in long-term studies.

Acknowledgements This study was funded by Department of Agriculture, Food and the Marine of ROI.

Note

The original version of this summary was published with S. Stergiadis's name incorrectly spelled. A notice detailing this has been published

Concentrate supplementation of a grass silage based diet during the dry period: effects on cow performance, metabolism and immune function

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Application *Pre partum* concentrate supplementation of a grass silage diet did not affect subsequent milk production, but improved neutrophil function during week 1 post calving.

Introduction The transition period, defined as the last three weeks of the dry period and the first three weeks of lactation, is characterised by significant physiological changes and an increased disease risk (Friggens *et al.*, 2004). A 'stressful' transition can increase the risk of both metabolic and infectious diseases, and may have a detrimental impact on milk production during the subsequent lactation. Therefore, nutritional and management strategies which encourage a successful transition, and result in improved performance and health during the subsequent lactation, are desirable. The present study was designed to examine the impact of offering dairy cows a concentrate supplement during the dry period on feed intake, milk production, metabolism and immune function. It was hypothesised that supplementing a grass silage with concentrates during the dry period would have a beneficial effect on immunity during the transition period.

Material and methods Multiparous Holstein Friesian dairy cows (n=51) were offered a medium quality grass silage, either alone (S) or mixed with concentrates (mean concentrate DM intake, 3.0 kg/cow/day: SC) for eight weeks *pre partum*. Cow performance was measured for the first 70 days of lactation, during which time all cows were offered a common diet comprising grass silage and concentrates (40:60 DM ratio). Blood samples taken at weeks 5, 3 and 1 prior to the predicted calving date and at weeks 1, 2, 3, 4 and 8 post-calving, were analysed for non-esterified fatty acid (NEFA) and haptoglobin concentrations, and for interferon gamma production by pokeweed mitogen (*Phytolacca Americana*) stimulated whole blood culture. Blood samples taken at week 3 prior to the predicted calving date, and at week 1 and 2 post-calving, were used to determine the *in vitro* phagocytic index of neutrophils (percentage of phagocytic positive neutrophils multiplied by their phagocytic activity). Mean weekly data were analysed using one-way ANOVA. Continuous data were analysed using REML repeated measures analysis.

Results Pre-calving treatment (S vs. SC) had no effect of on mean daily *post partum* DM intake (21.9 vs. 21.0 kg: $P=0.210$), mean daily milk yield (39.0 vs. 39.4: $P=0.830$), milk protein content (32.1 vs. 32.5 g/kg: $P=0.475$) or milk fat + protein yield (2.97 vs. 3.11 kg: $P=0.239$). Cows on treatment SC had a higher mean daily *pre partum* DM intake (10.2 vs. 12.6: $P<0.001$), gained more liveweight (36 vs. 86 kg: $P<0.001$) and body condition (0.1 vs. 0.2: $P<0.001$) *pre partum*, lost more liveweight (-11 vs. 33 kg: $P<0.001$) and body condition (0.0 vs. 0.1: $P=0.010$) *post partum*, produced milk with a higher fat concentration (44.3 vs. 46.5 g/kg: $P=0.072$), and tended to have a higher serum NEFA concentration (0.52 vs. 0.63 meq/L: $P=0.056$) *post partum*, compared with cows on treatment S. Pre-calving treatment (S vs. SC) had no effect on serum haptoglobin concentrations (0.74 vs. 0.76 mg/mL: $P=0.681$) or on interferon gamma production by pokeweed mitogen stimulated whole blood culture (7.1 vs. 8.5 ng/mL: $P=0.290$) *post partum*, although there was a significant change in these parameters over time ($P=0.005$ and $P=0.018$). Cows on treatment SC had a higher ($P=0.045$) neutrophil phagocytic index than cows on treatment S during week 1 *post partum* (Figure 1), largely due to a greater ($P=0.005$) phagocytic activity with the former treatment, although the phagocytic index was unaffected by treatment at weeks 3 pre calving and at week 2 post calving.

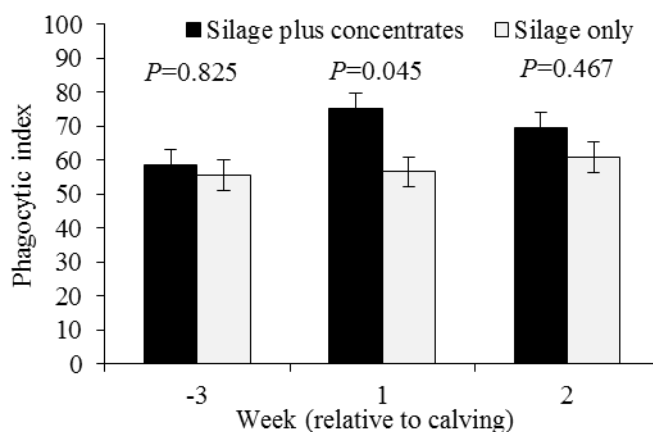


Figure 1 Effect of concentrate supplementation during the dry period on the mean phagocytic index of neutrophils

Conclusion Supplementing dairy cows with concentrates during the dry period resulted in greater liveweight and body condition gain pre-calving, greater liveweight and body condition loss post-calving, but had no effect on milk production. Cows offered concentrates during the dry period had an increased phagocytic activity at week 1 post-calving compared to those offered silage only, which supports the initial hypothesis of a beneficial impact on transition period immunity.

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Seasonal variation of milk fatty acid profiles from dairy cows crossbred with US Brown Swiss in low-input farms in Switzerland

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Application Using more Original Braunvieh (OB) genetics in crossbred dairy cows in low-input systems may improve milk FA profiles, especially during the grazing period.

Introduction The three main Brown Swiss populations in Switzerland are US Brown Swiss (BS), Braunvieh and Original Braunvieh (OB). Stergiadis *et al.* (2015) showed that summer milk fatty acid (FA) profile varied between crossbred cows, with genetics from these populations with the relative effect influenced by pasture intake. Cows' diets, in particular pasture intake, has been suggested to be the main reason for seasonal variation in milk fat composition (Butler, 2011). This study investigated the interaction of season and the contribution of BS in cows' genetics on milk FA profile, focusing both on nutritionally undesirable saturated FA (SFA) and desirable monounsaturated FA (MUFA), such as vaccenic acid (VA, t11 C18:1), and polyunsaturated FA (PUFA), such as omega-3 FA (n-3), omega-6 FA (n-6), rumenic acid (RA, c9t11 C18:1 conjugated), α -linolenic (ALN, c9c12c15 C18:3) and eicosapentaenoic acid (EPA, c5c8c11c14c17 C20:5).

Material and methods Milk samples (n=1,976) were collected from 1,220 cows on 40 low-input farms twice over one year, to represent winter (indoor feeding) and summer (grazing) periods. Animal crossbred type details were provided by Braunvieh Schweiz (Zug, Switzerland) and cows were allocated into four crossbred categories, based on the contribution of BS genes (BS1, 75-99%; BS2, 50-74%; BS3, 25-49%; BS4, 0-24%). Data on management and feeding practices were recorded at milk collection, using questionnaires. Milk FA profiles were analysed in a Varian CP-SIL 88 fused silica column (100 m x 0.25 mm internal diameter x 0.2 μ m film thickness) and 68 FA were identified using external standards. Analysis of variance, by linear mixed effects models in R, used crossbred type (BS1, BS2, BS3, BS4) and season (winter, summer) as fixed factors and cow as random factor.

Results In winter, yield was higher in BS1 cows than BS4 cows but the same was not observed in summer. When compared with milk from BS1 cows, (i) milk from BS3 and BS4 cows had more PUFA, VA, ALN and EPA and a higher n-3:n-6 ratio and (ii) milk from BS2 cows had higher contents of n-3 and ALN (Table 1). Summer milk had less SFA and ALN and more MUFA, PUFA, VA, RA and EPA (Table 1). Differences between genotypes were not consistent between seasons, generally being less extensive or showing non-significance over summer. For example, the nutritionally desirable increase of n-3:n-6 ratio when decreased BS genetics were used was more pronounced in summer, when animals grazed (Figure 1).

Table 1 Relative proportions of FA (%) in crossbred types and summer, compared with BS1 and winter respectively.

	BS2	BS3	BS4	P-value	SummerP-value
SFA	0.15	-0.05	-1.00	ns	-5.8 ***
MUFA	-0.8	-0.9	1.5	ns	15.1 ***
PUFA	2.0	5.2	6.4	***	11.8 ***
n-3	6.0	16.3	15.9	***	1.2 †
n-6	0.4	0.7	2.5	ns	0.9 ns
n-3:n-6	5.6	13.0	10.5	**	-1.0 ns
VA	1.7	11.7	11.0	*	60.5 ***
RA	2.3	1.5	2.0	ns	56.6 ***
ALN	6.8	19.0	16.8	***	-7.9 ***
EPA	-6.2	19.2	31.9	***	22.9 ***

Significant differences to BS1 or winter are shown in bold case. ***, $P < 0.001$; **, $P < 0.01$, *, $P < 0.05$; †: $0.05 \leq P < 0.10$; ns, $P \geq 0.10$.

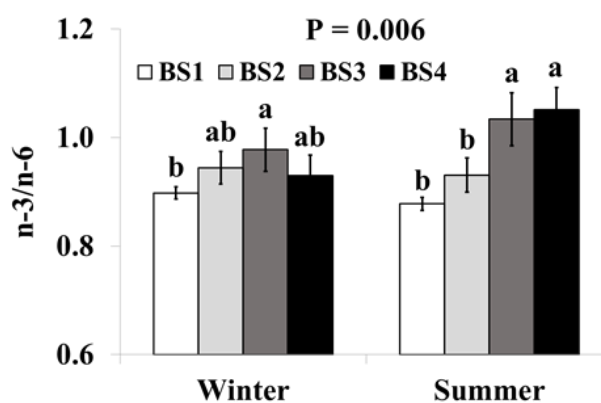


Figure 1 Interactions between crossbred type (BS1, BS2, BS3, BS4) and season on milk n-3:n-6 ratio. Bars with different lower case letter within season differ significantly.

Conclusion Using OB genetics for crossbreeding in low-input farms improved milk FA profile, with differences being more pronounced over summer possibly as a result of different diets (especially higher fresh grass intake) between seasons.

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The impact of field bean inclusion level in dairy cow concentrates on cow performance

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Application With mid lactation dairy cows, a field bean inclusion level in the concentrate of up to 500 g/kg can be adopted without loss of performance.

Introduction As a result of price volatility and instability in supply, the UK livestock sector is seeking to reduce its reliance on imported protein feedstuffs. Consequently, there is increasing interest in the use of locally-grown forage and grain legume crops. Field beans (*Vicia Faba*) are a grain legume of particular interest as they can be grown successfully in many areas across the UK. However, there is limited information available on the optimum and maximum inclusion levels of field beans in dairy cow diets. This experiment was conducted to examine the impact of four different field bean inclusion levels in dairy cow concentrates on performance parameters. It was hypothesised that up to 500g/kg of the concentrate component of the diet could be composed of field beans with no adverse effects on performance.

Material and methods Sixty multiparous Holstein-Friesian dairy cows (mean lactation number, 3.3) were used in a continuous design 10-week experiment involving four treatments. Cows were a mean of 133 (s.d., 3.6) days calved at the start of the experiment, and had a mean pre-experimental milk yield of 34 kg per day. Treatments examined (FB0, FB166, FB333 and FB500) differed in concentrate type offered, with the experimental concentrates differing in field bean (*Vicia Faba*. Var. *Fuego*) inclusion level, namely zero, 166, 333 and 500 g/kg on a fresh basis, respectively. Cows on each treatment were offered 10.0 kg concentrate per day through an out-of-parlour feeding system. Fresh silage was offered daily at approximately 09.00 h (at 1.1 of the previous days intake), with uneaten silage removed the following day at approximately 08.00 h. The ingredient composition (g/kg, fresh basis) of the concentrate offered with FB0 was as follows: maize meal, 250; soya hulls, 175; wheat, 175; soya-bean meal 150; rapeseed meal, 150; maize gluten feed, 75; mineral and vitamin mix, 25; field beans 0; while the respective values for the concentrate offered with FB500 was 167, 125, 58, 50, 50, 25, 25, and 500. The concentrate offered with FB166 was produced by mixing the FB0 and FB500 concentrate in a 1 : 2 ratio, while that offered with FB333 was produced by mixing the FB0 and FB500 concentrate in a 2 : 1 ratio. The silage component of the diet was offered via a series of feed boxes, which cows accessed via Calan gates linked to an electronic identification system, thus enabling individual cow intakes to be recorded daily. 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 10-week experimental period. Data were analysed in GenStat using an ANOVA.

Results Including field beans in dairy cow concentrates at inclusion levels of up to 500 g/kg had no effect on forage intake, milk yield, milk composition, or fat + protein yield ($P>0.05$). In addition, neither cow live weight nor body condition score at the end of the experiment were 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 10 week period

Treatment					S.E.M	P-Value
	FB0	FB166	FB333	FB500		
Silage DMI (kg/day)	12.8	12.9	13.1	12.7	0.24	0.646
Total DMI (kg/day)	21.7	21.9	21.8	21.5	0.25	0.637
Milk yield (kg/day)	28.0	29.0	27.4	28.0	0.63	0.383
Milk fat (g/kg)	43.5	44.6	45.1	44.5	0.93	0.662
Milk protein (g/kg)	34.9	35.2	34.9	34.1	0.35	0.150
Milk fat + protein yield (kg/day)	2.05	2.23	2.16	2.16	0.075	0.375
Final live weight (kg)	670	670	663	654	6.67	0.262
Final body condition score	2.56	2.52	2.44	2.43	0.062	0.364

Conclusion With mid lactation dairy cows, up to half of the concentrate component of the diet can be composed of field beans without loss of performance as hypothesised, thus providing the UK with an opportunity to substantially reduce its reliance on imported protein feedstuffs.

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

Effect of inclusion rate and chop length of lucerne (*medicago sativa*) silage in a total mixed ration with maize (*zea mays*) on milk yield and composition in dairy cattle

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Application Increasing lucerne inclusion rate in the ration from 25 to 75% of forage dry matter, combined with a short chop length, can be a viable compromise between reducing intake and milk yield but increasing feed conversion efficiency.

Introduction Utilising legume silages in dairy systems reduces the need for inorganic fertiliser use for forage production, which has both economic and environmental benefits. Combining lucerne, which is high in rumen degradable protein, with maize as a source of fermentable energy in the ration can enhance microbial protein synthesis. Furthermore, lucerne chop length can alter the rate of particle breakdown in the rumen. Longer chop lengths increase physically effective neutral detergent fibre (peNDF) and stimulate chewing and saliva production, which benefits rumen pH (Zebeli *et al.*, 2006). However, shorter chop lengths increase dry matter intake (DMI), which can increase milk yield and energy balance (Kononoff and Heinrichs, 2003). The hypothesis of this study was that lower lucerne inclusion and shorter chop length would increase DMI and milk yield.

Material and methods Sixteen Holstein Friesian dairy cows in mid lactation (144 d in milk, s.e.m. \pm 4.3) were blocked according to milk yield and randomly assigned to one of 4 initial treatments within each block in a 4x4 Latin square design experiment with 3 week periods. A total mixed ration (TMR) with 50:50 ratio on a dry matter (DM) basis of forage:concentrate blend was fed. The forage was comprised of maize and lucerne silage in proportions of either 25:75 (HL) or 75:25 (LL) on a DM basis, respectively. First cut lucerne was harvested simultaneously from the same field and stored as silage in concrete walled clamps at either a long (L) or short (S) chop length. These variables were combined in a 2x2 factorial design to give four treatments (HLL, HLS, LLL, LLS). Diets were formulated to be isonitrogenous (170g crude protein/kg DM) and contain similar levels of fibre (330 g neutral detergent fibre/kg DM). Cows were housed and individually fed in a cubicle yard and milked twice daily at 0630h and 1630h. In the final week of each period, DMI and milk yield were recorded daily and milk composition measured for four consecutive milkings. Least squares means for each cow and treatment were analysed using Mixed Model procedures.

Results Milk yield, DMI, milk protein concentration and yield (all $P < 0.001$) and milk fat yield ($P < 0.02$) were greater at the lower lucerne inclusion rate (Table 1). However, feed conversion efficiency (energy corrected milk yield/DMI) tended to be higher at the higher rate of Lucerne inclusion ($P < 0.07$). The shorter chop length of lucerne increased milk and milk protein yield ($P < 0.001$) and DMI ($P < 0.02$). There was a tendency ($P < 0.07$) for milk protein concentration to be differentially affected by chop length for LL versus HL diets.

Table 1 Effect of lucerne silage inclusion rate (IR), chop length (C) and their interaction (IRxC) on DMI and milk yield

	Diets				SEM	P value		
	LLS	LLL	HLS	HLL		IR	C	IRxC
Dry matter intake, kg/d	26.4	26.0	23.7	22.3	0.74	0.001	0.017	0.172
Milk yield, kg/d	35.2	33.9	32.5	30.6	1.04	0.001	0.001	0.449
Feed conversion efficiency, L/kg	1.34	1.37	1.42	1.42	0.040	0.068	0.744	0.698
Milk composition								
Fat, g/kg	36.9	37.7	37.6	38.3	1.37	0.263	0.242	0.705
Protein, g/kg	30.2	30.5	31.2	30.9	0.68	0.001	0.962	0.066
Fat yield, g/d	1287	1279	1211	1207	65.6	0.017	0.844	0.954
Protein yield, g/d	1101	1061	997	946	36.5	0.001	0.003	0.706

Conclusions The inclusion of lucerne silage in the TMR at the higher rate as fed in the present study reduced DMI, milk yield, and milk protein concentration. This may be partly attributable to greater rumen fill. A shorter lucerne chop length increased DMI, milk yield, and milk protein yield, which may be due to effects on rate of passage.

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The effect of white or coloured flower forage pea silage as a replacement for grass silage on the performance and whole tract digestibility of high yielding dairy cows

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Application The replacement of grass silage with forage peas reduced intake and milk performance in high yielding dairy cows, and there was no effect of pea flower colour on performance or protein digestibility.

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. Peas (*Pisium sativum*), are of interest due to their high protein content that can complement the lower protein levels found in cereal based forages such as whole crop wheat and maize silage (Fraser *et al.*, 2001). The protein in forage peas is however, highly degradable, and as a consequence may not supply sufficient undegradable protein to meet the requirements of high yielding dairy cows (Sinclair *et al.*, 2009). Tannins are polyphenolic compounds of high molecular weight that can bind to protein to form a complex that is resistant to microbial degradation at pH levels found within the rumen, but dissociate at the lower pH levels found in the abomasum (Aerts *et al.*, 1999). In forage peas the presence of condensed tannins is linked to flower colour, with coloured varieties having a lower rumen degradability than white varieties, which increases dietary undegradable protein supply (Sinclair *et al.*, 2009). The study objectives were to determine the effect of inclusion of forage peas differing in flower colour (and therefore tannin content) as a replacement for grass silage in the diet of high yielding dairy cows on intake, performance, and whole tract digestibility.

Material and methods Eighteen multiparous dairy cows that were 100 ± 23 days in milk received one of 3 diets in each of 3 periods of 28 day duration, in a Latin square design with measurements taken in the final 7 days of each period. The white and coloured pea silages were harvested at growth stage 206, the grass silage was a second cut from a ryegrass sward, and the maize silage was harvested at approximately 350 g DM/kg. The three dietary treatments were: grass and maize silage (C), low tannin pea silage and maize silage (LT), and high tannin pea silage and maize silage (HT). The proportion of grass, low tannin or high tannin pea silage to maize silage was 40:60 (DM basis), and all dietary treatments had a forage to concentrate ratio of 55:45 (DM basis), and were formulated to be isonitrogenous. The concentrates and forages were fed as a total mixed ration once daily at approximately 0800h. Cows were milked twice daily with yield recorded at each milking. Blood samples were taken once during the collection week at 0700, 0900, 1100 and 1300 h. Acid insoluble ash was used to estimate whole tract digestibility. Performance data and digestibility were analysed as a Latin square design, and blood metabolites as repeated measures using Genstat (v.17).

Results The grass, low tannin, high tannin and maize silages contained 376, 333, 389 and 365 g DM/kg, with a protein content of 178, 195, 208 and 82.5 g DM/kg respectively. Intake and milk yield was highest in cows when fed C, but there was no effect on milk fat content or weight change (Table 1). Compared with C, cows receiving LT or HT had a lower milk protein content and increased plasma urea concentrations, whereas nitrogen digestibility was lower in HT than C.

Table 1 Intake, performance, plasma urea and whole tract digestibility in dairy cows fed diets containing grass and maize silages (C), low tannin pea and maize silages (LT) or high tannin pea and maize silages (HT).

	C	LT	HT	s.e.d	P-value
DM intake, kg/d	21.4 ^b	19.9 ^a	20.7 ^{ab}	0.37	0.002
Milk yield, kg/d	40.6 ^b	38.4 ^a	38.3 ^a	0.75	0.005
Milk fat, g/kg	34.5	36.7	34.5	0.11	0.110
Milk protein, g/kg	30.8 ^b	30.2 ^a	30.1 ^a	0.26	0.045
Live weight change, kg/d	0.53	0.06	0.24	0.205	0.088
Plasma urea, mmol/l	4.10 ^a	5.34 ^b	5.43 ^b	0.301	<0.001
Digestibility, kg/kg					
Organic matter	0.564	0.468	0.430	0.063	0.103
Nitrogen	0.686 ^b	0.585 ^{ab}	0.523 ^a	0.059	0.032

^{a,b} Means with different superscript differ ($P < 0.05$).

Conclusion The inclusion of low or high tannin pea silage reduced intake, milk performance and diet digestibility. Any commercial advantage from feeding forage peas will therefore be based on savings in N from fertiliser or dietary protein.

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Effects of fresh grass, grass hay and vitamin E supplementation on the rumen function *in vitro*

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Application Fresh grass should be used as much as possible, instead of grass hay, in order to improve rumen function and to decrease the environmental impact of livestock agriculture. These findings must be further demonstrated *in vivo*.

Introduction Fresh ryegrass represents the main forage consumed by ruminants in the UK. Livestock intensification has however required the use of grass hay (or silage) during the winter period to maintain production levels, and some farmers based on intensive systems use grass hay throughout all year. Nonetheless, this decision is often arbitrary based on the farm management, breeding program and feed price, without taking in consideration the impact of the feeding strategy on rumen function, feed efficiency and ultimately on the environment. In contrast to hay, fresh grass represents an important source of vitamin E to satisfy the requirements of the ruminant. Hence the use of vitamin E as a feed additive is gaining interest to improve animal health and product quality. As a result, high levels of vitamin E are often used in ruminants regardless the type of forage used. The aim of this *in vitro* experiment was to investigate the effect of using fresh ryegrass or ryegrass hay on the rumen function and methane emissions when used alone or when supplemented with vitamin E.

Material and methods Fresh ryegrass (G) and ryegrass hay (H) from the same pasture (AberMagic) and harvest (3rd cut) were used. Fresh ryegrass was immediately frozen while hay was left to dry in the field for 2 days and finished in an air-force oven at 25°C. Experimental diets consisted of two types of forage (G vs H) supplemented with 20% of concentrate containing zero (-) or 50 IU/d (+) of added vitamin E (as dl- α -tocopheryl acetate with adsorbate). The experiment was carried out using a rumen simulation technique consisting of 16 vessels (800mL each) inoculated with rumen fluid from four rumen-cannulated cows. Forages were chopped and incubated in the fermenters for 48h. The liquid dilution rate was maintained at 3.35%/h by continuous infusion of artificial saliva. After 9 days adaptation, feed digestibility, gas and methane emissions and production of fermentation products were measured over 4 days. Microbial protein synthesis was then measured over a 2 day period using ¹⁵N as a microbial marker. Data were analysed by ANOVA, blocking by cow according to a 2x2 factorial design.

Results Feed digestibility of OM and NDF, as well as the total production of VFA was unaffected by the experimental treatments; however the hay diet increased N digestibility and propionate production. Grass hay also promoted greater production of gas and methane emissions per day or per unit of digested OM or VFA production in comparison to fresh grass. On the contrary fresh grass increased the flow of non-ammonia N and microbial N and ultimately the flow of AA available for absorption by the ruminant. Supplementation with vitamin E at 50 IU/d had no substantial effects on rumen function.

Table 1 Effect of fresh grass or grass hay and vitamin E supplementation on the rumen function *in vitro*

Forage Vitamin E	Fresh Grass		Grass Hay		SED	P-value		
	G-	G+	H-	H+		Forage	Vitamin E	Interaction
Disappearance (%)								
OM	55.7	62.1	59.4	63.1	4.68	0.494	0.162	0.692
N	69.3	73.1	77.6	78.7	2.39	0.003	0.177	0.437
NDF	43.8	52.3	50.1	53.2	5.27	0.356	0.155	0.486
Gas emissions								
Total gas (L/d)	1.72	1.71	1.82	2.03	0.09	0.009	0.167	0.133
Methane (mmol/d)	5.10	5.01	6.04	7.59	0.79	0.012	0.223	0.180
Methane (mmol/gDOM)	0.90	0.79	0.98	1.16	0.10	0.012	0.666	0.081
Methane:VFA (mol/mol)	0.16	0.15	0.17	0.21	0.02	0.033	0.405	0.069
Fermentation products								
Total VFA (mmol/d)	31.2	33.8	35.1	35.5	2.37	0.131	0.383	0.536
Acetate (mmol/d)	14.2	15.7	15.7	16.8	1.25	0.182	0.164	0.794
Propionate (mmol/d)	8.02	8.93	9.60	9.96	0.80	0.047	0.293	0.631
Butyrate (mmol/d)	4.50	4.94	5.51	5.04	0.48	0.139	0.975	0.211
Ammonia N (mg/d)	58.8	58.3	48.8	46.5	3.86	0.003	0.616	0.755
Non-ammonia N (mg/d)	140	152	122	115	6.92	<0.001	0.609	0.084
Microbial N (mg/d)	101	97.7	84.9	86.3	4.71	0.003	0.840	0.536
Microbial N : DOM (g/kg)	17.5	15.3	13.8	13.3	0.81	<0.001	0.040	0.180

Conclusions Use of ryegrass hay had a negative impact on the rumen function by increasing methane emissions (+35%) and feed proteolysis (+10%). On the contrary fresh ryegrass improved the microbial protein synthesis (+16%).

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Effect of a saponin extract from *Hedera Helix* on protozoa activity and rumen fermentation *in vitro*

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Application A saponin extract from ivy (*Hedera Helix*) could be used as a native feed additive for ruminants to reduce protozoa in the rumen increasing nitrogen utilization which may lead to an enhanced animal production.

Introduction In recent years there has been a major interest in the use of plant secondary compounds as antiprotozoal agents with a particular emphasis on saponins. Some saponin sources have been shown to modify rumen fermentation, enhancing animal production (Wina *et al.*, 2005). The most common sources of saponins used in ruminant nutrition are *Yucca schidigera*, rich in sterol saponins, and *Quillaja saponaria* which contains triterpene saponins. Fruits of common ivy or English ivy (*Hedera Helix L.*) are rich in triterpene saponins; however its potential as a feed additive for ruminants has not yet been explored. The aim of this study was to evaluate the effect of an ivy fruit extract on protozoa activity and fermentation parameters, as compared with *Y. schidigera* and *Q. saponaria* extracts.

Material and methods Ivy fruit extract, rich in saponins, was obtained after three subsequent extractions with ethanol, petrol ether and n-butanol. *Yucca* and *Quillaja* extracts were obtained from commercial suppliers. The *in vitro* effect of the three saponin extracts on protozoal activity was measured from the breakdown of [¹⁴C]labelled bacteria by rumen protozoa as described by Wallace and McPherson (1987). Strained rumen fluid from four cows (four replicates) was diluted in Simplex-type Salt Solution and incubated at 39°C with [¹⁴C]leucine-labelled *Streptococcus bovis* in the presence or absence of 1 g/L of *Yucca*, *Quillaja* or Ivy extracts. 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 measure the influence of the saponin extracts on fermentation parameters, strained rumen fluid from four cows (four replicates) was diluted 1:2 in artificial saliva solution. Aliquots of 30 mL were incubated with 0.3 g of a TMR diet (60:40, ryegrass silage: concentrate) and 1 g/L of *Yucca*, *Quillaja* or Ivy extracts, under CO₂ and at 39°C for 24 h. Volatile fatty acids and ammonia concentrations were determined and pH measured at 24 h. Data were analysed statistically by ANOVA considering treatments as main factor and cow as the block term. Fisher's unprotected least significance difference test was used to establish comparisons between treatments.

Results Protozoa activity decreased (P<0.001) in the presence of the three saponin extracts with the ivy extract having the strongest effect. Saponin extracts did not affect the concentration of total VFA although the percentages of propionate and butyrate produced increased (P<0.001) and decreased (P=0.055), respectively. The latter effect was greater in the presence of Ivy extract. Ammonia concentration was reduced (P=0.001) by 26% only in incubations with Ivy extract.

Table 1 Effect of *Yucca*, *Quillaja* and Ivy extracts on protozoa activity and rumen fermentation *in vitro*

	Control	<i>Yucca</i>	<i>Quillaja</i>	Ivy	SED	P
Protozoa activity (% radioactivity/h)	4.08 ^c	3.19 ^{bc}	2.69 ^b	0.65 ^a	0.409	<0.001
pH	6.30 ^c	6.25 ^b	6.22 ^{ab}	6.20 ^a	0.010	<0.001
NH ₃ -N (mmol/L)	12.29 ^b	11.35 ^b	12.16 ^b	9.14 ^a	0.426	<0.001
Total VFA (mmol/L)	75.16	80.60	77.95	80.89	3.490	0.371
Acetate (%)	59.73 ^{ab}	60.30 ^b	60.49 ^b	59.06 ^a	0.403	0.023
Propionate (%)	20.53 ^a	21.59 ^c	21.10 ^b	23.50 ^d	0.191	<0.001
Butyrate (%)	14.83 ^b	13.78 ^{ab}	13.94 ^{ab}	13.24 ^a	0.483	0.055
BCFA (%)	3.12 ^b	2.67 ^a	2.73 ^a	2.53 ^a	0.099	0.001

Conclusion The effect of Ivy extract on protozoa and fermentation parameters was greater than that observed for *Yucca* and *Quillaja* extracts. Ivy extract had a strong antiprotozoal effect, reducing ammonia concentration and shifting the fermentation pattern towards higher propionate and lower butyrate. 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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Reduction of anti-nutritional enzyme inhibitors present in Adzuki bean and Soyabean by using different processing methods

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Application Utilising processing to reduce the quantities of anti-nutritional enzyme inhibitors in underutilised legumes will improve the digestion of plant nutrients when they are incorporated into aquafeed.

Introduction The progressive increase in demands of the carnivorous fish farming industry for the finite resource of fish meal and fish oil has stimulated investigations into the potential of using plant-based aquafeed as an alternative (Hill *et al.*, 2013). Although legumes can be a source of protein and other nutrients, they also contain anti-nutritional factors (ANFs) such as enzyme inhibitors that might reduce nutrient availability, which may impact on their use as aquafeed. Hence, prior to the application of underutilised legumes as potential aquafeed ingredients, the aim of this study was to identify the most effective processing methods for reducing the activity of trypsin, chymotrypsin and α -amylase inhibitors found in adzuki bean and the soyabean.

Material and methods Adzuki bean or soyabean were subjected to 12 combinations of processing methods which included soaking for 6 h (S) at room temperature (25°C), wet heating at 50°C for 1 h (WH), autoclaving at 121°C for 15 min at 15 psi (A) or dry freezing at -80°C for 24 h (DF). The raw and processed legumes were then analysed for trypsin, chymotrypsin and α -amylase inhibitors, using enzymatic assays. IBM SPSS Statistics software (Version 20, IBM Corporation, USA) was used to perform Analysis of Variance (ANOVA) and *post hoc* Duncan's Multiple Comparison test, significance was accepted at $P < 0.05$.

Results Among the 12 combinations of processing methods, only the dry freezing and autoclaving (DF + A) method reduced trypsin inhibitory activity to no detectable level in adzuki bean and soyabean. In adzuki bean, 6 methods (including DF + A) reduced chymotrypsin inhibitor to no detectable levels, but only A + DF also achieved this for soyabean. For α -amylase inhibitor the processing methods reduce adzuki bean α -amylase inhibitory activity by 28% to 98%, whereas for soyabean, only 5 methods were successful in reducing the α -amylase inhibitory activity while 3 methods (S+WH, WH+DF and WH+S) had enhanced the inhibitory activity.

Table 1 The trypsin, chymotrypsin and α -amylase inhibitory activity of the adzuki bean and soyabean

Processing methods	Trypsin inhibitory activity (TIA/mg)		Chymotrypsin inhibitory activity (CIA/mg)		α -amylase inhibitory activity (AIA/mg)	
	Adzuki bean	Soyabean	Adzuki bean	Soyabean	Adzuki bean	Soyabean
Raw	0.07 ± 0.01 ^{hi}	0.06 ± 0.01 ^{cde}	1.07 ± 0.05 ^b	1.53 ± 0.03 ^{cd}	0.124 ± 0.002 ^b	1.991 ± 0.149 ^a
S + WH	0.15 ± 0.01 ^{de}	0.03 ± 0.01 ^{de}	0.89 ± 0.08 ^{bc}	2.01 ± 0.15 ^b	0.058 ± 0.001 ^d	2.711 ± 0.266 ^{cd}
S + A	0.06 ± 0.02 ⁱ	0.12 ± 0.02 ^{bc}	nd ^f	0.47 ± 0.06 ^f	0.019 ± 0.003 ^f	0.013 ± 0.002 ^f
S + DF	0.31 ± 0.03 ^a	0.18 ± 0.01 ^a	0.18 ± 0.06 ^{cd}	3.01 ± 0.14 ^a	0.077 ± 0.004 ^c	1.958 ± 0.138 ^e
WH + A	0.12 ± 0.01 ^{efg}	0.11 ± 0.02 ^{bc}	nd ^f	0.26 ± 0.07 ^{fg}	0.028 ± 0.001 ^{ef}	0.030 ± 0.000 ^f
WH + DF	0.20 ± 0.00 ^{bc}	0.12 ± 0.02 ^{bc}	1.33 ± 0.11 ^a	1.88 ± 0.20 ^b	0.060 ± 0.003 ^d	2.953 ± 0.111 ^c
WH + S	0.11 ± 0.01 ^{fg}	0.03 ± 0.01 ^{de}	0.54 ± 0.09 ^e	1.74 ± 0.14 ^{bc}	0.058 ± 0.005 ^d	2.576 ± 0.056 ^d
A + DF	0.14 ± 0.02 ^{def}	0.09 ± 0.01 ^{bcd}	nd ^f	nd ^h	0.034 ± 0.002 ^e	0.017 ± 0.002 ^f
A + WH	0.10 ± 0.01 ^{gh}	0.07 ± 0.01 ^{cd}	nd ^f	0.37 ± 0.09 ^f	0.037 ± 0.002 ^e	0.024 ± 0.002 ^f
A + S	0.11 ± 0.01 ^g	0.06 ± 0.02 ^{cde}	nd ^f	0.83 ± 0.09 ^e	0.038 ± 0.003 ^e	0.023 ± 0.002 ^f
DF + S	0.23 ± 0.01 ^b	0.15 ± 0.02 ^{ab}	0.67 ± 0.13 ^{de}	1.28 ± 0.14 ^d	0.089 ± 0.003 ^b	4.944 ± 0.254 ^a
DF + WH	0.17 ± 0.01 ^{cd}	0.16 ± 0.05 ^{ab}	0.76 ± 0.11 ^{cd}	2.03 ± 0.05 ^b	0.086 ± 0.006 ^{bc}	4.231 ± 0.101 ^b
DF + A	nd ^j	nd ^e	nd ^f	0.21 ± 0.05 ^{fg}	0.037 ± 0.002 ^e	0.031 ± 0.003 ^f

Notes: values are presented in mean ± SEM (n=9). nd represented not detectable. ^{a-j} Within a column, values with different superscripts are significantly different at $P < 0.05$.

Conclusion Out of 12 combinations of processing methods, DF + A method is the most effective method to reduce trypsin inhibitor in adzuki bean and soyabean. Most of the methods used were able to reduce the chymotrypsin and α -amylase inhibitors significantly. Further study will need to be carried out to incorporate these processed legumes into aquafeed to allow further investigation on the digestibility of this legume by carnivorous fish.

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Effect of Hederagenin bissuccinate, a chemically modified triterpene, on protozoa and rumen fermentation *in vitro*

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Application Hederagenin bissuccinate, a novel chemically modified triterpene, could potentially be used in ruminant diets as an effective defaunation agent to, ultimately, increase nitrogen utilization and enhance animal production.

Introduction In recent years there has been a major interest in the use of plant secondary compounds as antiprotozoal agents with a particular emphasis on saponins. 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 a chemically modified saponin without the natural glycoside bond would not be enzymatically cleaved, maintaining its activity. The aim of this work was to test the effect of hederagenin bissuccinate, a compound synthesized from the aglycone hederagenin which constitute saponins in Ivy (*Hedera Helix*), on rumen protozoa and fermentation parameters.

Material and methods The *in vitro* effect of hederagenin bissuccinate on protozoal activity was measured from the breakdown of [¹⁴C] labelled bacteria by rumen protozoa as described by Wallace and McPherson (1987). Strained rumen fluid from four cows (four replicates) 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 bissuccinate at 0.5 or 1 g/L. Samples were taken at 0, 1, 2, 3 and 4 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 measure the influence of hederagenin bissuccinate on fermentation parameters, strained rumen fluid from four cows (four replicates) 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 on DM basis) under CO₂ and at 39°C for 24 h. Treatments consisted of control incubations (diet only) and incubations with hederagenin bissuccinate at 0.5 and 1 g/L. Protozoa numbers, volatile fatty acids and ammonia concentrations were determined and pH measured at 24 h. Data were analysed statistically by ANOVA with treatments as main factor and cow as the block term. Fisher's unprotected LSD test was used to establish comparisons between treatments.

Results The rate of bacterial degradation and protozoa numbers after 24 h incubation resulted decreased (P<0.001) with hederagenin bissuccinate at 0.5 and 1 g/L. Ammonia concentration was reduced (P=0.001) by 34-42% in the presence of hederagenin bissuccinate compared with the control. Hederagenin bissuccinate caused an increase in the concentration of total VFA (P=0.018), increasing propionate (by 28-43%) and decreasing butyrate (by 34-43%) (P<0.001).

Table 1 Effect of hederagenin bissuccinate on protozoa and *in vitro* rumen fermentation

	Concentration (g/L)			SED	P
	0	0.5	1		
Protozoa activity (% radioactivity/h)	5.62 ^a	0 ^b	0 ^b	0.399	<0.001
Protozoa (log cells/mL)	4.97 ^a	4.48 ^b	4.35 ^b	0.075	<0.001
pH	6.33 ^a	6.10 ^b	6.17 ^b	0.055	0.015
NH ₃ -N (mmol/L)	9.56 ^a	6.28 ^b	5.56 ^b	0.589	0.001
Total VFA (mmol/L)	97.50 ^a	112.90 ^b	109.80 ^b	4.000	0.018
Acetate (%)	65.74	66.60	65.25	0.741	0.259
Propionate (%)	17.20 ^a	22.11 ^b	24.70 ^c	0.754	<0.001
Butyrate (%)	13.44 ^a	8.81 ^b	7.61 ^c	0.302	<0.001
BCFA (%)	2.11 ^a	1.31 ^b	1.25 ^b	0.063	<0.001

Conclusion Hederagenin bissuccinate had a strong antiprotozoal effect, reducing ammonia concentration, as bacteria predation is decreased, and shifting the fermentation pattern towards higher propionate and lower butyrate. Further longer term experiments to study the persistence of these effects are needed.

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The effects of resistant starch from underutilised legumes on the growth of potential probiotic *Lactococcus lactis* isolated from barramundi (*Lates calcarifer*)

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Application Resistant starch (RS) extracted from legumes has the potential to act as a prebiotic for lactic acid bacteria so could be used to improve gastrointestinal microbiota, thereby improving fish health in aquaculture production systems.

Introduction: Legumes are an important source of resistant starch (RS), which can be improved further through the process of hydrolysis, gelatinisation and retrogradation. As a prebiotic, RS contributes to improved gastrointestinal health in humans (Fuentes-Zaragoza, *et al.*, 2011). Human prebiotics have also shown benefits in fish (Ringø *et al.*, 2010). The aim of this study was to investigate the effect of RS-enhanced legume starch on a potential probiotic bacterium isolated from the intestine of barramundi (*Lates calcarifer*).

Material and methods Starches were isolated from six legumes (adzuki beans (*Vigna angularis*), mung beans (*V. radiata*), black-eyed peas (*V. unguiculata* subsp. *Unguiculata*), pigeon peas (*Cajanus cajan*), Bambara groundnuts (*V. subterranea*) and red lentil (*Lens culinaris*)) and were subject to acid or enzyme hydrolysis, gelatinisation via autoclave and retrogradation at 4°C prior to lyophilisation. The RS were produced in a single batch and were labelled as acid-hydrolysed resistant starch (AH), enzyme-hydrolysed resistant starch (EH) and untreated starch (ST), which was used as the control. RS content of samples from each treatment and legume were quantified and supplemented at 5% (w/v) into nutrient broth which were then inoculated with 24h-old active *L. lactis* and incubated at 37°C for 24 h. The samples were prepared in triplicates and the viable cell count of *L. lactis* and pH of the nutrient broth were determined at 0 and 24 h incubation as an assessment of bacteria growth rate and acid production ability, respectively. All assays were conducted in triplicate and the results obtained were analysed using two-way ANOVA (Genstat) and *Post hoc* Duncan's test, error bars are standard deviation of the mean. Significance was accepted at $p < 0.05$.

Results The types of legumes and the treatments applied led to significant interaction for both growth and acid production in the bacteria ($P < 0.001$). The EH treated RSs of Adzuki bean, Lentils and Bambara groundnut significantly enhanced the bacterial growth. No significant difference was observed in the bacterial growth for the remaining legumes treated with EH and ST processing methods. AH processing method was the least effective method to enhance the bacterial growth, only the AH treated RSs of Bambara groundnut showed significant higher bacterial growth than ST treatment (Figure 1). Accompanying the enhanced bacterial growth of EH treated RSs of Adzuki bean, Lentils and Bambara groundnut there was significantly reduced the pH of the nutrient broth which indicated the production of acid (Figure 2).

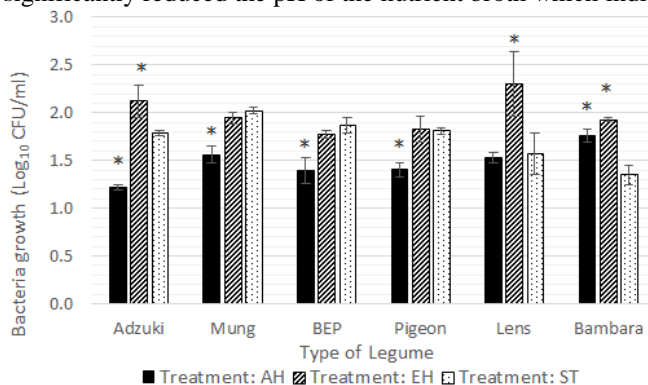


Figure 1 Effect of processed resistant starches and the control starch of legume on the growth of *L. lactis*. Asterisks (*) above each bar refer to treatments significantly different compared to ST ($p < 0.05$).

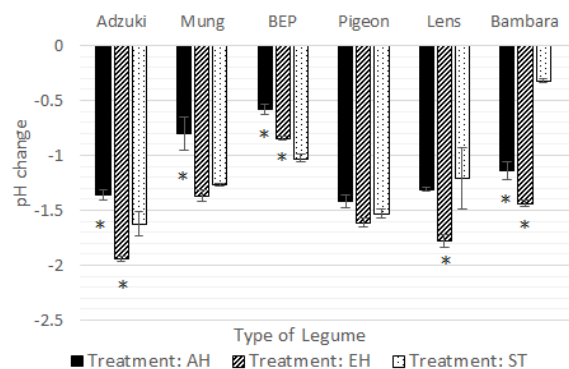


Figure 2 Effect of processed resistant starches and the control starch of legume on the acid production of *L. lactis*. Asterisks (*) above each bar refer to treatments significantly different compared to ST ($p < 0.05$).

Conclusion The EH treated RSs of Adzuki bean, Lentils and Bambara groundnut were found to enhance the growth of *L. lactis* isolated from the intestine of barramundi. These RSs also increased acid production which indicated growth of *L. lactis*, as its growth will lead to the production of lactic acid, and hence could be applied as putative prebiotic in aquafeed to promote intestinal health of fish.

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Manipulation of rumen nitrogen flows by use of Festulolium hybrids

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Application The Festulolium forage grass hybrids have the potential to improve ruminal nitrogen use efficiency in a fresh feeding system. This would help to increase production efficiency as well as decreasing the environmental impact of livestock agriculture.

Introduction Inefficient capture of forage N by ruminants is a significant problem for livestock farming resulting in the need for protein supplementation and release of nitrogenous pollutants to the land. We have shown previously that endogenous plant responses to rumen conditions contribute to inefficient protein use, and hence forage-based solutions based on genetic improvement of the feed are possible. *Lolium perenne* (perennial ryegrass) is a commonly used forage grass that would benefit from enhanced post-ingestion protein stability. One technique that has shown promise is to combine *Lolium* with *Festuca* genomes to generate Festulolium hybrids. Here we have used rumen simulation to explore the extent to which swards containing Festulolium populations previously shown to have decreased rates of endogenous protein degradation can confer advantageous protein utilisation in comparison with a currently market leading diploid ryegrass.

Material and methods Four forage genotypes; Lp (*Lolium perenne* var AberMagic), and three festulolium hybrids: LpxFg (*Lolium perenne* × *Festuca glaucescens*), LpxFm (*Lolium perenne* × *Festuca mairei*) and LmxFg (*Lolium multiflorum* × *Festuca glaucescens*) were randomly allocated to the fermenter vessels contained in 16 identical Rusitec vessels of 900mL volume (4 per treatment). All forages were cut into 1cm lengths before being weighed into nylon bags, introduced into the fermenters where they remained for 48h. The liquid dilution rate was maintained at 3.65%/h by continuous infusion of artificial saliva. After 9 days adaptation 2.3 mg ¹⁵N/d was continuously infused to label the microbial protein. Days 10 to 12 of the experiment were used to determine the diet degradability, gas production and rumen fermentation, while effluent was collected during days 13 and 14 and mixed with their correspondent bag residue to reconstitute the digesta flow. Finally, on day 15 total (TB), liquid- (LAB) and solid-associated bacteria (SAB) were isolated to determine microbial synthesis. Data were analysed by ANOVA blocking by cow.

Results There was no detectable difference in the total VFA production between genotypes. A significant difference was detected in the total nitrogen production fermentation process. LpxFg produced the lowest amount of total nitrogen as compared to the other three genotypes, which had relatively similar production. The amount of NAN produced was similar across all genotypes. Significant differences were detected in the production of ammonia. Fermentations with LmxFg and Lp had the highest amounts of ammonia while LpxFg recorded the lowest. No substantial differences among the four forages were observed in the overall proportion of both LAB-N and SAB-N derived from ammonia. Although non-significant LpxFg produced approx. 20% less methane and 15% less gas than the other genotypes.

Table 1 Rumen function

Rumen parameters	Lp	LpxFg	LpxFm	LmxFg	SED	p-value
Total VFA (mmol/d)	23.6	20.5	26.2	28.9	3.32	ns
Total N (mg/d)	168.3 ^a	144.1 ^b	167.5 ^a	169.8 ^a	7.3	*
Total Non-ammonia N (mg/d)	119.2	107.4	125.8	118.6	7.82	ns
Total Ammonia N (mg/d)	49.1 ^a	36.7 ^b	41.7 ^{ab}	51.2 ^a	4.46	*
Total Microbial N-LAB (mg/d)	70.2 ^{bc}	63.1 ^c	76.9 ^{ab}	85.8 ^a	5.01	**
Total Microbial N-SAB (mg/d)	68.8	65.8	80.9	81.6	7.65	ns
Total Microbial N-LAB from NH3	0.49	0.48	0.49	0.48	0.0285	ns
Total Microbial N-SAB from NH3	0.50	0.46	0.47	0.51	0.0628	ns
Total Gas produced (L/d)	1.89	1.69	2.04	1.85	0.224	ns
Methane (mL/d)	83.7	67.4	85.7	81.5	20.01	ns

**P<0.01, *P<0.05, , ns not significant (n=4)

Conclusion The Festulolium hybrids showed potential for improved nitrogen partitioning during fermentation of fresh forage compared with a commonly used ryegrass. Further work is underway to determine the basis for this differential and the extent of genetic control of the trait.

Acknowledgements This experimentation was funded by Aberystwyth University DCDS (SKW), BBSRC grant BBS/E/W/10964A01 (AB, TED, AK-S).

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Effect of different forms of vitamin E on rumen fermentation and protozoal activity *in vitro*

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Implications Inclusion of high levels of vitamin E, as α -tocopheryl acetate, had a positive impact on rumen fermentation *in vitro*. Further *in vivo* trials are needed before recommending its systematic inclusion at high levels in ruminant's diets.

Introduction Alfa-tocopherol is the major vitamin E compound found in chloroplasts and it accumulates as part of the plant's response to stress. Thus, fresh forages represent an important source of vitamin E to satisfy the ruminant's requirements. The use of antioxidants as feed additives is however gaining interest to improve animal health and product quality. As a result, high levels of the vitamin E derivative, α -tocopheryl acetate, are often used in ruminant diets. While it is known that α -tocopherol has a higher bioavailability than α -tocopheryl acetate this latter form is widely used in the feed industry due to its lower susceptibility to oxidative destruction. However, little is known about the differences between the forms of vitamin E on rumen function. This experiment investigated the effects of supplementing with these two forms of vitamin E on rumen fermentation *in vitro*.

Material and methods A dose-response experiment was conducted to identify the effects of increasing doses (0, 0.5, 5, 50 and 500 IU/L) of α -tocopherol and dl- α -tocopheryl acetate on gas production and fermentation pattern. Treatments were conducted in quadruplicate using rumen fluid from four rumen-cannulated cows fed ryegrass hay and concentrate at 67:33 on DM basis. Rumen contents were filtered through a double layer of muslin, diluted 2:1 with anaerobic buffer and anaerobically dispensed to 120-mL Wheaton bottles (50mL per bottle) containing 500mg DM of mixed diet ground at 1mm² sieve pore size (90% ryegrass, 6% barley, 2.4% corn, 1.6% soya). Bottles were sealed and held in an incubator at 39°C. Fermentation pattern, in terms of pH, ammonia, VFA and methane emissions, were determined after 24h incubation. Gas production (GP) was measured at 2, 4, 6, 9, 12, 24, 48, 72 and 96h using a semi-automated pressure transducer. Cumulative GP data were fitted to the predictive equation $y = a [1 - e^{-ct}]$ (France *et al.*, 2000) where y (mL) is the cumulative GP at time t (h), a is the asymptotic or potential GP (mL) and c is the GP rate (μ L/h). Protozoal activity was measured from the breakdown of ¹⁴C-labelled bacteria by the rumen protozoa and expressed as a percentage of the initial radioactivity released per hour. Data were analysed by ANOVA blocking by cow.

Results Incubation of α -tocopherol in rumen fluid had a moderate but positive effect on rumen fermentation tending to increase total VFA production, OM fermentation (FOM), asymptotic GP as well as promoting a significant increase in GP rate when used at concentrations above 50 IU/L. In comparison to α -tocopherol, α -tocopheryl acetate had a stronger effect on the rumen fermentation promoting a significant increase in the total VFA production and OM fermentation when incubated at concentrations above 5 IU/L. This acetate form also tended to increase GP rate and tended to increase the percentage of acetate in detriment to butyrate. As a result of this, α -tocopheryl acetate decreased methane production per unit of FOM when used at concentrations above 50 IU/L. Protozoal activity was however not affected by any of the vitamin E forms tested.

Table 1 Effect of different forms of vitamin E on the rumen function and protozoal activity *in vitro*

Dose (IU/L)	α -tocopherol							α -tocopheryl acetate						
	0	0.5	5	50	500	SED	P-value	0	0.5	5	50	500	SED	P-value
pH	6.36	6.34	6.36	6.36	6.34	0.02	0.64	6.36	6.37	6.35	6.35	6.34	0.02	0.80
NH ₃ -N (mg/dL)	19.6	21.0	22.2	20.2	21.3	2.60	0.87	19.6	22.1	19.9	20.6	21.4	2.23	0.79
Total VFA (mM)	62.0	73.4	78.1	72.6	68.5	4.80	0.053	62.0 ^c	69.7 ^{bc}	73.3 ^{ab}	80.6 ^a	75.6 ^{ab}	4.64	0.018
Acetate (%)	67.7	68.2	68.1	67.9	67.6	0.39	0.56	67.7	67.8	67.3	68.5	68.5	0.44	0.071
Propionate (%)	16.4	16.5	16.6	16.6	16.7	0.11	0.16	16.4	16.5	16.7	16.5	16.4	0.16	0.23
Butyrate (%)	11.6	11.3	11.1	11.3	11.5	0.24	0.40	11.6	11.5	11.5	11.0	11.1	0.22	0.064
FOM (mg)	269	319	338	315	297	20.9	0.056	269 ^c	303 ^{bc}	318 ^{ab}	350 ^a	328 ^{ab}	20.0	0.019
Asymptotic GP (mL)	116	119	120	120	124	2.39	0.096	116	117	120	117	119	1.44	0.18
GP rate (μ L/h)	64.5 ^b	64.8 ^b	67.3 ^{ab}	69.0 ^a	68.3 ^a	1.58	0.047	64.5	65.6	67.0	64.5	67.5	1.23	0.095
Methane (mL/d)	21.3	21.5	22.2	22.0	22.2	0.67	0.57	21.3	21.5	22.1	21.1	21.9	0.43	0.20
Methane (mL/g FOM)	81.1	67.9	65.6	70.3	74.7	6.77	0.23	81.1 ^a	71.7 ^{ab}	69.6 ^{ab}	60.4 ^b	67.3 ^b	5.80	0.046
Protozoal activity (%/h)	9.95	9.58	9.36	9.46	9.53	0.44	0.72	9.95	10.26	10.41	9.95	9.66	0.39	0.38

Conclusions Supplementation of ruminants' diets with vitamin E had a positive effect on the rumen fermentation by increasing feed fermentation *in vitro*. These effects seems however to be more obvious using α -tocopheryl acetate rather than α -tocopherol, possibly as a result of its greater stability in the rumen.

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

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Effects of rate of inclusion of marine algae and fish oil on the biohydrogenation of 20:5*n*-3 and 22:6*n*-3 polyunsaturated fatty acids *in vitro*

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Application Marine algae is a rich source of C22:6*n*-3 although the biohydrogenation of this fatty acid is rapid and extensive. In contrast, the rate of biohydrogenation of C20:5*n*-3 in algae is lower than in fish oil.

Introduction Long chain (LC) *n*-3 polyunsaturated fatty acids (PUFA) have beneficial effects on human health but the majority of the human population, especially those in Western countries are failing to meet the recommended daily intake (>0.2g a day) (Meyer, *et al.* 2003). Increasing the content of the two main LC *n*-3 PUFA, *eicosapentaenoic acid* (EPA; C20:5*n*-3) and *docosahexaenoic acid* (DHA; C22:6*n*-3), in milk and cheese would enhance the availability and consumption of these essential FA. Fish oils and marine algae are particularly rich sources of these LC *n*-3 PUFA but may be extensively biohydrogenated in the rumen (Sinclair *et al.*, 2005). The objective of this study was to investigate the effect of rate of inclusion of LC *n*-3 PUFA in fish oil and a novel source of marine algae on the rate and extent of biohydrogenation *in vitro*.

Material and methods The study used an *in vitro* batch culture technique (Sinclair *et al.*, 2005) with a 2 x 4 factorial design, with two oil sources; fish oil (FO) and marine algae oil (ALG; SP1, Alltech UK Ltd.), which were supplemented to a basal diet of ground grass nuts at inclusion rates of 20, 40, 60 and 80 mg/100g DM. The samples were incubated in 250ml duran bottles containing 200ml of buffer/rumen fluid mixture (80: 20, v/v; pH 6.7), and fermentation was terminated by freezing after 6, 12, 24 and 48 hrs. Samples were freeze dried prior to FA analysis by gas chromatography (Jenkins 2010). Biohydrogenation was calculated as the quantity of the individual FA in the residue as a proportion of the amount added to each vessel, and expressed as g/kg. The experiment was replicated four times, and data was analysed as repeated measures analysis of variance using Genstat 17. Differences were reported as significant when $P < 0.05$.

Results A greater amount of EPA was detected at most time points in the FO treatments ($P = 0.007$; Table 1). In contrast the vessel content of DHA was greater in ALG at all time points ($P < 0.001$). Biohydrogenation of EPA was lower at the lower inclusion rates in ALG but not FO, whereas the biohydrogenation of DHA was high in both sources at all times points ($P < 0.01$; Fig.1, $P < 0.03$; Fig.2).

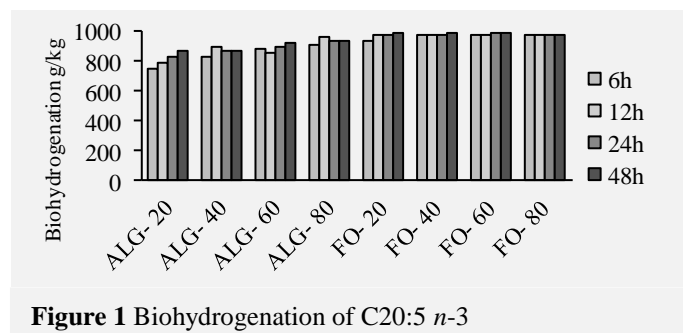


Figure 1 Biohydrogenation of C20:5 *n*-3

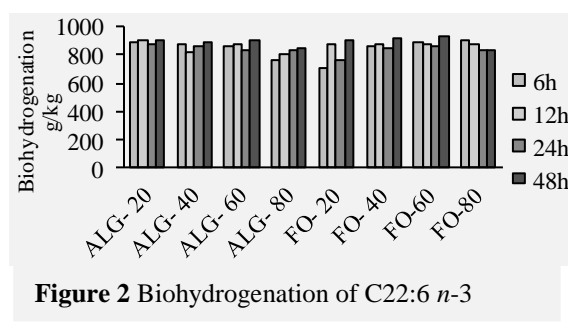


Figure 2 Biohydrogenation of C22:6 *n*-3

Table 1 Total vessel content (mg) of C20: 5*n*-3 and C22: 6*n*-3 with added marine algae (ALG) or fish oil (FO)

Fatty Acid	Time	ALG- 20	ALG- 40	ALG- 60	ALG- 80	FO- 20	FO- 40	FO- 60	FO- 80	s.e.d	
C20: 5 <i>n</i> -3	0	3.22	6.48	9.71	12.97	31.10	62.11	93.17	124.22		
	EPA	6	0.80	1.06	1.05	1.18	1.76	0.91	1.29	1.86	
		12	0.66	0.67	1.31	0.51	0.69	0.98	1.46	1.73	0.464
		24	0.54	0.83	1.03	0.79	0.72	1.13	1.19	2.59	
		48	0.43	0.81	0.72	0.81	0.50	0.75	0.71	1.92	
C22: 6 <i>n</i> -3	0	25.37	50.98	76.35	101.97	3.66	7.32	10.99	14.65		
	DHA	6	2.84	6.49	10.33	24.83	1.08	0.97	1.16	1.43	
		12	2.54	9.33	9.29	19.31	0.43	0.90	1.33	1.84	2.617
		24	3.00	6.94	13.01	16.91	0.89	1.12	1.48	2.44	
		48	2.47	5.69	7.19	16.07	0.67	0.68	0.95	2.44	

Conclusion The biohydrogenation of EPA was lower in algae than FO. The marine algae was a rich source of DHA, but the biohydrogenation was extensive at all time points and inclusion levels in both the algae and fish oil.

Acknowledgements The authors gratefully acknowledge Alltech for funding this study.

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Evaluation of two modified Butanol-HCl methodologies for the analysis of free condensed tannins in conjunction with their quantity occurrence in alfalfa (*Medicago sativa* L.) dried at various temperatures

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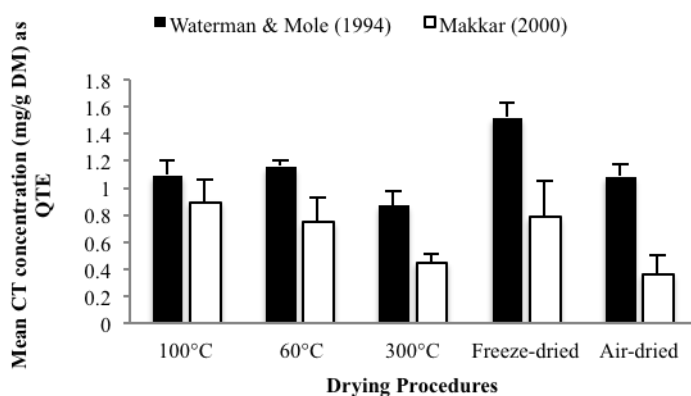
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Application Condensed tannin concentration in *Medicago sativa* L. is insufficient to induce beneficial or detrimental effects on animal production, whilst the effects of drying procedure on condensed tannin concentration are variable.

Introduction Consumption of tannins, especially condensed tannins (CTs), have beneficial effects on animal production if ingested at a concentration of 20-50mg/g DM. Beneficial effects include increased growth rate of liveweight and wool, increased milk yields, fertility, utilisation of dietary proteins and animal welfare due to their bloat preventative activity and antiparasitic properties. However, elevated CT levels present in feed may lead to detrimental or fatal consequences. *Medicago sativa* L., commonly known as alfalfa, is one of the most nutritious preserved forage sources for livestock, although its CT concentration varies (Arrigo and Dohme, 2009). The effect of high-temperature drying on CTs has not been evaluated to date, so the aim of this study was to investigate the free CT quantity occurrence and the effect of different forage drying procedures on free CT concentration in *Medicago sativa* L. based on two established Butanol-HCl assays due to contradictory results during preliminary experiments.

Material and methods Pre-bloom *Medicago sativa* L. samples were subjected to a) oven drying at 100°C, b) oven drying at 60°C, c) high temperature drying (300°C), d) air drying (room temperature) and e) freeze drying (-30°C). Samples were



extracted using a 7:3 (v/v) acetone: distilled water solvent including 0.1% ascorbic acid as an antioxidant for 48h at room temperature and further analysed for free CTs using two modified Butanol-HCl methodologies based on the instructions of Waterman and Mole (1994) and Makkar (2000). Quebracho tannin was used as a standard substance. Differences in mean CT concentration (mg/g DM Quebracho tannin equivalents (QTE)) of differently dried *Medicago sativa* L. for each chemical analysis were ascertained using a one-way ANOVA with a Games-Howell *post hoc* test. A paired t-test tested for any differences between the two modified methodologies (SPSS v20).

Figure 1 Mean CT concentration (mg/g DM) expressed as QTE

Results Results indicate the presence of free CTs in *Medicago sativa* L.. The application of the modified Waterman and Mole (1994) assay revealed that freeze-dried material was statistically significantly higher ($P < 0.001$) and high temperature-dried samples were statistically significantly lower ($P < 0.05$) than all other samples. Results obtained by the modified Makkar (2000) method revealed highest values in the oven-dried at 100°C material and lowest in the air-dried samples. Mean CT levels of air-dried material was statistically significantly different from oven-dried at 100°C ($P < 0.05$) and 60°C ($P < 0.05$). Drying procedures fit for agriculture use (high-temperature and air drying) revealed lowest CT concentrations in both methods and therefore are not optimal for CT preservation compared to laboratory drying procedures (freeze drying and oven drying). Modified Waterman & Mole (1994) method exhibited statistically significantly higher mean value than the modified Makkar (2000) method ($P < 0.001$).

Conclusion Although there is evidence of CTs in pre-bloom *Medicago sativa* L., concentrations are insufficient to induce beneficial or detrimental effects in animals. The findings regarding the effect of drying procedure on free CTs present in *Medicago sativa* L. are controversial and due to the significant difference between methods are therefore not representable.

Acknowledgements The authors gratefully acknowledge the provision of *Medicago sativa* L. samples from Dengie Crops Ltd. and the provision of the Quebracho tannin standard substance (UNITAN ATO) from UNITÁN SAICA.

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Effect of grass – concentrate ratio and different levels of cashew nut shell liquid inclusion on *in vitro* fermentation products

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Application Essential oils (EOs) such as Cashew Nut Shell Liquid (CNSL) have been identified to be a potential alternative to feed antibiotics and growth promoters.

Introduction Cashew nut shell liquid (CNSL) is an extract from cashew nut shell and this liquid contain a phenolic compound known as anacardic acid which has been reported to have antimicrobial activities. However, very little information is available on its potential as rumen modifiers and the optimal dose at which efficient utilization of nutrients in ruminant can be obtained is scarce and varied.

Material and methods The extraction of CNSL was carried out using a Soxhlet extractor and petroleum ether (laboratory reagent grade with boiling range of 40 – 60°C) as solvent. The procedure outlined by Edoga *et al.*, (2006) was followed. The extracted CNSL was then included in a complete concentrate (140g/kg CP, DM) at four levels viz: 0ml, 5ml, 10ml and 15ml/kg DM. Three diets were then prepared by taking different grass (*Panicum maximum*, 8.12% CP, DM) and concentrate ratio i.e high forage low concentrate (**HFLC, 75G:25C**) diet, medium forage concentrate (**MFC, 50G:50C**) diet and low forage high concentrate (**LFHC, 25G:75C**) diet. Diets were milled to pass through 0.5mm sieve and used as substrate for the *in vitro* experiment. Approximately 500mg (n=3) of each sample was weighed into separate fibre bag that was carefully placed into 100ml calibrated transparent glass syringes fitted with silicon tube and thereafter, filled with 40ml inoculums (rumen fluid and Buffer at 1:2) under continuous flow of oxygen free CO₂. Syringes were thereafter incubated at 39°C for 48 h. The experiment was laid out as a 3 x 4 factorial arrangement in a completely randomized design and *in vitro* fermentation of the feeds was done according to the principles of Tilley and Terry (1963). At the end of incubation, inoculums were analyzed for volatile fatty acids (VFA) and ammonia concentration. Data was analysed using SPSS while difference of mean were separated using Duncan multiple range test at 5% significant level.

Table 1 *In vitro* fermentation products of three diets at four levels of CNSL inclusion

Main Effect	Forage – Concentrate Ratio				CNSL* Inclusion Level					Contrast		
	HFLC	MFC	LFHC	SEM	0ml	5 ml	10 ml	15 ml	SEM	L	Q	C
TVFA (mMol)	67.8	64.9	65.73	0.72	68.00 ^{ab}	68.98 ^a	65.07 ^{bc}	62.53 ^c	0.73	0	0.16	0.26
AA (mol/100 mol)	57.92	55.31	56.98	0.59	58.00 ^{ab}	58.53 ^a	55.62 ^{ab}	54.80 ^b	0.59	0.02	0.55	0.28
PA (mol/100 mol)	21.37 ^b	23.27 ^a	23.52 ^a	0.33	23.44	23.13	22.6	21.69	0.33	0.06	0.65	0.95
BA (mol/100 mol)	11.12	10.85	11.67	0.19	10.87	11.23	11.32	11.43	0.84	0.33	0.77	0.87
NH ₃ N (%)	4.76 ^c	6.03 ^b	6.32 ^a	0.02	6.09 ^a	5.81 ^b	5.72 ^c	5.21 ^d	0.03	0	0	0

	HFLC				MFC				LFHC				Contrast			
	0ml	5ml	10ml	15ml	0ml	5ml	10ml	15ml	0ml	5ml	10ml	15ml	SEM	L	Q	C
TVFA	70.40 ^b	75.60 ^a	64.40 ^{cd}	60.80 ^d	66.80 ^{bc}	65.60 ^{bcd}	63.60 ^{cd}	63.60 ^{cd}	66.80 ^{bc}	65.73 ^{bcd}	67.20 ^{bc}	63.20 ^{cd}	0.72	0.02	0.01	0
AA ¹	60.40 ^b	64.27 ^a	54.40 ^d	52.60 ^d	56.80 ^{bcd}	55.60 ^{cd}	53.60 ^d	55.27 ^{cd}	56.80 ^{bcd}	55.73 ^{cd}	58.87 ^{bc}	56.53 ^{bcd}	0.59	0.14	0	0.01
PA ¹	21.27 ^{bc}	20.73 ^c	22.93 ^{abc}	20.53 ^c	24.53 ^{ab}	23.73 ^{abc}	22.40 ^{abc}	22.40 ^{abc}	24.53 ^{ab}	24.93 ^a	22.47 ^{abc}	22.13 ^{abc}	0.33	0.01	0.08	0.32
BA ¹	10.7	10.61	12.07	11.09	10.85	10.96	10.38	11.21	11.07	12.11	11.51	12.01	0.19	0.24	0.28	0.39
NH ₃ N ²	3.65 ⁱ	4.85 ^h	4.95 ^h	5.60 ^g	5.75 ^f	6.35 ^{bc}	5.84 ^{ef}	6.20 ^d	6.23 ^{cd}	5.95 ^e	6.63 ^a	6.49 ^b	0.05	0	0	0

* CNSL contain Anacardic acid (90%) and Cardol (10%), ¹ - mol/100 mol, ² - %

Results LFHC Diet containing 5ml CNSL/kg DM feed had the highest (p<0.05) VFA production while ammonia nitrogen was highest (p<0.05) in LFHC Diet containing 10ml CNSL/kg DM feed and lowest (p<0.05) in HFLC Diet containing 0ml CNSL/kg DM feed.

Conclusion HFLC diet at 0ml (control) and 5ml/kg DM, LFHC diet at 5ml/kg had increased VFA production for efficient energy production while MFC at all levels of CNSL inclusion had a similar performance.

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***In vitro* gas production of *Panicum maximum* as influenced by cutting height and stage of growth**

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Application Identification of the optimum defoliation heights and growth stage that aids net forage accumulation, delays senescence and stem accumulation, will favour the efficient production of large quantities of highly nutritious forage.

Introduction Feed constraint is the most important impediment to improved livestock production in most of tropical countries as a result of seasonal shortages in the quality and quantity of forage from natural pastures that supply most of the feed for animals (Babayemi and Bamikole, 2008). Most pastures in Africa lack proper management in terms of optimum defoliation. This research work aims at determining the *in vitro* digestibility of *Panicum maximum* grass under different heights and growth stage.

Material and methods The experimental site was cleared, ploughed and harrowed before establishment. The *P. maximum* vegetative materials (Tillers) were sourced from the natural pasture around the Teaching and Research Farm, Federal University of Agriculture, Abeokuta, Nigeria. They were carefully separated into crown splits; and planted with spacing of 0.5m x 0.5m, so that each treatment had nine (9) stands with plot dimension of 1m x 1m. The experiment was a 3 x 3 factorial arrangement consisting of three stage of growth: (8, 10, and 12) weeks, Three cutting heights: (10cm, 15cm and 20cm) and was laid out in a Randomized Complete Block Design (RCBD) with three replicates. Samples were harvested at 8, 10 and 12 weeks after planting and at required height (10, 15 and 20cm), weighed and oven dried at 65°C to a constant weight for *in vitro* gas production according to the technique of Menke and Steingass (1988). Data were analysed using the general linear model and treatment means separated using Duncan's Multiple Range Test of the SAS software package (SAS 1999).

Results Grasses harvested at 8 weeks after planting and at 20 cm above ground level resulted in the highest (P<0.05) gas production throughout the incubation period except at 3hours. The interaction between cutting heights and stage of growth was significant through the hours of incubation period.

Table 1 Effect of cutting height and stage of growth on the *in vitro* gas production of *Panicum maximum*

Factors	Hours of incubation								
	3	6	9	12	18	24	30	36	48
Cutting Height									
10cm	2.13	4.29	6.78 ^b	8.90 ^b	13.64 ^b	17.56 ^b	21.57 ^b	24.15 ^b	28.19 ^b
15cm	1.90	4.20	6.15 ^b	8.37 ^b	12.32 ^c	15.83 ^c	19.01 ^c	21.07 ^c	24.93 ^c
20cm	1.88	4.65	7.60 ^a	10.60 ^a	15.97 ^a	20.97 ^a	25.20 ^a	27.63 ^a	32.88 ^a
SEM	0.25	0.39	0.53	0.68	0.96	1.17	1.33	1.40	1.59
Stage of Growth									
8 WAP	2.16 ^a	4.46	7.11	9.90 ^a	15.11 ^a	19.96 ^a	23.81 ^a	26.72 ^a	31.31 ^a
10 WAP	2.30 ^a	4.32	6.45	8.15 ^b	12.13 ^b	15.14 ^b	18.39 ^b	20.54 ^b	24.35 ^b
12 WAP	1.44 ^b	4.36	6.97	9.79 ^a	14.70 ^a	19.25 ^a	23.58 ^a	25.58 ^a	30.33 ^a
SEM	0.24	0.39	0.53	0.68	0.96	1.17	1.33	1.41	1.61
CH x SOG	***	***	***	***	***	***	***	***	***
P-value	0.0030	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002

abcd: means on the same column with different superscript are significantly different; **: p<0.01; ***:p<0.001

Conclusion *Panicum maximum* grass harvested at eight weeks after planting and at 20 cm above ground level had the highest gas volume which suggests greatest is perceived to be more digestibility potential.

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Ingestive behaviour of three novel forages in horses

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Application Pea haulm may be suitable for inclusion in the diet of horses where minimal nutrient intake but maximal chewing time is required, whereas whole crop oat and sainfoin deliver more nutrition for relatively less chewing time.

Introduction Horses have many diverse roles in current society and thus require different nutrient and energy intakes although their evolution as a hind-gut fermenter dictates that frequent feeding and forage should form the mainstay of their diets (Hale, Hemmings & Bee, 2011). Forage intake is associated with physiological factors such as dental health, saliva production and its resultant impact on stomach lining as well as moderating undesired behaviours (Sarrafchi & Blokhuis, 2013). The aim of the study was to evaluate three novel forages, pea haulm (PH), sainfoin (S) and whole crop oat (WCO) through proximate analysis and assess preferences and bite/chew rates of individual forages and blends thereof.

Material and methods Proximate analysis was conducted on the novel forages, including dry matter (DM), acid detergent (ADF) and neutral detergent fibre (NDF). Using a stratified sample of healthy horses from the on-site college equine centre ($n = 18$), individual preference tests with randomised presentation of 200g of forage were conducted between three single forages (PH¹, S², WCO³) and three forage blends (S:PH⁴, WCO:PH⁵, S:WCO⁶) in a 1:1 ratio. Preference presentations were limited to five minutes and first taste, subsequent preferences and amount remaining recorded. Chew and bite rates for eight forage blends (as for preference testing plus 2:1 ratio each of S:WCO⁷ and WCO:S⁸) were assessed using 16 horses in a replicated, balanced Latin square. Each horse was fed 500g of forage blend and presentations timed for 10 minutes. The amount remaining, number of bites and chews, and time taken to eat were recorded and used to calculate time taken to eat 1kg/DM, chews and bites/kg DM, chew/minute and chews/kg NDF. The level of significance was set to 0.05 and the non-parametric data analysed in Minitab 17 using Friedman's test for differences and Spearman's rho for correlations.

Results PH had a higher DM and ADF and NDF values than WCO and S respectively. Preference test results (Table 1) indicated significant differences for the amount consumed between single forages ($S = 21.52$, $p < 0.05$) and forage blends ($S = 18.97$, $p < 0.05$). Significant differences were found for the time taken to eat 1kg of DM ($S = 41.40$, $p < 0.05$), total amounts eaten ($S = 67.50$, $p < 0.05$), chews/kg DM ($S = 19.78$, $p < 0.05$), chews per minute ($S = 69.70$, $p < 0.05$) and chews/kg NDF ($S = 23.93$, $p < 0.05$). A negative correlation was found between NDF content and DM intake ($r_s = -0.55$, $p < 0.05$) and chews/minute and NDF content ($r_s = -0.34$, $p < 0.05$).

Table 1 Ingestive behaviour expressed through preference testing (mean and s.d.)

Forage/forage blend	PH	S	WCO	S:PH	WCO:PH	WCO:S
Amount consumed (g)	8.2 (± 12.7)	125.5 (± 69.0)	79.2 (± 69.4)	47.2 (± 53.3)	13.8 (± 30.9)	126.1 (± 75.1)

Conclusion Horses expressed strong individual preferences between forage blends and preference trials should be replicated to account for any feeding-side preferences. Forage blends containing WCO and S were consumed faster than those containing PH. PH may be suitable for inclusion in the diet of horses where minimal nutrient intake but maximal chewing time is required. Conversely, S and WCO appear to maximise nutrient intake with less chewing effort. These findings are in line with Ellis, Thomas, Arkell and Harris (2005) who reported a decreased eating time when lucerne was added to a meal, but an increased eating time when short-chopped straw was added. Further long-term studies may be warranted to assess voluntary intake and chew rates across a wider sample of horses and ponies but commercial production of custom-blended forages which are convenient to feed, provide occupational therapy through chewing and form the mainstay of a balanced diet supplemented with concentrates where necessary, could eventually be realised.

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Effects of feeding frequency on nutrient digestibility and feeding behaviour in the Turkmen horse

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Application Feeding more frequently improves nutrient digestibility and horse health. Feeding 4 or more times per day resulted in higher nutrient digestibility and the intake of alfalfa, but increase the concentrate intake than other feeding frequencies.

Introduction Intensively managed horses are often only provided with meals twice a day and this means that feed is consumed rapidly which may lead to the development of gastric ulcers and other health problems (Søndergaard *et al.*, 2004). Increasing feed frequency increases chewing activity, stimulating salivation and breaking the forage into smaller pieces improving the action of enzymatic and microbial digestion (Müeller *et al.*, 1998). Finding the right feeding frequency to control physiological, digestive, and behavioural issues is crucial for the health of the horse (Clauss *et al.*, 2009). Therefore, the aim of this trial was to assess the effect of increasing the feeding frequency, from 2 up to 8 times per day, on the feeding behaviour (i.e., chewing and rate of intake) and nutrient digestibility changes in Turkmen horses.

Material and methods Sixteen Turkmen horses with average weights of 430 ± 46 kg and age 7 ± 3 years were used. Four feeding frequencies of 2, 4, 6 and 8 meals per day were used for a period of 28 days with diets based on 70% alfalfa and 30% concentrate (NRC, 2007). Lignin content of feed and faeces was used as an internal marker to determine apparent nutrient digestibility and individual nutrient digestibility was also measured. Chewing and total intake rates for alfalfa and concentrate in horses were observed and recorded. Data were statistically analysed based on a completely randomized design using General Linear Models procedure in SAS software.

Results Apart from ether extract digestibility, the results of this experiment showed that by increasing feeding frequency above 2 times/day, the nutrient digestibility increased ($P < 0.05$) (Table 1). For alfalfa, there was a decrease in the number of chews/kg DM and total intake time when feeding 2 or 4 meals per day compared to the other treatments. However, for concentrate, there was an increase in the number of chews/kg DM at 2 and 4 times per day feeding compared to other treatments and total intake time was reduced at 8 meals per day compared to other treatments.

Table 1 Effect of feeding frequency on nutrient digestibility (g/kg DM)

	Feeding frequency per day				SEM	P value	Contrast	
	×2	×4	×6	×8			L	Q
Digestibility								
Dry matter	426 ^b	476 ^a	480 ^a	501 ^a	7.4	0.001	0.001	0.06
Organic matter	479 ^b	485 ^a	491 ^a	497 ^a	2.6	0.01	0.004	0.93
Crude protein	476 ^b	501 ^a	535 ^a	518 ^a	11.4	0.018	0.09	0.09
Ether extract	421	415	427	422	7.4	0.03	0.31	0.19
NDFom	228 ^b	276 ^a	270 ^a	278 ^a	4.5	0.001	0.01	0.05
ADFom	204 ^b	247 ^a	266 ^a	271 ^a	10.9	0.004	0.001	0.10
DE (MJ/ kg DM)	6.71 ^c	7.38 ^b	7.38 ^b	7.89 ^a	0.27	0.03	0.01	0.80
No. of chews/kg DM of alfalfa	1972 ^b	1989 ^b	2449 ^a	2548 ^a	51.0	0.01	0.01	0.43
Total alfalfa intake time for all meals (min)	164 ^b	163 ^b	198 ^a	201 ^a	0.76	0.03	0.08	0.73
No. of chews/kg DM of concentrate	629 ^a	609 ^a	564 ^b	588 ^b	14.2	0.02	0.01	0.13
Total concentrate intake time for all meals (min)	20.2 ^a	20.0 ^a	19.8 ^a	18.7 ^b	0.17	0.01	0.01	0.11

L: linear effect of feeding frequency, Q: quadratic effect of feeding frequency, SEM: standard error of means.

Conclusion Feeding 4 or more times per day resulted in an increase in nutrient digestibility compared to feeding twice daily. Increasing the number of feeds tended to increase chewing and intake time for forage (alfalfa) but tended to reduce chewing and intake time for concentrate feed.

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The effect of novel yeast strain *Saccharomyces cerevisiae* Y1242 on apparent digestibility in ponies

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Application Improving diet digestibility using yeast products is advantageous for any horse feed producer and horse owner. The effect of higher concentrations of yeast product supplementation warrants further investigation.

Introduction There is increasing interest in feeding higher energy, fibre-based diets to horses as an alternative to rations composed of high levels of starch-rich cereal grains. Yeast supplementation has been shown to increase fibre digestibility in ruminants (Desnoyers *et al.*, 2009) and horses (Agazzi *et al.*, 2009; Jouany *et al.*, 2008). This appears to be due to yeasts having an important role in the microbial digestion process (Medina *et al.*, 2002). The precise mode of action has not been identified; however, the most common finding in yeast research studies is an increase in the fibrolytic bacterial population and fibre digestibility (Agazzi *et al.*, 2009; Jouany *et al.*, 2008). *In vivo* data is crucial for evaluation and validation of any potential feed additive for horses used for this purpose. The aim of the experiment reported here was to examine the effects of *Saccharomyces cerevisiae* Y1242 on diet digestibility in ponies.

Material and methods Six gelding ponies were used in a digestibility study to evaluate the effect of a new strain of live yeast *Saccharomyces cerevisiae* Y1242 on diet digestibility. The yeast was supplied to the treatment group as a top dressing on the concentrate feed at a dose rate of 1.8g yeast/horse/day. Animals were fed a ration consisting of mature grass hay and concentrate mix fed twice daily at 1.75% of their bodyweight on a dry matter (DM) basis. Concentrate inclusion rate in the diet was preset at 20% of daily digestible energy requirements. *In vivo* evaluation consisted of two phases in a double-blind randomized crossover design. All animals were randomly assigned to one of the two experimental groups and each group received their respective diet (control or treatment) during the first phase; the groups crossed over from one treatment to another during the second phase of the trial. During each of the two phases described previously all animals were subject to an adaptation period of 21 days followed by a 7 day collection period. Total daily DM intake and excretion were ascertained. Apparent digestibility of DM, crude fibre, neutral detergent fibre (NDF), acid detergent fibre, hemicellulose, crude protein and organic matter were determined by total collection method. All data are presented as mean \pm SEM, $n=6$. Data were analysed using the general linear model (GLM) procedure (Minitab 17, Minitab Inc., State College, PA). Faecal pH was also determined.

Results Yeast supplementation at a dose rate of 1.8 g/horse/day did not significantly affect nutrient digestibility in ponies. However, there were trends ($p<0.10$) for higher NDF apparent digestibility with yeast supplementation in comparison to the control diet (Table 1). Furthermore, faecal pH was not affected by yeast supplementation ($p>0.05$).

Table 1 Total diet *in vivo* apparent digestibility coefficients of nutrients

	Treatment	SEM	Control	SEM	p-value
Dry Matter	0.482	0.0035	0.483	0.0061	0.850
Organic Matter	0.490	0.0042	0.491	0.0066	0.786
Crude Fibre	0.362	0.0124	0.365	0.0166	0.801
Neutral Detergent Fibre	0.373	0.0071	0.358	0.0102	0.087
Acid Detergent Fibre	0.340	0.0112	0.341	0.0173	0.911
Hemicellulose	0.414	0.0185	0.388	0.0069	0.143
Crude Protein	0.573	0.0193	0.574	0.0110	0.915
Gross Energy	0.418	0.0062	0.413	0.0114	0.428

Conclusion In the current study *Saccharomyces cerevisiae* Y1242 did not significantly improve diet apparent digestibility in ponies. However, given the trend for higher NDF apparent digestibility with yeast supplementation it is possible that this lack of effect may be due to insufficient level of yeast supplementation. Therefore, further work is required to evaluate optimal yeast inclusion rates and also investigate factors such as level of exercise and diet composition on the efficacy of yeast supplementation.

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Temporal changes in the faecal volatile organic compound (VOC) metabolome of healthy mares *pre-* and *post-partum*

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Application Understanding normal changes in the faecal metabolome will act as benchmark for disease.

Introduction The *post-partum* mare has an increased risk of colic. Changes in the faecal microbiome have been identified in mares prior to a colic episode (Weese *et al.*, 2015). Faecal volatile organic compound (VOC) profiling is an economical technique to study the metabolome (functional microbiome). Horses with colic have been shown to have a different faecal VOC profile from healthy horses (Turner *et al.*, 2013). The aim of this study was to characterise the faecal VOC changes in healthy mares *pre-* and *post-partum* to understand the normal fluctuations in the metabolome around this time.

Material and methods A faecal sample was collected from seven mares two (-2) and one week (-1) prior to foaling and at one (1) and two weeks (2) post foaling. Mares were stabled overnight with *ad lib* hay and turned out into a paddock during the day throughout the sampling period. Post collection, samples were stored at -80°C before analysis. Aliquots of 1000 mg were placed in 10 ml glass vials with a septum in the lid, each sample was analysed in triplicate giving a total sample size of 21 per time point. VOCs were extracted using headspace solid-phase micro extraction (HS-SPME) at 60°C for 20 minutes. Prior to extraction vials were incubated at 60°C for 30 minutes. The SPME fibre used was divinylbenzene/carboxen/polydimethylsiloxane (DVB-CAR-PDMS) 50/30µm (Sigma-Aldridge, Dorset, UK). Gas chromatography mass spectroscopy (GC-MS) analysis was carried out using a Perkin Elmer Clarus 500 GC/MS Quadruple bench top system (Beacons field, UK). The GC-MS method and conditions are detailed elsewhere (Reade *et al.*, 2014). Data was analysed using the Automated Mass Deconvolution System (AMDIS-version 2.71, 2012) and the NIST mass spectral library (version 2.0, 2011) to identify VOCs. Statistical analysis was performed in R, version 3.1.2. A one-way analysis of variance (ANOVA) followed by Tukey's HSD test for pairwise comparisons or a Fisher's exact test was applied to the data where appropriate. Final p-values were adjusted using Bonferroni correction.

Results A total of 102 VOCs were identified across all four weeks; these were mainly carboxylic acids, ketones and aldehydes. The means and SEM of VOC numbers were 79 ± 1.4 , 80 ± 1.3 , 74 ± 1.4 , 79 ± 1.8 detected at -2, -1, 1 and 2 weeks, respectively. Significantly fewer VOCs were found 1 week *post-partum* than at 1 and 2 weeks *pre-partum* ($p < 0.05$). Fig. 1 shows numerical range of VOCs identified at each week *pre-* and *post-partum*. Two compounds (butanoic acid, 2-methylbutyl ester and propanoic acid, 3-methyl-butnyl ester) were significantly less frequent in samples one week post foaling ($p < 0.05$).

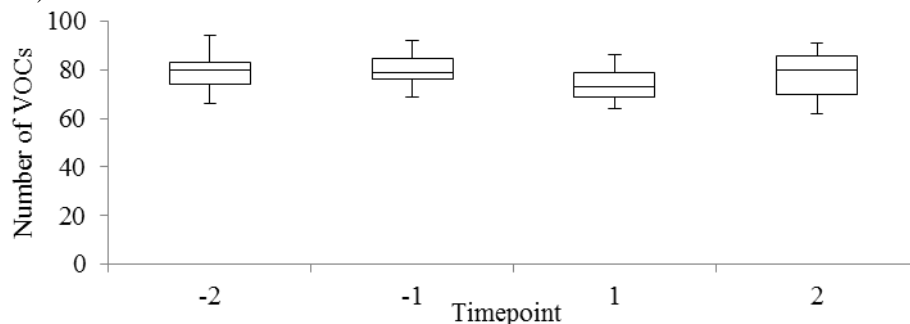


Figure 1 The number of VOCs obtained from the faeces of mares two weeks prior to and two weeks post foaling using HS-SPME-GCMS analysis.

Conclusion The faecal metabolome of the mare appears to alter one week after foaling before returning to baseline levels by the second week post foaling. Further research is necessary to establish the mechanisms underlying this finding.

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Incidence of Caslick's vulvoplasty operations in a population of Thoroughbred mares

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Application Of the 602 Thoroughbred (TB) broodmares sampled, 46.7% had the Caslick vulvoplasty operation. The likelihood of Caslick vulvoplasty operations in TB mares is increased with increased age, multi-parity and the country in which the mare resides, in this case, Great Britain (GB) *versus* Ireland and Northern Ireland (IRL and NI).

Introduction Caslick vulvoplasty operations are used to correct pneumovagina in mares with poor vulva conformation (Pascoe, 1979). Both poor conformation and treatment by Caslick's vulvoplasty are common in the TB, as selection of broodmares is predominantly for performance, not reproductive competence. Limited research exists about the frequency of the procedure and the exact reasons that lead to the treatment. This study aimed to determine the frequency of the operation in a sample of TB mares residing in GB and IRL/NI, identify if there are certain factors that contribute to whether the mare had a Caslick vulvoplasty operation and the affect these factors have on observational grading of the performed vulvoplasty (is the Caslick vulvoplasty efficient).

Material and methods 602 TB broodmares aged 2-26 years were recruited; mares were sourced via online search engines and personal contacts. Information obtained for each mare included; presence or absence of Caslick operation, year of first Caslick operation, reason why the mare was given the Caslick operation, age, whether she had ever been bred from, if she had a race career and country of residence. Fifty-four broodmares were given a perineal examination via observation by the researcher to enable the mares to be placed into a 5-tiered grade system (1. Neat and tidy suture – 5. Suture is of the lowest quality).

Results From a total of 602 mares in both areas 46.7% had been given a Caslick vulvoplasty, with the percentage of Caslick operations being significantly ($p<0.001$) higher in GB (59%) than in IRL/NI (34%). The largest sole factor for performing Caslick's across GB and IRL/NI was poor conformation. Significant associations were identified in the effect of age ($p<0.001$), reproductive status ($p<0.001$), country of residence ($p<0.001$) and race career ($p<0.02$) to the likelihood of a Caslick operation. There was a higher incidence of Caslick vulvoplasty operations in mares $\geq 15+$ years than mares aged <5 (81.4% *vs.* 10.3% respectively; $p<0.001$). Reason for performing the Caslick (mainly poor conformation and injury) and increased age had a detrimental effect upon the grading of the Caslick ($p<0.05$ and $p<0.01$, respectively), with Caslick's more likely to be graded between 2-5, signifying a more inefficient Caslick.

Conclusion Caslick's vulvoplasty is routinely used to combat pneumovagina and decrease conformational fault in TB broodmares, with heightened use in GB compared with IRL/NI. Reasons provided for performing the operation varied, however it is still predominantly used to correct faults that are sufficiently treated with a vulvoplasty. In support of the literature, the results reiterate that as broodmares get older and have foaled there is an increased chance of having the operation (Saastamoinen and Barrey, 2000). Some of the reasons listed as to why the Caslick vulvoplasty operation was performed including urovagina, endometritis and as a breeding measure (a non-specific diagnosis) should be reviewed as a Caslick vulvoplasty will not treat these problems but may exacerbate them.

Acknowledgements

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Anatomical differences in the equine pineal gland between gender and breed: a pilot study

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Application Advancement in knowledge of pineal gland anatomy could aid commercial breeding programs in manipulating reproductive cycles in both the mare and stallion.

Introduction The equine pineal gland plays an essential role in the relaying of photoperiodic information to allow for the production of the neurohormone melatonin (Reiter *et al.*, 2010). Melatonin is multifunctional and effects not only sleep-wake cycles but also reproductive function in seasonal breeders (Aurich 2011). Manipulation of seasonal breeding in thoroughbred racehorses is common practice to ensure offspring are born at the optimum time for racing success (Satue and Gardon 2013). Ovine studies show that both the mass of the pineal gland and the number of pinealocytes in contains has a direct effect on melatonin concentrations produced (Gomez Brunet *et al.*, 2002), however this has not been followed up in the horse. This pilot study aimed to determine differences in the mass of the equine pineal gland between genders and breed types.

Material and methods Horse head cadavers (n=16), comprising of Thoroughbred females (n=4), native females (n=4), Thoroughbred males (n=4) and native males (n=4) all between the ages of 3-20 years were collected. These horses were slaughtered for human consumption of which these cadavers were a by-product. Brain extraction was done as described by McBride and Hemmings (2005). The brain was carefully removed and in order to expose the pineal gland a scalpel blade was used to make a incision down the centre of the brain to locate the pineal gland. Once removed the pineal gland was weighed and its mass recorded. Data was analysed using a Two-Way ANOVA with breed and sex as factors and LSD test.

Results When comparing all Thoroughbreds against all native types there was a significant difference in pineal mass (P=0.043), with mean TB pineal mass 0.166g +/- 0.039g and native pineal mass 0.113g +/- 0.047g. No significant difference was seen between males or females with male pineal size 0.140g +/- 0.04g and female 0.138g +/- 0.061g (P =0.923) across both breeds.

Conclusion These results suggest that Thoroughbred types may require a higher volume of melatonin to enter into periods of anoestrus than Native types due to pineal mass and this is supported by evidence in ovine research where melatonin output was found to be proportional to pineal gland mass (Coon *et al.*, 1999, Gomez Brunet *et al.*, 2002). Melatonin plays an important role in the reproductive physiology and function of both males and females potentially explaining why there is no significant difference in their pineal mass. A more extensive study is required using a larger sample size so that factors such as age may be taken into consideration. Notwithstanding the small sample size used in this study this information adds value to our understanding of pineal mass in the horse and could help to explain the impact of selective breeding for homogeneity in the Thoroughbred breed and reduced seasonality in Thoroughbreds.

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A preliminary comparison of semen quality between competing and non-competing stallions

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Application Equine Artificial Insemination practices in the UK have increased from only 18 in 2006 to over 50 in 2015 (BEVA, 2015). Competition records of stallions often determine their breeding popularity, as stallions can be competed at high levels in order to make them more desirable to potential clients. These stallions have to meet the double intensity requirements of performance and breeding activities, a process which may require further optimisation.

Introduction Amann *et al.* (1987) suggested that moderate daily exercise is important for the wellbeing of stallions, but may lead to reduced libido compared to stallions that are not in exercise. More recent research suggests that training of stallions at moderate exercise intensity does not affect semen quality (Gordon *et al.*, 2014). In contrast, Janett *et al.* (2006) found strenuous repeated treadmill exercise dramatically decreases both quality and freezability of the semen. Rosenberg *et al.* (2013) found that a rise in body temperature during exercise induces thermal stress to stallion testes, resulting in reduction of the stallion's reproductive capacity. The aim of the study was to preliminarily investigate whether stallions with concurrent breeding and performance careers have lower semen quality compared to non-competing stallions.

Material and methods Retrospective data was collected for 625 collections over six breeding seasons (from a total of 76 stallions), with 394 collections from competing stallions and 230 collections from non-competing stallions. Data recorded included ejaculate volume, progressive motility (PM) and concentration. An independent-samples t-test was conducted to compare volume, PM and concentration in competing and non-competing stallions, using SPSS 23.0.

Results There was a significant difference in the volume between both groups, with competing stallions producing higher volume ejaculates ($p=0.003$), at a mean 5.96 ± 24.3 ml more per ejaculate than non-competing stallions. There was a significant difference in PM ($p=0.006$), with PM in competing stallions recorded at a mean $3.28\% \pm 10.4\%$; which was lower than the non competing stallions. There was also a significant difference for the concentration ($p= 0.009$) between both groups, with competing stallions showing a lower concentration by a mean 2.7×10^6 compared to non-competing stallions.

Conclusion The differences between the two groups descriptively do not seem to be large, but as all three areas showed significant differences between groups, some concerns are raised. Whilst semen collection with a concurrent competition career remains a viable option, care must be taken to manage both performance areas for stallions carefully, to ensure the highest possible semen quality is collected and distributed. Where semen quality is strongly affected, stallions may not be able to cope with concurrent careers, and semen collection should be planned out of the competition season, or the early stages of the competition season, to limit the travel and competition stress that may have had an effect on semen quality. Follow up research should consider the intensity or level of competition, discipline and age of the stallions to produce more practical guidance for practitioners.

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Thermographic imaging study into the comparative efficiency of cooling agents on the equine limb post exercise

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Application A large range of leg cooling products are widely available, for use at home and in competition, and have replaced cold hosing widely where access to cold hosing is not possible, or time is limited, as cold hosing takes around 20 minutes (Wilson & Goodship, 1994). The actual cooling effect of these products remains to be ascertained; alongside optimum application times and improved effects compared to traditional cold hosing.

Introduction When a horse is exercised, heat is naturally produced in the limbs due to increased circulation to the area and the release of stored energy (Sander *et al.*, 2013), which is beneficial during exercise to keep structures pliable. After exercise, residual heat can damage tissues through secondary inflammatory processes, the use of external cooling may reduce the risk of damage. The aim of the study was to investigate the cooling effect of three commercially available products in comparison to traditional cold hosing, using thermal imaging technology.

Material and methods Five horse received each treatment in a randomised, cross over trial, acting as their own controls (left leg cooled, right leg untreated). Three commercially available products; Barrier White Ice (BWI), Lincoln Cool Gel (LCG) and Equine Products relax (EPR), were compared with cold hosing. All horses were subjected to a standard exercise test (SET) in a fully enclosed indoor arena (ambient temperature recorded), and leg temperature was assessed by thermal imaging before and immediately after exercise, once product was applied, 15 minutes into treatment, at the end of treatment, and 1 hour after treatment cessation. Data was analysed using Factorial Repeated Measures ANOVA (SPSS 23.0).

Results There was a significant main effect of the type of product on the temperature of the limb, ($F(4,16) = 4.89$, $p = 0.009$), indicating that EPR had the same cooling effect as using cold hosing, whilst LCG and BWI were less efficient at cooling the limb than cold hosing. The cooled leg was significantly cooler than the control leg for all treatments ($F(1,4) = 13.59$; $p = 0.021$). The leg is at its coldest 1 hour after treatment, with a mean temperature of 0.5°C lower than the initial mean leg temperature, which is significantly lower than 15 mins into treatment, and at the end of treatment ($F(1,4) = 22580$, $p = 0.000$ and $F(1,4) = 31.67$, $p = 0.005$ respectively). On average, legs are 0.5°C cooler after treatment compared to the pre exercise temperature.

Table 1 Temperatures recorded for all time points and all products

T- Treatment C- Control	Cold Hosing	Barrier White Ice	Lincoln Cool Gel	Equine Products Relax
T pre exercise	22.96±5.91	23.42±5.97	22.51±5.95	22.11±5.77
C pre exercise	22.74±5.98	23.20±5.99	22.29±5.85	21.95±5.72
T post exercise	22.91±5.91	23.39±5.67	22.48±5.94	22.07±5.77
C post exercise	22.96±5.98	23.16±5.97	22.27±5.84	21.92±5.72
T 15mins into cooling	22.82±5.94	23.37±5.95	22.47±5.93	22.01±5.77
C 15mins into cooling	22.62±5.97	23.13±5.94	22.24±5.93	21.88±5.67
T end of cooling	22.82±5.94	23.04±5.91	22.42±5.90	21.99±5.75
C end of cooling	22.55±5.96	23.06±5.98	22.18±5.77	21.84±5.64
T 1hr post cooling	22.81±5.96	23.22±5.98	22.36±5.89	21.94±5.73
c 1hr post cooling	22.55±5.96	23.04±5.90	22.17±8.45	21.84±5.63

Conclusion Cold hosing remains a viable option, with EPR having an equal cooling effect, and LCG and BWI showing less effective in this trial. All products had a cooling effect overall, with initial heating observed in early treatment, which warrants further investigation.

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Neural modulators of temperament: a multivariate approach to personality trait identification in the horse

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Application Temperament traits ‘Anxiety’ and ‘Docility’ have a correlative relationship with inferred neural dopamine levels. This could provide the basis for identifying horses undergoing hyper- or hypodopaminergic neural adaptations.

Introduction Whilst temperament is widely studied in the horse, this has yet to be linked with brain function. This is interesting given that temperament is a key production characteristic of the horse, as well as the fact that temperament is a behavioural manifestation governed by neural circuitry. A number of neural regions utilise the neurotransmitter dopamine for cognitive processes. Considering the horse can present with hyperdopaminergic (e.g. stereotypy) and hypodopaminergic (e.g. pituitary pars intermedia dysfunction) pathologies, the overall aim was to determine whether there was a relationship between equine temperament and inferred measurements of dopamine.

Material and methods A sample of 99 horses were sought using a non-discriminative approach to ensure a wide range of equine backgrounds were represented. Each horse was observed for the dopamine correlates spontaneous blink rate (SBR) and behavioural initiation rate (BIR) for 30 minutes for each dopamine correlate, with this repeated over three consecutive days. Mean SBR and BIR was then calculated for each individual. No management or environmental changes were implemented by the observer. Temperament data was collected utilising a questionnaire consisting of 41 temperament variables, adapted from previous horse personality questionnaires (Momozawa *et al.*, 2003; Lloyd *et al.*, 2007). A principal component analysis (PCA) with varimax rotation was conducted to produce distinct temperament traits. Only components with eigenvalues greater than 1 were retained. Similarly, any temperament variable with component loadings less than 0.40 or more than -0.40 were suppressed. Component scores were attributed to each individual animal using the Anderson-Rubin method on SPSS v.22. A Spearman Rank Correlation Coefficient was conducted to correlate SBR and BIR with the extracted temperament components from the PCA. Statistical significance was set at $p < 0.05$.

Results Following examination of the Kaiser-Meyer-Olkin (KMO) statistic during the PCA, five temperament variables were removed. The remaining 36 temperament variables gave rise to 9 components, accounting for 72.4% of the total variance. By examining the component loadings for each of the 9 components, it could be concluded that the components consisted of the following temperament traits; ‘Anxiety’, ‘Trainability’, ‘Excitability’, ‘Docility’, ‘Inquisitiveness’, ‘Irritability’, ‘Self-Reliance’, ‘Horse-Horse Interaction’ and ‘Horse-Human Interaction’. The temperament trait ‘Anxiety’ was found to have a weak positive relationship with SBR ($R = 0.202$, $p = 0.045$) (Fig.1). Furthermore, ‘Docility’ demonstrated a weak negative relationship with SBR ($R = -0.215$, $p = 0.032$) (Fig.1).

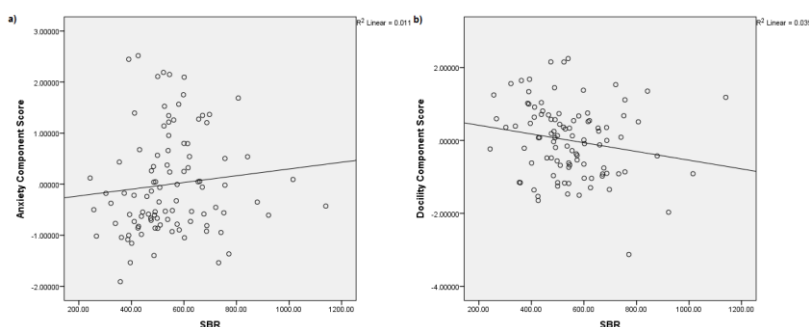


Figure 1 Spearman Rank Correlation Coefficients for a) ‘Anxiety’ versus SBR and b) ‘Docility’ versus SBR

Conclusion The temperament traits ‘Anxiety’ and ‘Docility’ have a relationship with dopamine levels, as revealed by SBR measurement. This could allow the identification of horses undergoing hyperdopaminergic or hypodopaminergic alterations respectively, prior to the emergence of classic symptoms by simply observing alterations in equine temperament.

Acknowledgements The authors would like to express their thanks to the RAU Research Committee for funding this study as part of an ongoing PhD project.

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Effect of selenium and vitamin E supplements on blood lactate and serum lactate dehydrogenase activity in horses undergoing moderate exercise

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Application Working horses at high altitudes are exposed to an important oxidative stress and free radical formation, which could damage their skeletal muscle. Oral antioxidants could reduce these effects, using the appropriate doses.

Introduction Exercise in horses is directly associated with O₂ uptake by tissues, mainly by skeletal muscle. Mitochondria use O₂ to produce ATP. However, this generates oxidation and free radical formation (Piccione *et al.*, 2012), which could alter the sarcolemma permeability. This fact could increase serum lactate dehydrogenase (LDH) activity and lactatemia (LAC). The secondary antioxidant system prevents cellular membrane damage caused by free radicals. The objective of this study was to evaluate the effect of selenium (Se, selenium methionine) and vitamin E (E, α -tocopheryl acetate) supplementation on lactatemia and LDH serum activity in horses under moderate exercise.

Materials and methods Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico approved the experimental protocol. The study lasted 77 days and was carried out in the Mounted Police Unit of Mexico City (2,240 m above mean sea level) in winter 2014. The trial involved 24 clinically healthy horses, without physical labour the month prior to this study. The horses were 5 to 15 years old (450 kg BW), and were randomly allocated in 4 experimental treatments with 6 animals each one. They were assigned in a factorial design [2 Se levels \times 2 E levels, high (H) and low (L)] with repeated measures. The supplementation levels followed NRC recommendations (2007). Therefore, the experimental treatments were: LSeLE, HSeLE, LSeHE and HSeHE (LSe, 0.1; HSe, 0.3 mg Se/kg of DM and LE, 1.6; HE, 2 IU vitamin E/kg of BW). Selenium was undetectable (<2 μ g/kg DM), while vitamin E reached 14.4 IU/kg DM in the daily ration used. Thus, Se and E were fully provided using oral supplements. Day zero (d0) corresponded to the baseline measurement of LAC and LDH. The trial was formed by an adaptation period (d0 to d32); from d33 to d56 the exercise period consisted of 3 consecutive days and 4 without exercise sequences per horse. Moderate exercise included 5:20:5 min (warm up:gallop:cool down). Horses were readapted from d57 to d77. At d64, supplementation was ended. Once a week, jugular blood samples were taken. During the exercise period, samples were taken at the end of the 3rd day, 10 min after physical activity. LAC was immediately quantified (Reflectance photometry, Roche) in blood, whilst LDH activity was quantified in serum by spectrophotometry (Randox DaytonaTM). Data were analysed using the 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. Statistical significant levels were set at P<0.05, while a trend was defined at P<0.10. Regression analysis between LDH and LAC were applied to each experimental day.

Results LAC and LDH in horses that received 0.3 mg Se/kg DM and 2.0 IU vitamin E/kg of BW supplementation, were not different from those who received 0.1 mg Se/kg DM and 1.6 IU vitamin E/kg of BW; therefore, all treatments were pooled together. Experimental day affected both LAC and LDH (P<0.05; Table 1). Noticeable differences for LAC (P<0.05) were established between d0 and d35 to d56. LDH increased at d21, which was not different (P>0.05) from that of d77. The lowest value was observed at d63. Day effect was explained by experimental periods (adaptation, physical activity and no supplementation). A trend (P<0.06) for vitamin E effect was observed on LDH activity. LAC at d56 was 2.4 times higher than LAC at d0 in all treatments. At d42 a positive relationship (P<0.05) between LDH and LAC was found for horses who received 1.6 IU vitamin E/kg BW [LAC (mMol/L) =0.003 \times LDH (U/L); n=12, R²(adj)=0.9]. At d63 (rest period), the lowest values of LDH were observed (P<0.05), while LAC returned to basal values. Both LAC and LDH increased at d70 and d77.

Table 1 Lactate blood (mMol/L) and LDH serum activity (U/L) in horses with moderate exercise

	Days of adaptation period					Days of moderate exercise					No supplementation	
	0	7	14	21	28	35	42	49	56	63	70	77
LAC ¹	0.846*	0.975	1.012	1.250	1.058	1.571*	1.800*	1.645*	2.067*	1.096	1.354	1.600*
LDH ¹	469.6	469.5	504.5	606.8*	514.5	506.5	524.3	490.0	454.2	391.8*	473.7	563.3*

¹SEM, LAC, 0.12; LDH, 24.8. Significant differences are marked with * (P < 0.05)

Conclusion Moderate physical activity affected both LAC and LDH values. The trend for a vitamin E effect on LDH activity and the relationship observed at d42 between LDH and LAC in horses from LE group requires further research.

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Equicoll: a product for selecting robust stallion sperm for artificial insemination or freezing

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Product description Equicoll is a species-specific colloid for selecting robust stallion sperm for artificial insemination or freezing, thus helping to improve pregnancy rates in inseminated mares.

Industry need Artificial insemination (AI) with cooled semen is being used more and more frequently in equine breeding in Europe. The per cycle pregnancy rate after inseminations with approximately 1×10^9 spermatozoa (cooled semen) is around 65% and is dependent on the sperm quality of the ejaculate. Thus approximately one third of mares inseminated with cooled semen do not become pregnant, due to a variety of reasons, including sperm quality, handling factors, or mare factors. Improving sperm quality could thus be an effective method of increasing pregnancy rates.

The solution A method for improving the sperm quality of semen doses for AI has been developed in which the most robust spermatozoa are selected by centrifugation through a colloid, Equicoll (previously known as Androcoll-E). This technique enables the most motile spermatozoa, and those with normal morphology, intact plasma membranes and good chromatin integrity to be separated from the rest of the ejaculate. These spermatozoa retain their motility, viability and chromatin integrity for longer than unselected spermatozoa, and show normal fertilizing capability at 5 days post-collection. This method has been shown not only to improve pregnancy rates for problem ejaculates but also to improve pregnancy rates from “normal” stallions in a multicentre study. Finally, the selected spermatozoa may freeze better than unselected samples. The technique can be performed easily at any stud where there is a bench centrifuge and is easy to learn.

Supporting evidence or validation The product has been tested extensively by the inventors in controlled trials and also by other research groups and stud personnel. The method is easy to use and can be done on any stud where there is a bench centrifuge. There is an extensive list of more than 80 scientific articles documenting the beneficial effects of colloid centrifugation on sperm quality.

Commercial applications and availability Androcoll-E is currently only available from the inventors (contact jane.morrell@slu.se for details) but this situation will change soon. Another version, Androcoll-Equine, was temporarily available from Minitüb International.

Future development There is scope to develop similar products for other species

Opportunities for collaboration/networking We seek collaboration with manufacturers of equine products who are interested in marketing and distributing the product.

Acknowledgements We thank BSAS for permission to present our product here.

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Gastro-G, a functional food/nutraceutical additive to help alleviate the underlying causes of Gastric Ulcers in horses

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Product description Gastro-G is a daily food additive which can be given for the modulation of the underlying causes/symptoms of Equine Gastric Ulcer Syndrome.

Industry need Equine Gastric Ulcer Syndrome (EGUS) is reported to affect up to 90% of horses (Nadeau and Andrews, 2010). Effective therapies for treatment in horses are namely prescription-only-medicines (POMs), Omeprazole or Ranitidine, that block the production of hydrochloric acid. These therapies do not address the underlying inflammatory response of gastric or duodenal cells. Moreover, these synthetic compounds are expensive, administered only under direction of a veterinary surgeon in the UK, and their effects may not persist beyond medication withdrawal (Reese and Andrews, 2009). Many horse owners rely on their insurance to pay for initial investigations and to cover the short term cost of ulcer treatment, when this runs out they are often unable to cover the ongoing expense. Following treatment for ulcers many insurance policies issue an exemption against further ulcer treatment and owners of young horses are faced with many years trying to manage the high cost of diagnosis and treatment themselves.

Therefore, there is a pressing clinical and commercial need for discovering new, non-toxic compounds with effective anti-inflammatory and anti-ulcerative properties for use in veterinary medicine. Moreover, natural biological compounds rather than synthetic drugs confer an advantage when it comes to market acceptability and licensing restrictions for use in a clinical setting.

The solution Gastro-G contains a plant compound which is used as a primary folk health care preparation for human peptic ulcer treatment in Brazil and Japan.

Maytenus ilicifolia is a Celastraceae plant native to Brazil, which for many years has been anecdotally reported to have anti-inflammatory and anti-ulcerative properties (Balbach, 1980; Cruz, 1982; Born, 2000). Scientific studies of *Maytenus ilicifolia* efficacy have been conducted using mouse or rat models and, although few specifically investigate its effects relating to gastrointestinal ulcers, those that do, report positive results (Jorge *et al.*, 2004; Cipriani *et al.*, 2009; Leite *et al.*, 2010;). There are no published data that support the anti-inflammatory or anti-ulcerative functioning of *Maytenus ilicifolia* specifically in cells of the gastrointestinal tract for humans or horses.

Supporting evidence or validation A study was designed to validate the use of the *Maytenus* using human Caco-2 cells; this cell line is used as an *in vitro* intestinal model in pharmacokinetics and pharmacodynamic studies.

The Caco-2 cells exhibit a measurable inflammatory response when stimulated by bacterial molecules. As such the ability of putative anti-inflammatories to abrogate this reaction may also be assessed using this model. Specifically, when challenged with the bacterial antigen lipoteichoic acid (LTA) Caco-2 cells undergo an inflammatory response by up-regulating gene expression and secretion of cytokines and chemokines, including interleukin 1 β (IL-1 β) and IL-8 respectively. The effect of putative anti-inflammatory agents can thus be measured by co-culturing stimulated cells with the compound under investigation and determining whether the gene expression and/or secretion of cytokines and chemokine's are abrogated. Therefore, the Caco-2 cell line offered the first opportunity to assess the anti-inflammatory and anti-oxidant effects of *Maytenus* specifically in relation to the human/horse gastrointestinal tract.

Maytenus ilicifolia did not cause Caco-2 cell death in differentiated cells (concentrations 0 to 200 $\mu\text{g}/\text{mL}$) determined by gene expression of the cell apoptosis marker, Caspase-3, and did not alter the permeability properties of the epithelia monolayer demonstrated by Apparent Permeability and Trans Epithelia Electric Resistance. *Maytenus ilicifolia* was able to abrogate the response at concentrations of 100 $\mu\text{g}/\text{mL}$ or greater. The inflammatory response, and abrogation of it by *Maytenus ilicifolia*, was measured using an extensive range of markers relating to the Toll-like receptor (TLR) pathway: gene expression of TLR2 itself and inflammatory cytokines IL-1 β and TNF α . Protein secretion of the inflammatory chemokine IL-8 by Caco-2 cells was also abrogated by high concentrations (200 $\mu\text{g}/\text{mL}$) of *Maytenus ilicifolia*. The study provides preliminary yet promising evidence that *Maytenus ilicifolia* does not accelerate death of human differentiated intestinal cells and indeed, exhibits anti-inflammatory properties effective at abrogating inflammation in the event of immune challenges to these cells.

Commercial applications and availability Gastro-G is currently sold in bulk 25kgs directly into the horse racing industry. This product is also for sale on line at www.superfix.net or through www.freestepsuperfix.co.uk

Future development On the basis of this trial *Maytenus ilicifolia* is a strong candidate for further study into its anti-inflammatory properties with regard to ulcer therapy and with potential for development towards the human dietary supplement market.

Opportunities for collaboration/networking We seek collaboration with other interested parties (universities/feed companies) with regard to further research and sales development.

Acknowledgements We thank Dr Debbie Nash and Dr Manuela Natoli from Aberystwyth University for their contribution to making this product commercially viable.

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Association of warm up and competitive placing at BE100 and Novice level eventing

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Application Identification of common warm up routines and their effect on competitive placing in British Eventing may inform riders of ideal techniques to ensure maximum performance.

Introduction Warm up has been shown to be an important element of success in human athletes (Kapnik *et al.*, 1992), yet there has been little research into the types and effect of warm up routines in any equestrian sport. Eventing is an extremely popular, but little researched equestrian discipline in the UK, in which hundreds of horse and rider combinations compete every year (Singer *et al.*, 2008). There have been a few studies that look at injury in event horses in training and competition at national and international level (Dyson, 2002). Yet, despite the majority of competitors competing at one day events, there is little research concentrating on these lower levels. This study aims to characterise warm up routines of competitors at lower levels of British Eventing (BE100 and Novice) and identify any effect they may have on competitive placing. In particular, rider status (professional or amateur) and rider and horse placing were compared to different warm up routines.

Material and methods Data were acquired anonymously by online survey using SurveyMonkey (SurveyMonkey Inc., www.surveymonkey.com) after permission was granted by British Eventing. Completion of the survey included permission to use data. Riders were recruited at competitions and online using social media (@Twitter, @Facebook, @Instagram) over the space of seven weeks. A total of 208 surveys were completed. Only complete surveys were used in the statistical analysis (n=203). The data were transferred into Microsoft Excel (www.microsoft.com), coded and Chi-Square analyses performed. Significance was set at P < 0,05.

Results Professional riders (defined as someone who makes a living from producing/competing/training horses) were shown to use pre-tack warm-up techniques such as massage pads or magnetic rugs significantly more commonly than amateur riders (X²=3.92; P=0.048). In addition, competitive placing was shown to be associated with the following events: cross country warm-up time in trot (P=0.0021), number of jumps performed before cross country (P=0.0026), show jumping warm-up time in trot (P=0.0079) and overall dressage warm-up time (P=0.038).

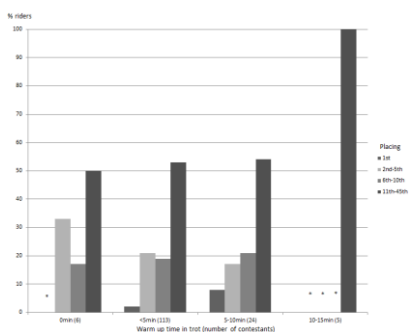


Figure 1 Competitive placing associated with warm up time in trot in cross country (* sign. less likely to place)

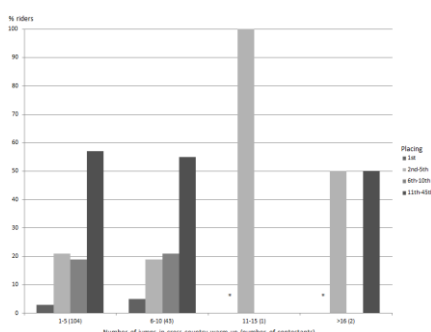


Figure 2 Competitive placing associated with jumps performed in cross country warm-up (* sign. less likely to place)

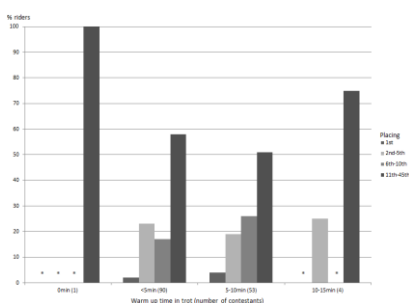


Figure 3 Competitive placing associated with warm up time in trot in show jumping (* sign. less likely to place)

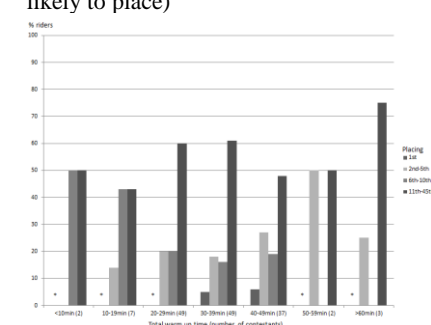


Figure 4 Competitive placing associated with total warm-up time in dressage (* sign. less likely to place)

Conclusion This study identified that too short (<30min) or too long (>49min) a warm-up before the dressage was significantly associated with a lower overall placing. Similarly, trotting less than 5 minutes or more than 10 minutes during the warm-up for show jumping and cross country was associated with a lower overall placing. In addition, during the cross country warm-up, performing 1-10 jumps was most advantageous for competitive placing. These results show that elements of warm-up routines do influence outcome for competitors. More research into this area could identify ideal warm-up routines to maximise performance during eventing.

Acknowledgements The authors gratefully acknowledge the support of British Eventing and their members.

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The implications of piglet temperature at birth and at 24 hours of age on survival

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Application Piglet temperature at birth and at 24 hours of age are a good indicator of survival and hence could be used to identify at risk piglets.

Introduction Pre-weaning mortality is a growing problem in industry. Increased selection for large litters has negative side effects such as increased litter weight variation and more piglets born with anoxia (Alonso-Spilsbury *et al.*, 2005). Low birth weight and anoxic piglets have reduced chance of survival, lower growth rates and lower viability compared to higher birth weight piglets (Milligan *et al.*, 2002). Temperature can be an indicator of vitality and survival; piglets with lower birth temperatures often have reduced vigour. Piglets with reduced vigour take longer to gain access to the sow and therefore do not get the energy they need for thermogenesis (Herpin *et al.*, 2002). The aim of this study was to determine whether the temperature of piglets at birth and 24 hours of age affected their survival.

Material and methods Eighty-two (Large White × Landrace) sows were housed indoors in groups of 8 until day 109 of gestation. Sows were moved into the farrowing house on day 109 of gestation and kept in conventional farrowing crates within individual farrowing pens measuring 4.2 m². Sows were fed diets throughout gestation and lactation which met all nutrient requirements (NRC, 2012). A total of 1,252 piglets were born and of these 90 were still born. Cross fostering to even up litter size commenced between 12 and 48 hours post fostering. At birth and 24 hours, piglet temperatures were taken using a tympanic ear thermometer. All deaths were recorded. Birth and 24 hour temperatures were each divided into categories. In order to do this piglets were sorted according to birth temperature and split into four equal groups, creating four temperature ranges. The same was done for 24 hour temperatures. The number of piglets that died in each category was then analysed for both birth and 24 hour temperatures using Pearson's Chi Squared tests.

Results Piglets with a birth temperature below 34 °C had a higher chance of mortality than piglets with a birth temperature above this ($P < 0.01$) (Figure 1). Piglets with a 24 hour temperature of 36.3 °C or below had an increased chance of mortality compared to those with a 24 hour temperature of 38.3 °C or above ($P < 0.001$) (Figure 2).

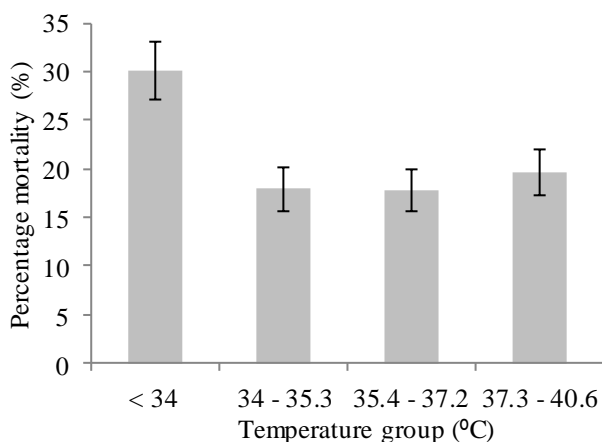


Figure 1 Percentage mortality in each birth temperature category \pm S.E

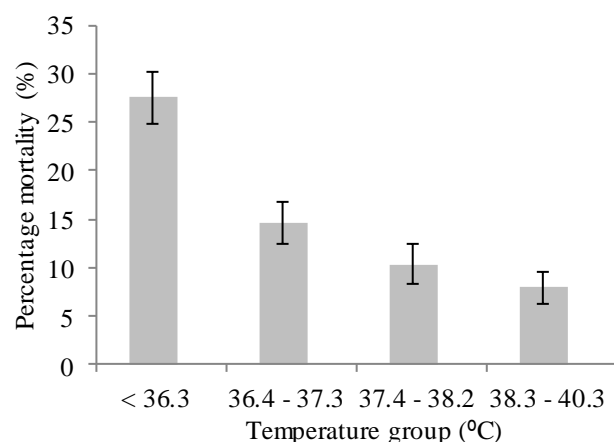


Figure 2 Percentage mortality in each 24 hour temperature category \pm S.E

Conclusion Piglets with birth temperatures below 34 °C and 24 hour temperatures below 36.3 °C had reduced chance of survival compared to piglets with temperatures higher than this, demonstrating temperature is a good indicator of survival.

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Efficacy of a novel efficient phytate degrading bacterial 6-phytase in gestating and lactating sows

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Application Excretion of phosphorous (P) by pigs has some negative impact on the environment in areas with a high density of pig production. The use of highly efficient phytases in animal nutrition is a promising tool to restrict that impact.

Introduction Phytate is regarded as the primary storage form of P in plants and accounts for 60–80% of P found in plant-derived feedstuffs (Crosgrave, 1966; Ravindran *et al.*, 1995). Since there are no significant amounts of intrinsic phytate degrading enzymes in monogastric animals it can be assumed that most of the phytate bound P is not absorbed, but excreted. Due to this, diets for pigs and poultry have been traditionally supplemented with inorganic P sources (Rodehutsord, 2001). On average 500 FTU/kg of phytase added to feed was assumed to generate 0.8g digestible P (dP) (Poulsen *et al.*, 2007). However, due to the increasing livestock density in many regions, manure has been applied to the soil at rates exceeding plant needs, resulting to accumulation of phosphate in the soil (CAST, 2002). Long lasting that bears the risk of eutrophication of surface waters and leaching of phosphate into ground water (Furrer and Stauffer, 1987). The objective of the study was to investigate the potential of a novel bacterial 6-phytase (6-Phy) that is different from established phytases by being a composite of various bacterial sources, in order to evaluate the potential of a possible reduction of inorganic P sources in the diet. The effects of this phytase on Ca, P, ash and DM digestibility in gestating and lactating sows was measured in addition to some performance parameters in sows and piglets.

Material and methods A total of 27 sows [*Duroc X Landrace*] with confirmed gestation (week 7) from three consecutive batches were distributed into groups (blocks) of three sows as similar as possible according to parity number and body weight. Sows in each block were randomly assigned to three experimental treatments that consisted of a negative control basal diet (NC), NC + 100 FTU (phytase unit) 6-Phy/kg of feed (6-Phy-100) and NC + 250 FTU 6-Phy/kg of feed (6-Phy-250). Basal diet was a common corn/SBM-based but digestible P (dP) was formulated to be marginal at amounts of 0.13 and 0.11 g/kg of feed in the gestation and lactation phase, respectively. Feed was restricted (3.6 kg/d) during gestation and offered *ad libitum* during lactation. Water was available *ad libitum* during the complete trial. Sows were weighed at day 2 and 26 *post partum*, piglets were weighed directly after farrowing and at weaning (day 26). Fresh faeces from sows for determination of the digestibility parameters was taken from day 85 to 88 of gestation and from day 18–22 of lactation. For that purpose, feed was supplemented with the indigestible marker TiO₂ (5kg/MT). The bacterial 6-Phy prototype was produced and supplied by BASF SE, Ludwigshafen, Germany. The P-Phy gene is assembled from sequences of various phytase producing bacteria of the species *Hafnia ssp.*, *Yersinia ssp.* and *Buttaxiella ssp.* Production was done by fermentation in *Aspergillus niger*. Phytase units (FTU) in feed were analysed according to Engelen *et al.* (1994).

Results No statistically significant differences were found for sow's final body weight and body weight changes among the treatments. Similarly, no significant improvements in litter size and weight of new born piglets were observed. However, a better performance relating to litter size ($p=0.08$) and litter weight ($p\leq 0.05$) was suggested at farrowing in phytase supplemented groups compared to NC. Apparent total tract digestibility (ATTD) of Ca was numerically improved by phytase addition in both periods. In both periods ATTD of P was significantly improved in 6-Phy-100 compared to NC ($p\leq 0.05$) and was further significantly improved in 6-Phy-250 ($p\leq 0.05$) (15.3 vs 25.2 vs 32.7% in gestation, 17.5 vs 23.6 vs 36.3% in lactation, respectively). For 6-Phy-250 increases of 0.70 and 0.83g dP/kg of feed compared to NC were calculated. ATTD of ash was significantly improved by the phytase treatment ($p\leq 0.05$).

Conclusion It is concluded that the novel bacterial 6-Phy improves P digestibility in gestating and lactating sows at rather low dose rates. The indicated positive impact on litter size and performance as well as the impact at higher dose rates needs further work in consecutive studies for confirmation.

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Comparative utilisation of two dietary fibre sources supplemented with direct-fed microbials in growing pigs

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Application Direct-fed microbials supplementation enhanced growth performance in growing pigs fed high fibre diets based on palm kernel meal, but had no effect in high fibre diet based on rice bran.

Introduction Dietary inclusions of Direct-fed microbials (DFM) may modulate gastrointestinal bacterial populations (Kerr *et al.*, 2013), which may be beneficial for gut health and nutrient metabolism. However, there is little or no study on the comparative influence of DFM on the ability of growing pigs to digest fibre from rice bran and palm kernel cake. Hence, this study was designed to investigate the effect of supplementing DFM in diets with rice bran or palm kernel cake on the growth performance and nutrient digestibility of growing pigs.

Material and methods In an 84-day feeding trial, 24 growing pigs with an average initial weight of 12 ± 0.5 kg (\pm SE) were assigned to six dietary treatments (TRT) in a 2×3 factorial design. The factors were two sources of dietary fibre namely palm kernel meal (PKC) and rice bran (RB) and three DFM inclusions which were without any additive; yeast based DFM and multi-strain DFM. Each treatment was replicated twice with two pigs per replicate. Pigs on TRT 1 were fed 30% rice bran-based diet containing no DFM, while pigs on treatments 2 and 3 were fed the same diet in TRT 1 supplemented with either yeast or multi-strain DFM respectively. Similarly, pigs on TRT 4 were fed 30% PKC-based diet without DFM addition, while TRT 5 and 6 were fed PKC-based diet supplemented with either yeast or multi-strain DFM respectively. The Multi-strain DFM contained 99.9% water, 1×10^8 CFU/g *Lactobacillus sp*, 4×10^{12} CFU/g *Bacillus sp* and 11×10^5 CFU/g *Saccharomyces cerevisiae*, while the yeast-based DFM contained yeast culture and dried brewer's yeast. Multi-strain DFM was supplemented at 2ml/kg diet and yeast based DFM at 2g/kg diet respectively. Diets and water were offered to the pigs on *ad libitum* basis. At the end of the feeding trial, three pigs per treatment were used to determine the nutrient digestibility of the test diets.

Results There was a significant ($P < 0.05$) effect of fibre source on final body weight and daily weight gain, while daily feed intake and feed conversion ratio were not significantly affected. There was also a significant interaction of fibre sources by direct fed microbial on final body weight, daily weight gain and daily feed intake (table 1). Also, DFM had no significant effect on the digestibility of proximate constituents. However, PKC-based diets had significantly higher dry matter and ether extract digestibility than for RB-based diets.

Table 1 Effect of rice bran and palm kernel cake based diets supplemented with direct-fed microbials on the performance of growing pigs

Parameters	Treatments						Pooled SEM	P (ANOVA)		
	T1	T2	T3	T4	T5	T6		DFM*	FS*	D×F*
Initial weight (kg)	13.63	14.88	13.38	14.25	13.63	15.25	0.72	0.95	0.69	0.46
Final weight (kg)	37.25 ^{bc}	36.25 ^{bc}	32.50 ^c	38.00 ^{bc}	40.88 ^{ab}	44.38 ^a	1.02	0.86	0.001	0.02
Daily gain (g)	281 ^{bc}	255 ^{bc}	228 ^c	283 ^{bc}	324 ^{ab}	448 ^a	19.60	0.26	0.004	0.02
Feed intake (g/day)	1089 ^{ab}	1118 ^{ab}	1013 ^{ab}	989 ^b	1123 ^{ab}	1238 ^a	27.20	0.28	0.38	0.04
FCR	3.90 ^{ab}	4.46 ^a	4.53 ^a	3.60 ^{bc}	3.47 ^{bc}	2.88 ^c	0.16	0.61	0.0003	0.07

*FS - fibre source, DFM - direct fed microbial, D×F - two way interactions between fibre sources and direct fed microbial.

Conclusion It was concluded that, there was an interaction between direct fed microbials and the fibre sources, as the growth performance of the growing pigs was enhanced and this was more evident on PKC-based diets than RB-based diets. Similarly, multi-strain DFM was more effective in enhancing growth performance of pigs than yeast direct fed microbial.

Acknowledgements The authors are grateful to AK Research Farm, Ibadan, Nigeria for facility to conduct this study and Best Environmental Technologies, Accra, Ghana for Multi-strain direct fed microbial.

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QTL mapping for Cell Mediated Immunity in chicken

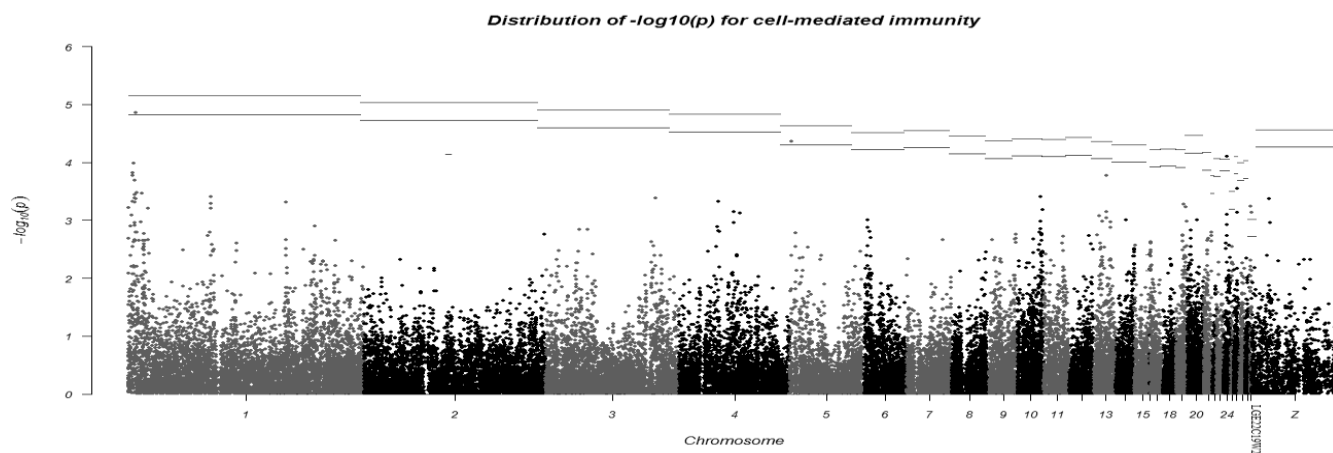
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Application Improvement of immunity system responses to disease and pathogens in domestic animals is achievable by gene identification and selective breeding in favour of immunity related traits. Selection of animals based on their genomic impact in immunity related traits results in producing animals with strong capacity to disease resistance.

Introduction Availability of genetic map for farm animals has enabled animal breeding community to seek for associations between genomic variables and phenotypes of complex traits and diseases, commonly called genome wide association studies (GWAS). Application of genomic variables for low heritable or hard to measure traits such as immunity related traits is an extra advantage of genomic selection. CMI refers to various immune cells, including neutrophils and cytotoxic T cells but humoral immunity mainly refers to antibodies (Harnett, Katz *et al.* 2005).

Material and methods a GWAS is performed to identify genomic regions associated with cell-mediated immunity in chicken using chicken 60k high density single nucleotide polymorphism (SNP) array. Phenotypes of 198 animals for cell mediated immunity (CMI) was available in a F2 population. In order to account for multiple testing a chromosomal false discovery rate was applied as significant thresholds. Based on genomic inflation factor, comparison of the power of fixed and mixed linear models (MLM) is done. Since MLM had better inflation rate its results is used for subsequent analysis. In order to account for multiple comparison, *P-values* were adjusted based on chromosomal false discovery rate (FDR) of 5% as significant and suggestive threshold respectively (Benjamini and Hochberg 1995).

Results 3 significantly associated SNPs on chromosome 24 and linkage group E22C19W28_E50C23 and 3 suggested associated SNPs on chromosome 1, 5 and 16 were identified. Pathway analysis showed that 2 biological pathways which are related to immune responses were strongly associated with the candidate genes surrounding identified SNPs and their influences were mostly on antigen processing and presentation and cellular structure.



Conclusion This study specifically focused on CMI in chicken and showed that it might be possible to predict genomic estimated breeding values (GEBV) and use it to improve immune response in poultry populations through genomic selection and marker assisted selection.

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Principal component factor analysis of the morphostructural traits of West African dwarf sheep

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Application Isolation of best traits to predict body weight of West African Dwarf (WAD) sheep from orthogonal body shape characteristics from multicollinear predictors

Introduction Livestock supplies contribute immensely to protein consumption, mainly estimated at 27.9 percent worldwide and 47.8 in developed countries (FAOSTAT, 2011). Sheep classified as small ruminant is raised commonly by farmers with low income (Kurnianto *et al.*, 2013). WAD sheep is a multipurpose predominant breed of humid tropics primarily reared for its meat. The Principal Component Analysis (PCA) is a multivariate statistical methodology for assessment of morphometric traits (Yakubu *et al.*, 2009).

Material and methods Evaluation of seven morphostructural traits across age range 0-2 and 2-4 years of 200 West African Dwarf (WAD) sheep, was employed in the prediction of body weight character. Collinearity effect was orchestrated with computations of eigenvalues and variance proportions. Component solution through VARIMAX orthogonal rotation generated one and two principal components for 0-2 and 2-4 years respectively. The principal component based regression models revealed, body weight was best predicted from heart girth, and combination of rump height and wither's height measurements

Results Evaluation of seven morphostructural traits across age range 0-2 and 2-4 years of 200 West African Dwarf (WAD) sheep was used in determining prediction of body weight character. Collinearity effect was orchestrated with computations of eigenvalues and variance proportions. Component solution through VARIMAX orthogonal rotation generated one and two principal components for 0-2 and 2-4 years respectively. The principal component based regression models revealed, body weight was best predicted from heart girth, and combination of rump height and wither's height measurements.

Table 1 Rotated component matrix for the age groups and body weight prediction with multiple regression of body measurements on the principal components scores of West African dwarf sheep

Model	Age group 0-2		Age group 2-4			
	Trait	PC 1	Community	PC 1	PC 2	Community
	BL	.775	.601	.693	.353	.605
	WH	.851	.725	.770	-.178	.625
	RH	.882	.777	.798	-.051	.639
	FCBL	.484	.234	.394	-.774	.755
	CD	.554	.307	.722	-.177	.552
	HW	.537	.288	.279	.843	.788
	HG	.816	.666	.748	.089	.567
	Eigenvalue	4.00		3.02	21.53	
	%	51.41		43.20	1.51	
	Variance	.63		.25	.14	
	R ²	-15.70		-12.80	1.08	
	Intercept	HG		(HG)(RH)	WH	
	Orthogonal Predictor					

BL: body length; WH: wither's height; RH: rump height; FCBL: fore cannon bone length; CD: chest depth; HW: hip width; HG: hearth girth.

Conclusion Selection of WAD sheep based on the PCA suggested traits for identification of early maturing animals is an appropriate tool for effective livestock farm management and breeding.

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Morphological changes induced by mineral supplements on *Bos indicus* × *Bos taurus* heifers in a tropical environment

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Application Manipulation of morphology by mineral supplementation could improve productive and reproductive performance in cattle as well performance in judging competitions.

Introduction Zoometric measurements of cattle could give information about the productive function of animals and thereby improve selection processes (Sañudo, 2009). On the other hand, manipulating morphology could affect the outcome of cattle judging shows, if induced modifications produce a better fit with standards. The aim of this work is to quantify the effect of an oral mineral supplement on the zoometric measurements of heifers.

Material and methods 12 Brahman × Holstein or Brahman × Brown Swiss heifers with 223±19 kg BW and ~10 months of age were housed in a very humid tropical environment in Costa Rica. They were fed at 0.025 BW with 0.35 banana peels, 0.35 green bananas, 0.1 forage (*Pennisetum purpureum*) and 0.2 concentrate (DM basis). Heifers were stratified by initial weight and assigned the following treatments: T1(n=6)- NaCl, T2(n=6)- *ad libitum* mineral supplements (115 g Ca, 90 g P, 20 Na, 15 g Mg, 4000 mg Zn, 1000 mg Mn, 250 mg Fe, 1000 mg Cu, 75 mg I, 15 mg Co and 32 mg Se per kg of supplement). Withers height (WH), hip height (HH), hip width (HW), pins width (PW), thoracic circumference (TC) and body length (BL) were measured each two months. Furthermore, Proportionality Index (PI=WH/BL×100), Anamorphosis Index (AI=TC²/WH×100), and Transversal Pelvic Index (TPI=HW/WH×100) were calculated. Data were analysed as repeated measures, using Proc MIXED by SAS/STAT (SAS Institute Inc. 2011), with initial measures as covariables for each trait.

Results Significant differences (p<0.05) were observed only for the hip width (+1.1 cm in the T2 group) (Table 1). This may mean that mineral supplemented heifers could give birth more easily than T1 heifers (Wójcik and Kruk, 2010). But there was a significant treatment*time interaction, and this shows that significant differences only occurred in the second, third and fourth measurement occasions (~12, 14 and 16 months of age, respectively), but differences were lost at 18 months (Figure 1). This implies that morphological changes are at very early age, not impacting calving ease, but could give insights on the early development in cattle. There were tendencies (p<0.10) to significance in other variables such as BL and PTI.

Table 1 Means, standard errors (SE) and p-values for the comparison of T1 and T2 treatments

Variable	T1 (n=6)		T2 (n=5 ^a)		P-value			
	Mean	SE	Mean	SE	Treatment	Time	Treatment*Time	Covariable
TC (cm)	167.8	0.87	168.0	0.95	0.850	<0.001	0.702	<0.001
HH (cm)	129.4	0.71	128.9	0.78	0.630	<0.001	0.124	0.003
WH (cm)	124.0	0.73	123.2	0.80	0.487	<0.001	0.899	<0.001
HW (cm)	41.4	0.31	42.5	0.34	0.045	<0.001	0.009	0.014
PW (cm)	27.8	0.19	27.7	0.20	0.786	<0.001	0.155	0.055
BL (cm)	120.2	0.91	123.2	1.01	0.066	<0.001	0.476	0.317
PI	103.0	1.14	100.3	1.25	0.158	0.439	0.634	0.031
AI	227.4	2.21	229.5	2.42	0.553	<0.001	0.768	0.228
PTI	33.4	0.30	34.4	0.32	0.067	<0.001	0.054	<0.001

^a A heifer was excluded from the study because of death

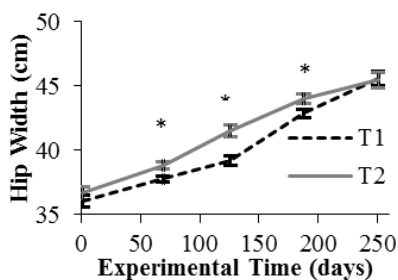


Figure 1 HW across evaluations for T1 and T2 groups. Asterisks represent differences in an evaluation (p<0.05)

Conclusion Mineral supplementation could induce changes in morphology in heifers, inducing wider hips, but although these differences could implicate easier calving, the existence differences disappear at ~18 months. No other variable registered significant treatment effects.

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Patulin in Maize Silage

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Application While patulin is not commonly reported in the UK, it is possible that in poorly stored silage patulin may occur in high quantities but only for a short period of time potentially making patulin mycotoxicosis difficult to diagnose from feed samples.

Introduction *Penicillium roqueforti* is found in silage, grain, and other plant materials stored under microaerobic conditions and produces several secondary metabolites, one of which is patulin. A cyclic lactone, patulin is toxic to plants and animals and has an antimicrobial effect on aerobic and anaerobic bacteria and protozoa. The effects of ingestion of patulin are relatively unknown with a few reports that it causes neurotoxic symptoms in animals, including tremors and ataxia and recumbency (Gallo *et al* 2015). Patulin is however widely recognised to alter the metabolism of nutrients by ruminal microbes and significantly reduce the degradation of fibre (Tapia *et al* 2005). While diagnoses of patulin toxicosis is infrequent in the UK, its occurrence would be detrimental to animal production.

Material and methods Maize silage samples from three farms in the South West of England were sampled during June 2015 from the face of well-maintained clamps in a typical W pattern. All three clamps had been inoculated with a silage improver: Farm A with maizecoolgold (Biotal, Cardiff, UK), Farm's B and C with solomaize (Biotal, Cardiff, UK). Three samples were taken from each farm and each sample was then split into three replicates of 1kg each and stored at 23°C ±1°C in low level light. Subsamples were taken at 0, 1, 2 and 3 weeks of storage for mycotoxins, DM and fungal counts. Mycotoxins were analysed by UPLCMS/MS by Micron-Biosystems (Somerset, UK) and for fungal counts a 5g subsample of silage was added to 45ml of PBS and stomached for 1 min before being spread on to PDA (Oxoid, Basingstoke, UK). The plates were incubated at 23°C ±1°C in low level light. The ten most predominant morphologies of fungi present on the PDA were sent for sequence analysis (MWG-Biotech, Germany) after amplification by panfungal PCR. Patulin levels were analysed by GLM Repeated Measures on SPSS V21.

Results Sequence analysis results showed that *P. roqueforti* was present at one week of storage onwards in samples from Farms A and B (Figure 1a) but at no time in Farm C. While no patulin was recovered from the fresh samples or after one week of storage, a high spike was evident after two weeks of storage in both Farms A and B where levels from Farm A were significantly higher ($P>0.01$) than from Farm B at two weeks of storage (Figure 1b). After three weeks of storage, the mean amount of patulin had fallen by 88% in the samples from farm A and was no longer evident in the samples from Farm B.

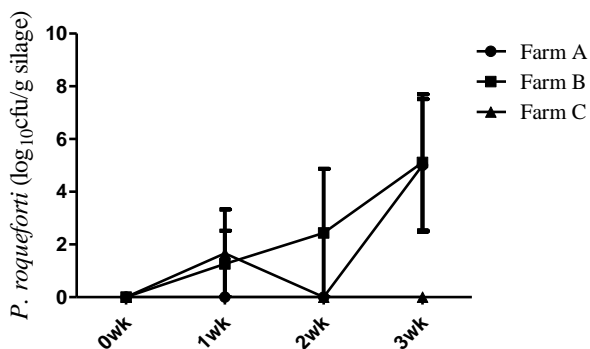


Figure 2 *Penicillium roqueforti* isolated from maize silage collected from three farms and stored over a three week period

Table 1 Patulin recovered from maize silage collected from three farms and stored over a three week period.

Patulin (mean ppm)	0wk	1wk	2wk	3wk
Farm A	0	0	106.40*	12.39
Farm B	0	0	0.14	0
Farm C	0	0	0	0

* denotes a significant difference ($P<0.01$) within a column

Conclusion While patulin is not common in the UK, it is possible that in poorly stored silage patulin occurs in high quantities for a short period (even when *P. roqueforti* is present in high levels), making it difficult to diagnose patulin mycotoxicosis. Further investigation is required as to why the patulin level dropped so suddenly.

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Chemical constituents of sown and naturally existing *Sesbania sesban* and *Tephrosia candida* in late wet season

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Application Screening of browse plants would assist livestock farmers to identify those with good potentials as livestock fodder and as supplements to the poor grasses, especially in dry season in Nigeria.

Introduction Shortage of nutrients prevents optimal performance of ruminants, particularly in the dry season when the quality and quantity of the natural pasture significantly drops. Shrubs and legume tree fodders have been important sources of supplementary nutrients for livestock at these times. Hence, this study aimed to assess the chemical composition in sown and naturally existing species of *Sesbania sesban* and *Tephrosia candida* in the late wet dry season.

Material and methods The study was conducted at Oyo State College of Agriculture and Technology, Igbo-Ora, located in the derived Savannah zone of Nigeria and lies on Latitude 7° 26' 0" N and Longitude 3° 17' 0" E (Google Earth, 2014). Treatments in the experiment were arranged as a Randomized Complete Block Design. 1kg fresh samples of leaves and twigs of naturally existing species of *T. candida* and *S. sesban* were randomly harvested during the late rainy season (August 2013) within the College premises, while the leaves and twigs of sown species of *T. candida* and *S. sesban* were harvested at the same period on a year-old sown pasture. Samples collected were dried at 65 °C and ground to pass through 1.0mm sieve screen. Dry matter (DM), crude protein (CP), ether extract (EE) and ash were determined using the procedure of A.O.A.C (2000). Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), Acid Detergent Lignin (ADL), were determined using the method of Van Soest *et al.* (1991). Cellulose (CELL) and hemicellulose (HEM) were calculated as follows: CELL= ADF-ADL and HEM= NDF-ADF. Data collected were subjected to Analysis of Variance and treatment means were separated using Duncan Multiple Range Test at 5% level of significance (SAS, 1999).

Results The results were significantly ($p < 0.05$) different for all investigated samples. Higher (20.12%) and least (7.14%) CP were obtained in sown and natural species of *T. candida*. Crude protein contents of the browse species were higher than the minimum level of 7% required for optimum rumen function (Van Soest, 1994). Roughage diets with NDF content of 45-65% and below 45% are generally considered as medium and high quality feeds, respectively (Singh and Oosting, 1992). Higher NDF (72.00%) was obtained from naturally existing *T. candida*, while sown *S. sesban* produced the least (59.00%).

Table 1 Chemical constituents of sown and naturally existing *S. sesban* and *T. candida*

Species	DM	CP	ASH	EE	NDF	ADF	ADL	CELL	HEM
<i>S. sesban</i> (S)	97.50 ^a	13.28 ^c	10.00 ^a	8.00 ^a	59.00 ^c	39.00 ^c	11.00 ^b	29.00 ^b	18.00 ^c
<i>S. sesban</i> (N)	96.50 ^b	15.20 ^b	6.00 ^b	7.00 ^{ab}	67.00 ^b	49.00 ^a	9.00 ^c	36.00 ^a	19.00 ^{bc}
<i>T. candida</i> (S)	97.50 ^a	20.12 ^a	6.00 ^b	5.50 ^c	66.00 ^b	47.00 ^b	11.00 ^b	35.00 ^a	20.00 ^b
<i>T. candida</i> (N)	95.50 ^c	7.14 ^d	4.00 ^c	6.50 ^{bc}	72.00 ^a	49.00 ^a	13.00 ^a	35.00 ^a	23.00 ^a
SEM	0.28	1.40	0.68	0.31	1.45	1.26	0.49	0.89	0.59

Mean on the same column with different superscripts differ significantly ($p < 0.05$); N: Naturally existing; S: Sown pasture.

Conclusion Sown *S. sesban* contained medially rated fibre contents and yielded higher inorganic contents (ash). Sown *T. candida* and naturally existing *S. sesban* were better protein sources respectively. The browse species were generally rich in crude protein which is difficultly or rarely met at that period of the season.

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Morphological changes induced by monthly injections of butaphosphane and cyanocobalamin or phosphorylcolamine, zinc, iodine and selenium on *Bos indicus* × *Bos taurus* heifers

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Application Manipulation of morphology by mineral supplementation could improve productive and reproductive performance in cattle as well performance in judging competitions.

Introduction Different parental solutions improved health, mineral hepatic concentration, immune response, antioxidant capacity and reproduction traits on cattle (Rodríguez and Ruiz, 2015). Mora *et al.* (2010) reported lower withers height growth in cattle injected with zinc gluconate. Instead, effects of injectable solutions on body conformation are scarcely evaluated. The aim of this study was to evaluate the effects of two mineral tonics on the zoometric measurements of heifers.

Material and methods 36 Brahman crossbred heifers with 223±19 kg BW and ~10 months of age were housed in Costa Rica and fed at 0.025 BW with 0.35 banana peels, 0.35 green bananas, 0.10 forage (*Pennisetum purpureum*) and 0.2 concentrate (DM basis). They were stratified by initial weight and assigned the following treatments: T1(n=12)- *ad libitum* mineral supplement (115 g Ca, 90 g P, 20 Na, 15 g Mg, 4000 mg Zn, 1000 mg Mn, 250 mg Fe, 1000 mg Cu, 75 mg I, 15 mg Co and 32 mg Se per kg), T2(n=12)- T1 plus a monthly injection of 100 mg butaphosphane and 50 µg of cyanocobalamin/20 kg BW. T3(n=12): T1 plus a monthly injection of 100 mg phosphorylcolamine, 13,19 mg ZnSO₄, 20 mg KI and 0.22 mg Na₂SeO₃/20 kg BW Withers height (WH), hip height (HH), hip width (HW), pins width (PW), thoracic circumference (TC) and body length (BL) were measured, and proportionality (PI=WH/BL×100), anamorphosis (AI=TC²/WH×100), and transversal pelvic (TPI=HW/WH×100) indexes were calculated, each two months. Data were analysed using Proc MIXED (SAS Institute Inc. 2011), with initial measures as covariables for each trait.

Results Significant differences (p<0.05) were observed only for PI (Table 1). As lower values are expected in beef animals (Sañudo 2009), T2 heifers showed an inferior conformation than T1 (Sañudo, 2009). There were significant treatment*time interactions for PI, because T2 had higher values at the beginning of the experiment, but they approximate to T1 in subsequent evaluations (Figure 1). In addition, HW tended to be lower in T3 than the other two treatments (P=0.08).

Table 1 Means standard errors and p-values for the comparison of T1, T2 and T3 treatments

Variable	T1 (n=11) ^a		T2 (n=12)		T3 (n=11) ^a		P-Value			
	Mean	SE	Mean	SE	Mean	SE	Treatment	Time	Treatment*Time	Covariable
TC (cm)	168.8	1.26	171.0	1.21	170.0	1.26	0.485	<0.001	0.620	<0.001
HH (cm)	129.2	0.56	130.2	0.54	129.3	0.56	0.407	<0.001	0.933	<0.001
WH (cm)	123.5	0.66	125.5	0.64	124.5	0.66	0.116	<0.001	0.240	<0.001
HW (cm)	43.2	0.28	43.0	0.27	42.2	0.28	0.080	<0.001	0.495	<0.001
PW (cm)	27.7	0.30	28.2	0.29	28.3	0.30	0.370	<0.001	0.725	0.002
BL (cm)	121.8	0.92	119.8	0.88	122.2	0.94	0.162	<0.001	0.214	<0.001
PI	101.1	0.87	105.0	0.81	102.4	0.87	0.008	0.013	0.039	0.001
AI	231.7	3.29	232.9	3.13	232.5	3.26	0.959	<0.001	0.371	<0.001
PTI	34.8	0.28	34.3	0.27	33.9	0.28	0.101	<0.001	0.292	<0.001

^aTwo heifer died and their data were excluded. ^{b,c} Different superscripts in a row indicate significant differences (p<0.05).

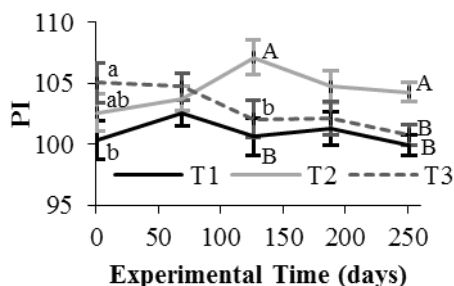


Figure 3 PI across evaluations for T1, T2, and T3 groups. Means with different letters indicate significant differences (lowercase p<0.05, uppercase p<0.01)

Conclusion The injected tonics differed in their effects on the morphology of animals: T2 induced higher values of PI and T3 did not significantly affect zoometric measures. There is a possibility to induce morphological changes in cattle using parenteral solutions, but mechanisms are unclear.

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Bioaccumulation of heavy metals, phenol and polycyclic aromatic hydrocarbons on skin-on red Sokoto buck goats differently singed

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Introduction The safety of skin-on meat obtained from singed carcasses is a matter of public health concern. This study was therefore carried out to investigate the concentration of heavy metals {Lead (Pb), Cadmium (Cd), Zinc (Zn), Manganese (Mn) and Copper (Cu)}, phenol and polycyclic aromatic hydrocarbons (PAH) in red Sokoto buck goat carcasses singed using fire wood, kerosene, scrap tyre and liquefied gas (LG).

Material and methods A total of twenty four good grade red Sokoto buck goats weighing between 18-20 kg were randomly distributed into each of the four treatments in a completely randomized design. Each treatment was replicated six times. The total PAH and phenol contents were determined by High Performance Liquid Chromatography (HPLC) while, the minerals were measured by Atomic Absorption Spectroscopy (AAS).

Results

PAH levels were highest ($P < 0.05$) in scrap tyre singed carcasses (0.040mg/kg) and least in LG singed carcasses (0.001mg/kg). Pb and Mn were below detectable limit in carcasses singed with LG while the concentrations were similar ($P > 0.01$) in the other treatments. Cd was not detected in any of the treatments. Zn concentration was highest ($P > 0.05$) in carcasses singed with kerosene (0.05mg/kg). The level of phenol ranged from 0.02 Gae/kg in LG singed carcasses to 0.38 Gae/kg when scrap tyre was used.

Table 1 Phenol, Hydrocarbons and heavy metals at the loin region of red Sokoto buck goats differently singed

Parameters	Singeing Materials				SEM
	Kerosene	Fire Wood	Scrape Tyre	Liquefied Gas	
Phenol,(Gae/kg)	0.040	0.210	0.380	0.020	0.020
Hydrocarbon(mg/kg)	0.006 ^c	0.025 ^{ab}	0.040 ^a	0.001 ^c	0.001
Heavymetals (mg/kg)					
Lead	0.013	0.013	0.017	ND	0.010
Cadmium	ND	ND	ND	ND	-
Zinc	0.050 ^a	0.023 ^b	0.020 ^b	0.023 ^b	0.020
Copper	0.200 ^a	0.200 ^a	0.100 ^b	0.100 ^b	0.010
Manganese	0.020	0.020	0.020	0.017	0.000

^{abc}Means along the same row with the same superscript are not significantly different ($P > 0.01$)

ND (Not detected)

Conclusion Materials used in singeing was found to have profound effect on heavy metal, phenol and PAH depositions on skin-on meat from red Sokoto buck goats.

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Growth performance of weaned rabbits reared under two different management systems in Nigeria

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Application Caged-housed rabbits had superior growth performance indices than pen-housed rabbits. Further research is however necessary to measure the welfare of rabbits in the different management systems in Nigeria.

Introduction Rabbit meat production has been on the increase in Nigeria in recent years. Rabbits (*Oryctolagus cuniculus*) are considered a fast growing and highly prolific species with a short generation interval, and represent a high potential for improving animal protein intake in the developing countries generally. Rabbit production in Nigeria is largely carried out in cages, and mainly in iron frame cages. Also, cages of different sizes are used with different stocking densities or group sizes. According to Dal Bosco *et al.* (2000) housing system affects body weight, some carcass parameters and sometimes the meat quality. Therefore, this study is aimed at evaluating the effects of two different management systems (pen-housed and caged-housed) on the growth performance of weaned rabbits.

Material and methods Twenty-four weaned heterogeneous breeds of rabbits in equal number of males and females, aged between 5 - 6 weeks with average initial live weight of 612 ± 14.72 g were procured from a rabbit farm. They were randomly divided into two groups (pen-housed and caged-housed) consisting of 12 rabbits per group. One of the groups was housed in well-ventilated room in three tier cages measuring $70 \times 60 \times 50$ cm per cage. The wire cages were fitted with earthen drinkers and feeders and aluminium tray for collection of faeces and urine. While the other group was housed in a pen according to housing standards for rabbits (0.4 sq meter/ rabbit) established by Hoy, (2005). Wood shaving was used as the litter material. The rabbits were pre-conditioned for two weeks during which they were treated with Ivermectin at the dose rate of 0.1ml per rabbit subcutaneously. Rabbits were fed a commercial diet supplemented with *Tridax procumbens* and given access to clean fresh water *ad libitum*. To evaluate the growth performance of the rabbits, initial weight (g), daily weight gain (g), daily feed intake (g) and final body weight (g) were measured. Weight gain for each rabbit was calculated by subtracting the initial weight (g) from the final body weight (g). The study lasted for 8 weeks.

Results The results from the study are presented in Table 1. Significant ($P < 0.05$) differences in daily body weight gain, feed intake and final body weight were observed. The cage-housed rabbits recorded higher daily weight gain (13.62 g/day), daily feed intake (72.24 g/day) and final body weight (1373 g) as against (9.92 g/day, 63.58 g/day and 1168 g, respectively for the pen-housed rabbits.

Table 1 Growth performance of weaned rabbit reared under two different management systems

	Pen-housed	Caged-housed	p
Initial body weight (g)	612.34±3.40	609.81±6.01	n.s
Daily Body weight gain (g/day)	9.92 ^b ±0.38	13.62 ^a ±0.70	<.0001
Daily Feed intake (g/ day)	63.58 ^b ±0.88	72.24 ^a ±0.70	<.0001
Final body weight (g)	1168 ^b ±23.36	1373 ^a ±41.57	<.0001

Conclusion Rabbits reared in cages had higher growth performance indices. The lower final body weight of the pen-housed rabbits could be attributed to high locomotor activity. Also, the lower daily feed intake recorded for pen-housed rabbits could be due to feed wastage associated with group rearing. Further research is however necessary to measure the welfare of rabbits in the different management systems in Nigeria. Keeping rabbits in cages has been associated with their poor welfare due to limited space.

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Breadfruit (*Artocarpus altilis*) flour as an ingredient in meat patties

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Application The use of a non-protein dried fruit flour as a binder in processed meat products to lower production cost without compromising nutritional qualities can improve income and make animal protein available at a lower cost.

Introduction Breadfruit flour (BFF) is much richer than wheat flour in lysine and other essential amino-acids, while it is similar to cassava flour in its carbohydrate and caloric value. It is also a relatively good source of iron, calcium, potassium, riboflavin (vitamin B2, the principal growth-promoting factor in the vitamin B complex) and niacin. Although, breadfruit is nutritious, cheap and available in abundance during its season, it has limited applications in the food industries (Adepeju *et al.*, 2011). The study seeks to determine the suitability, sensory characteristics and cost effects of BFF inclusion in meat products.

Material and methods The breadfruit (*Artocarpus altilis*) was harvested at the point of ripening, washed, sliced into small chunks and sundried for 48 h and milled using a hammer mill with a plate diameter of 1 mm, packaged and stored in a freezer until required. Frozen boneless lean pork (5 kg) were minced through a 5 mm sieve plate using a Mado Super Wolf (MEW 513, Maschinerfabrik Domhan, GmbH, Germany) meat mincer. Similarly pork fat (1 kg) trimmings, of shoulder, flank and thigh was also minced. The minced meat, fat and all the other ingredients were mixed thoroughly according to the four treatments in a Super Wolf MADO bowl cutter. Crushed ice was added during comminution to obtain the desired consistency. The products were formulated with breadfruit flour at three levels (T₂, 10 %; T₃, 20 % and T₄, 30 %) with the control T₁ containing 0 % of BFF; 100g of each treatment was weighed and placed on a meat patty machine and pressed to obtain a flat surface. Ten (10) patties were prepared for each of the four treatments. The patties were cooked in an oven at a temperature of 180 °C to a core temperature of 70 °C using a meat thermometer. The patties were cooled, labelled and stored in a freezer at a storage temperature of -2 °C for sensory and chemical analyses. Samples were taken from each treatment and weighed before cooking (broiling) and after cooking. The percentage cooking yield was obtained by dividing the cooked weight by the fresh weight and multiplying the answer by 100. A consumer panel made up of thirty five students of KNUST randomly selected evaluated the products for the appearance, mouth feel, texture, aroma, taste and acceptability using a five-point hedonic scale (5- like very much, 4- like moderately, 3- Neither like nor dislike, 2- dislike moderately, 1- Dislike very much). The products were thawed, wrapped in labelled Kitchen foil, warmed in an oven at 180⁰ C for 5 minutes, sliced into approximately equal sizes of 2.0cm² and served while hot. Water was offered each panellist to rinse the mouth in-between tasting the patties. All products were randomized. A completely randomised Design was used and each treatment was replicated five times. Chemical analysis was carried out on the pork, BFF and the patties for moisture, fat, protein and ash content using the procedures described by AOAC (1990). The data were analyzed using SAS 2000 package while means were separated with Duncan Multiple Range Test.

Results Panellists detected significant (P<0.05) differences in all the five attributes. Reduction in the cost of the patties increased with increased inclusion of the BFF. The appearance score of the patties decreased as the level of the BFF increased and this ran through all the attributes. Acceptability of the patties decreased significantly (P<0.05) for the 20 and 30 % inclusion levels.

Table 1 Sensory attributes and cooking yield of the sausages

Attributes	T ₁	T ₂	T ₃	T ₄	SEM
Appearance	4.37 ^a	4.00 ^{ab}	3.91 ^b	3.89 ^b	0.146
Taste	4.03 ^a	3.69 ^{ab}	3.46 ^{ab}	3.31 ^b	0.193
Aroma	4.26 ^a	3.83 ^{ab}	3.54 ^b	3.49 ^b	0.173
Texture	4.29 ^a	4.03 ^{ab}	3.66 ^b	3.77 ^b	0.159
Mouth feel	4.09 ^a	3.71 ^{ab}	3.43 ^b	3.46 ^b	0.182
Acceptability	4.03 ^a	3.77 ^a	3.54 ^b	3.40 ^b	0.178
*% change in cost/kg	NA	8.14	17.38	28.16	-

Means with different superscripts within a row are significantly (P<0.05) different; *% change in production cost relative to the control

Conclusion The results revealed that BFF could be used as an extender in meat patties at up to 30% inclusion as this had salutary effects on the sensory profile of meat patties and also might provide the consumer with food containing natural additives, which might be healthful at a lower cost though, acceptability at 20 and 30 % levels was low compared to the 10 %.

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Unfermented African locust bean flour (UALBF) *Parkia biglobosa* (jacq.) Benth as a non-conventional extender in frankfurter type sausages

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Implications Decreased production cost in processed meat products without compromising comparative eating qualities and nutritional benefits; improved income and making animal protein available at a lower cost can be achieved by using a non-meat protein extender.

Introduction Dawadawa the fermented seeds of the African locust beans is used as a flavouring agent and improves the nutritional quality of poor protein diets. As a protein additive in stews and soups it has a CP content of 23.5–33.4 % as well as containing riboflavin (Dike and Odumfa, 2003). The study seeks to evaluate the effects of substituting ground meat with unfermented african locust bean flour (UALBF) to produce affordable frankfurter sausage with comparable eating quality and nutritional benefits.

Material and methods Dawadawa (*Parkia biglobosa*) seeds were separated out of the pods and boiled overnight at 100 °C. The heat treated seeds were rubbed to remove testa, washed, sundried for 48 h and milled using a hammer mill with a plate diameter of 1 mm. The milled flour was packaged in polyethylene bags and stored in a freezer until required. Chilled lean beef (6 kg) and pork fat (1 kg) trimmings, of shoulder, flank and thigh, were minced through a 13 mm diameter (Mado Super Wolf meat mincer). The dawadawa flour, spices and water were weighed and mixed with the minced beef and pork fat after, which each treatment was stuffed into casings of uniform size and shape using a hand operated stuffer. The sausages were formulated separately for the five treatments, with five replicates each. The treatments were T₁ (control product), T₂ (5 % inclusion), T₃ (10 % inclusion), T₄ (15 % inclusion) and T₅ (20 % inclusion) of UALBF. They were linked at 10 cm length strands, smoked for 3 h using firewood and sawdust after, which they were scalded in water at 72 °C to a core temperature of 68 °C, cooled under tap water and placed under ceiling fan for 30 min. The sausages were stored at -18 °C for 24 h, re-packaged and kept at the same temperature till required. The sausages were thawed overnight at 10 °C and were heated in an oven at 180 °C to obtain a core temperature of 77 °C using a meat thermometer. Samples were taken from each treatment and weighed before cooking (broiling) and after cooking. The percentage cooking yield was obtained by dividing the cooked weight by the fresh weight and multiplying the answer by 100. The products were then sliced and kept in a microwave till they were served. A hedonic scale of 5 – 1 was used; where 5 means ‘like extremely’ and 1 means ‘Dislike extremely’. Forty five untrained regular consumers of processed meat products were used as panelists from Kwame Nkrumah University of Science and Technology. At each sensory evaluation session, the coded samples of the different treatments were served following a completely randomized design, which ensured that no two panelists at any time were served in the same order. They were each served with water, disposable cups and napkins. Chemical analysis was carried out on the beef, UALBF and the processed product for moisture, fat, protein and ash content using the procedures described by AOAC (1990). The data were analyzed using the one-way analyses of variance (ANOVA) in a Completely Randomized Design. Means and associated standard errors for measured parameters were computed using Genstat 2008 Version 7.2 DE for Windows and differences between means were compared using the Student-Newman-Keuls test.

Results The panelists detected significant ($P < 0.001$) differences in taste, flavor, cooking yield and overall acceptability of the test samples (Table 1). There was no significant ($P > 0.001$) difference between T₁ (control) and T₂ (5 % inclusion) for all the attributes (appearance, taste, flavor, texture and overall acceptance). The test products had significantly ($P < 0.001$) higher cooking yields T₂, T₃, (86.7 %), T₄ (92.9 %) and T₅ (93.3 %) when compared to the control T₁ (78.8 %), likewise the CP content (52.95 %) compared to the control (21 %) and these could be due to the moisture retention capacity and the high CP (44.7 %) content of UALBF.

Table 1 Sensory attributes and cooking yield of the sausages

Attributes	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Appearance	4.60 ^a	4.23 ^a	3.71 ^b	3.66 ^b	3.60 ^b	0.167
Taste	4.00 ^a	3.71 ^{ab}	3.46 ^{ab}	3.29 ^b	3.26	0.191
Flavour	4.23 ^a	3.91 ^{ab}	3.57 ^b	3.26 ^b	3.34 ^b	0.188
Texture	4.23 ^a	3.60 ^b	3.77 ^{ab}	3.63 ^b	3.66 ^{ab}	0.196
Acceptability	4.29 ^a	3.80 ^{ab}	3.43 ^{bc}	3.17 ^c	3.17 ^c	0.207
Cooking Yield	78.80 ^c	86.70 ^b	86.70 ^b	92.90 ^a	93.30 ^a	0.514

Means with different superscripts within a row are significantly ($P < 0.001$) different

Conclusion The study showed that UALBF can be used as an extender in frankfurter-type sausages at an inclusion level of 5 % with no effect on appearance, taste, flavor, texture and overall acceptance and also enhanced the CP content of the product.

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