

Effects of substituting sesame oil for barley grain on rumen fermentation parameters and blood metabolites in lambs

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Introduction Lambs need a high energy ration to assure high productivity and fast growth. Fat supplementation can effectively increase the energy density of rations. Our objective was to test the substituting energy from sesame oil for that from barley grain in diets on rumen fermentation parameters and blood metabolites for finishing lambs.

Materials and methods Eighteen male Chaal lambs (139 ± 6 days of age, BW 23.7 ± 0.73 kg) were used to evaluate three diets (six lambs per diet) containing 0, 2.5 and 5% sesame oil (SO). The diets were isocaloric and isonitrogenous and consisted of 33% forage and 67% concentrate. Lambs were housed in individual pens, and the TMRs fed twice a day to allow 5%orts. Sesame oil was substituted with barley grain. The experiment lasted for 84 days. Individual blood samples were taken before the morning feeding from the jugular vein on days 40 and 80 of experiment. Blood samples of each lambs were pooled and analyzed for serum triglycerides, total cholesterol, HDL, LDL, VLDL and glucose concentrations. At the end of fattening period, all lambs (with similar DMI and ADG) were slaughtered and samples of rumen fluid from each lamb were taken (in pre-feeding condition). Rumen fluid samples were analyzed for pH, ammonia-N, protozoal count and VFAs concentration. The data were analysed using the GLM procedure of SAS (2002) for a completely randomized design.

Results and discussion The results of this study are shown in Table 1. Under the conditions of this experiment, feeding sesame oil to lambs caused depression ($P < 0.05$) on the concentration of rumen propionate, but the acetate to propionate ratio of rumen fluid was linearly increased. This could be related to a reduced grain concentration in the diet, since the type and amount of carbohydrate digested by ruminants is a decisive factor in determining the proportions of the resultant rumen VFA. It seems that in sesame oil supplemented diets in which fat was used in replacement of barley grain, there was no reasonable amount of starch in sheep feeds to yield high proportion of propionate, hence propionate was decreased and acetate: propionate ratio was increased. Results from blood metabolites showed that substituting sesame oil for barley grain linearly increased ($P < 0.01$) serum total cholesterol and HDL concentrations. Also, the HDL cholesterol concentration was increased in sesame oil supplemented lambs to approximately the same extent as total cholesterol. Hence, the elevation in total cholesterol in sesame oil supplemented lambs was due primarily to an increase in HDL. No significant differences were found in glucose, triglyceride, LDL and VLDL levels in blood of lambs in three treatment. In this study, the reasons that sesame oil supplemented lambs shown differences in rumen fermentation parameters and blood metabolites could be due to the exchange of easily fermentable carbohydrates by sesame oil.

Table 1 Rumen fermentation parameters and blood metabolites of experimental lambs

Item	Diets			S.E.M.	P value	
	C	2.5%SO	5%SO		linear	quad
Rumen fermentation						
Parameters:						
Rumen pH	6.96	7.10	7.06	0.045	0.140	0.162
Ammonia-N (mg/l)	128	133	131	3.513	0.645	0.429
Protozoa numbers (n/ml) (10^5)	2.93	3.40	3.13	0.661	0.834	0.657
VFAs concentration (m mol/l)						
Acetate	36.98	35.62	39.89	2.026	0.325	0.274
Propionate	11.99	9.95	10.48	0.486	0.045	0.047
Butyrate	7.17	6.89	7.45	0.384	0.610	0.380
Valerate	0.71	0.57	0.68	0.072	0.169	0.785
Isovalerate	0.85	0.82	0.99	0.110	0.377	0.473
Total VFA	57.69	53.83	59.50	2.540	0.621	0.147
Acetate: propionate ratio	3.10	3.57	3.84	0.168	0.008	0.621
Blood metabolites (mg/dl):						
Total cholesterol	45.83	51.50	56.50	2.701	0.014	0.921
HDL cholesterol	24.33	27.83	32.50	1.623	0.003	0.773
LDL cholesterol	17.30	19.10	19.27	1.445	0.349	0.649
VLDL cholesterol	4.20	4.57	4.73	0.232	0.129	0.733
Triglycerides	21.00	22.83	23.67	1.173	0.129	0.733
Glucose	66.33	68.17	67.67	1.782	0.601	0.604

Effect significant $P < 0.05$.

Conclusions Substituting sesame oil, as a concentrated energy source, for barley grain in diets of lambs decreased propionate concentration and increased acetate: propionate ratio of rumen fluid, HDL and total cholesterol concentration of serum.

***In vitro* rumen fungi growth in medium containing sugarcane pith treated with low temperature steam and acid using QC-PCR assay**

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Introduction The key to maximising the nutritional value of lignocellulosic materials is in disrupting the plant cell walls to allow complete access to nutrients and not creating extra anti-nutritional factors (Castro *et al.*, 1994). In particular, steam and pressure treatments to disrupt lignocellulosics in a way which allows improved utilisation of cell wall polysaccharides by rumen microbes (Castro and Machado, 1990). Rumen fungi produce a wide range of polysaccharide degrading enzymes and are as primary colonisers of fibrous plant materials that degrade lignin-containing plant cell walls. With the advancement of molecular enumeration methods, in particular 18S rDNA gene probing methods, researchers were able to monitor fungal species within the rumen (Stahl *et al.*, 1998). Quantitative competitive PCR (QC-PCR) techniques play an important role in nucleic acid quantification because of their significant lower cost of equipment and consumables (Franz *et al.*, 2001). The aim of this study was to use QC-PCR to determine the effect of sugarcane pith treated with low temperature steam plus 0.9% H₂SO₄ on *in vitro* growth of rumen anaerobic fungi.

Material and methods Rumen fungi were isolated from pre-incubated wheat straw in the rumen of fistulated sheep and then method of Joblin (1981) was used to grown under anaerobic conditions at 39°C for 3 days. These isolates were used (1:9) as a source of fungi inoculum. Serum bottles (four replicate per sample) containing fungi culture medium, 1g of sugarcane pith as untreated (UTP) or treated with low temperature steam (at 135 °C for 80 min) and 0.9 % H₂SO₄ (STP) and 1ml antibiotic solution were used to culture the isolated fungi at 39°C in an incubator. To preparing fungi pure culture, sub-culturing was done three times. Total genomic DNA was isolated from pure culture samples using Guanidine Thiocyanate-Silica Gel method. A universal PCR primer pair GAF (F): 5'-GAG GAA GTA AAA GTC GTT AAC AAG GTT TG-3' and GAF(R): 5'-GAAATT CAC AAA GGG TAG GAT GAT TT-3' was used to amplify a specific region of 18S rDNA from anaerobic rumen fungi. Standard control DNA was constructed to use in the QC-PCR and was shown to amplify under the same reaction condition and the same amplification efficiency as the target DNA. The PCR was performed in a final volume of 25µl sealed in a capillary tip, and thermocycling was carried out in a model 2000 (Biometra). The PCR amplification condition was as follows: denaturation at 94°C for 4min followed by 35 cycles of 94 °C for 30s; 56°C for 30s; and 72°C for 1min followed by a final extension at 72°C for 5min. The PCR products were separated by electrophoresis on agarose gels, stained with ethidium bromide, and visualized by UV transillumination. The relative intensities of PCR products were used to compare fungal biomass under different samples. The signal intensity was quantified by Image J 1.29x and expressed in arbitrary units. The data was analysed using the GLM procedure of SAS for a completely randomized design.

Results The competitive PCR reaction for DNA extracted from fungi media is shown in Figure 1. The result of QC-PCR (Figure 2) showed that the growth of rumen fungi in the medium containing sugarcane pith treated with low temperature steam plus 0.9% H₂SO₄ was greater than the other treatment ($P < 0.05$).

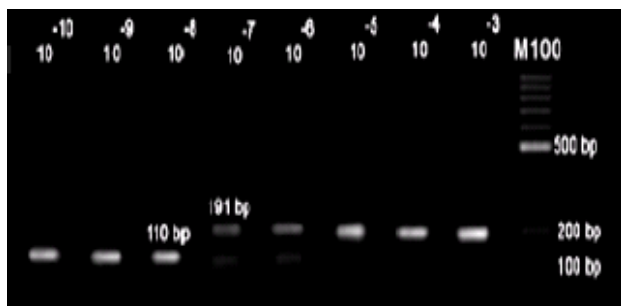


Figure 1 Competitive PCR reaction for DNA extracted from fungi medium. 110bp: belong to rumen fungi, 191bp: belong to competitor.

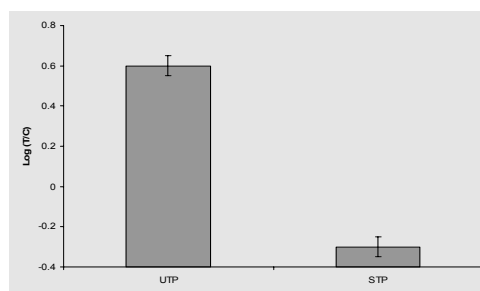


Figure 2 Quantitative changes of rumen anaerobic fungal population. UTP; untreated sugarcane pith, STP; sugarcane pith treated with low temperature steam plus 0.9% H₂SO₄, Log (T/C); log ratio of intensities of amplified target DNA to standard

Conclusions The method of QC-PCR for enumerating anaerobic rumen fungi in the present study demonstrated that sugarcane pith treated with low temperature steam plus 0.9% H₂SO₄ increased fungal growth. The increase in enzymic hydrolysis after steam treatment can be explained by the removal of the hemicellulose that caused to increase accessibility for rumen microbial enzymes (Castro *et al.*, 1994).

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The effect of soaking and urea treatments on the nutrient composition of wheat straw

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Introduction Numerous efforts have been made to improve the nutritive values of crop residues such as cereal straws by using chemical and biological treatments (Sundstol and Owen, 1984; Chaudhry, 1998). Although some of these treatments have been effective in improving the nutritive value of cereal straws, their farm scale use is limited due to the costs and relevant safety issues for humans, animals and the environment. Therefore, this study investigated the use of water (soaking) and urea as relatively cheap and safe alternatives to improve the nutritive value of wheat straw before their feeding to sheep.

Material and methods Eighteen batches of about 150 kg chopped (10-15cm) wheat straw were loaded into silo bags placed inside the galvanized mesh rings. A 2 x 3 factorial design, in triplicate, was used to apply different amounts of water and urea to prepare treated wheat straws as follows. Water representing 2 water to straw ratios (0.15:1 and 0.50:1; soaking) and 3 urea levels (0, 2.5 and 5%) were sprayed onto these straws in bags which were compressed to exclude air, sealed and left outdoors for ten weeks. At opening the silos, pH of these straws were immediately determined and replicated samples were dried, ground and analysed for their chemical compositions (AOAC, 1990). About 10 g fresh straw samples, in triplicate, were mixed with 30 ml of distilled water in beakers, filtered and 10 ml of the filtered solutions were acidified with 10 ml of 1M HCl before their NH₃ and VFA analysis as described by Chaudhry (2008). The data were statistically compared for the effect of soaking, urea and soaking x urea. Tukey's test was used to compare the treatment means for their significance.

Results Table 1 presents the mean chemical compositions of different straws for only the main effects of soaking and urea treatments as the soaking x urea interactions were not significant for any of these compositions. Both Urea and Soaking showed significant effects on DM (P<0.001) indicating the different amounts of water that were used to treat these straws. Urea treatment showed significant increases in the CP and ADF but significant decreases in the NDF and ADL contents especially at 5% urea (P<0.001). The pH, NH₃-N and acetate levels were significantly increased when urea was increased from 0 to 2.5 or 5% and the greatest increases were obtained when urea was used at 5% (P<0.001). Apart from the significant (P<0.001) reductions in DM, NH₃-N and acetate for the higher soaking ratio, there were no major changes in other components of treated straws for different soaking ratios.

Table 1 Mean chemical compositions of treated wheat straws with relevant standard errors (SEM) and significance (Sign)

Composition (g/kg DM)	Urea%			SEM	Sign	Soaking ratio		SEM	Sign
	0	2.5	5			0.15:1	0.50:1		
Dry matter (DM) (g/kg)	466 ^b	596 ^a	482 ^b	3.9	P<0.001	581 ^a	449 ^b	3.2	P<0.001
Organic matter (OM)	936 ^b	939 ^a	936 ^b	0.7	P<0.05	939 ^a	935 ^b	0.6	P<0.05
Crude protein (CP)	80 ^c	131 ^b	343 ^a	1.9	P<0.001	181 ^b	188 ^a	1.6	P<0.05
Ether extract (EE)	13	12	13	0.6	NS	13	12	0.5	NS
Neutral detergent fibre (NDF)	756 ^a	747 ^a	706 ^b	3.2	P<0.001	735	738	2.6	NS
Acid detergent fibre (ADF)	435 ^c	452 ^b	496 ^a	1.4	P<0.001	462	460	1.2	NS
Acid detergent lignin (ADL)	106 ^a	89 ^b	74 ^c	1.3	P<0.001	89	91	2.5	NS
pH	8.0 ^c	9.4 ^b	9.6 ^a	0.01	P<0.001	9.0	9.0	0.01	NS
NH ₃ -N mg/L	19 ^c	273 ^b	488 ^a	6.1	P<0.001	306 ^a	288 ^b	5.2	P<0.05
Acetate mmol/L	9 ^c	22 ^b	26 ^a	0.2	P<0.01	22 ^a	16 ^b	0.2	P<0.001

NS = Non-significant; Values with different subscripts in the same row indicate significance at P<0.001.

Conclusion Urea treatments at either 2.5 or 5% modified the chemical compositions of the treated wheat straws but the greatest change was observed at 5% urea after 70 days of conservation. The modified compositions were attributed mainly to the changes perhaps in the hemi-cellulose and lignin contents as reflected by the NDF, ADF and ADL contents of these straws. It appears from this study that the urea treatment can be used to modify the chemical composition of wheat straw but the level of water and treatment conditions would be critical in optimising the impact of urea treatments in our future studies of this kind.

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Effects of whole cottonseed on small intestine morphology of Chaal fattening male lambs

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Introduction Whole cottonseed (WCS) contains high levels of CP and TDN and requires no processing, which makes it a very desirable by-product feed. WCS may promote growth and stimulate functional development of the rumen (Anderson *et al*, 1982). Villus height (VH), Villus width (VW), crypt depth (CD) and ratio of villus: crypt (V:C) are direct representation of the intestinal environment and may be used as indicators of intestinal health (Wang *et al*, 2008). The objective of this study was to determine the effects of dietary WCS levels on small intestine morphology in Chaal fattening male lambs.

Materials and methods Twenty Chaal male lambs with similar body conditions (30.4±1.8Kg live weight and 5 months of age) were used in completely randomized design. The lambs were divided, at random in four groups (5 lambs in each pen). All lambs were housed in experimental pens and fed with the experimental diets as totally mixed diets twice daily at 0800 and 1600 h for 90 days. Four diets were formulated (Table 1). Treatments were (1) 0%, (2) 4%, (3) 8% and (4) 16% WCS (DM basis) in the ration. Water and mineral lick were available free choice. At the end of the experiment, the lambs were transported to a slaughterhouse, where they were weighed before sacrifice. The small intestinal sections were collected from the duodenum (10 cm distal to the pyloric sphincter), jejunum (10cm distal to ligament of trite) and ileum (10cm proximal to the ileocecal junction). The digesta in the lumen of each space was removed. Samples were placed into formaldehyde and glutaraldehyde mixing fluid. Cross-sections of intestinal samples preserved in formaldehyde and glutaraldehyde mixing fluid were prepared using standard paraffin embedding techniques. Samples were sectioned at 8µm thickness and stained with haematoxylin and eosin. VH, VW and CD were measured on the stained sections under microscope with 100× combined magnification (10× eyepiece and 10× objective) and an ocular micrometer. A minimum of 10 straight, intact villi in each intestinal position was measured in triplicate cross-sections for each lamb with in each treatment. VH was measured from the tip of the villi to the villus crypt junction, CD was defined as the depth of the invagination between adjacent villi and VW was measured at the mid of the villus. The V:C was determined as the ratio of VH to CD. Data were analyzed as a completely random design using the GLM procedures of SAS using the model $Y_{ij} = \mu + \tau_i + \epsilon_{ij}$. Duncan's test for treatment mean comparison to control at (P<0.05) was used.

Results Morphology results of the duodenum, jejunum and ileum are presented in Table 2. Four physiological parameters, including VH, VW, CD and V: C was used to reflect small intestine development. Dietary WCS had a significant effect on all investigated parameters (P<0.05), except VW in jejunum.

Table 1 Ingredient and chemical composition of the diets (DM basis)

Item	Diet			
	1	2	3	4
Ingredient (% of DM)				
Whole cottonseed	0	4	8	16
Barley grain	60	58	55	51
Cottonseed meal	7	5	4	1
Alfalfa hay	15	15	15	15
Barley straw	18	18	18	17
Chemical composition				
ME(Mj/kg DM)	11.05	11.22	11.3	11.51
CP (%)	13.62	13.52	13.64	13.74
Ether Extract (%)	2.01	2.7	3.39	4.78
NDF (%)	35.3	36.26	37.3	38.7
ADF (%)	21.3	22.3	23.4	25.1

Table 2 Influence of dietary WCS on small intestinal morphology

Item	Diet	Diet				S.E.M
		1	2	3	4	
Duodenum	VH, µm	246 ^c	290 ^{ab}	330 ^{ab}	363 ^a	15.4
	VW, µm	226 ^b	286 ^{ab}	303 ^a	313 ^a	9.1
	CD, µm	223 ^b	236 ^b	263 ^a	266 ^a	12.1
	V:C	1.10 ^b	1.22 ^{ab}	1.24 ^{ab}	1.39 ^a	0.03
Jejunum	VH, µm	253 ^b	276 ^{ab}	296 ^a	313 ^a	8.4
	VW, µm	273	270	263	266	7.1
	CD, µm	236 ^c	243 ^{bc}	263 ^a	256 ^{ab}	4.2
	V:C	1.07 ^b	1.13 ^{ab}	1.12 ^{ab}	1.22 ^a	0.02
Ileum	VH, µm	223 ^b	276 ^a	303 ^a	303 ^a	11.2
	VW, µm	206 ^b	263 ^a	270 ^a	273 ^a	15.2
	CD, µm	190 ^b	236 ^a	260 ^a	236 ^a	8.2
	V:C	1.17 ^b	1.17 ^b	1.16 ^b	1.28 ^a	0.02

ME: metabolizable energy, CP: crude protein
NDF: Neutral detergent fibre, ADF: Acid detergent fibre

Means in a rows with different superscripts are significant at P<0.05

Conclusions This study showed that dietary WCS level affected small intestinal morphology. Increasing WCS in diet, not only increased intestinal VH, VW and CD but also ratio of V: C increased.

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The effect of *Samanea saman* and *Stylosanthes hamata* supplementation on intake and NDF digestibility of a Nerica 1 rice straw basal diet in Djallonké sheep

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Introduction Rice straws generally have low nutritive value but recent improved varieties like Nerica1 have increased nitrogen (N) contents compared to other rice straws. Furthermore, the utilization of rice straw by sheep could be improved by supplementing with leaves of multipurpose trees like *Samanea saman* and *Stylosanthes hamata*. As leaves of *S. saman* have been shown to produce less methane in *in vitro* digestion studies (Soliva *et al.*, 2008) this makes it a tree foliage that needs further study. The purpose of this study was to assess the intake and digestibility when sheep were offered Nerica 1 rice straw with foliage of *S. saman* and *S. Hamata*.

Materials and methods This study was carried out at the Livestock Section of the Department of Animal Science, K.N.U.S.T, Kumasi, Ghana. *S. saman* and *S. hamata* were harvested and sun dried for three days and Nerica 1 rice straw, obtained from farmers' fields, was chopped into 60-80 mm length before feeding. Four rumen fistulated Djallonké rams, average weight 25 kg, were used in an intake and digestibility study in a 4 x 4 Latin Square Design with a 2x2 factorial arrangement of treatments. The factors were foliage type (*S. saman* and *S. hamata*) and level of supplementation (360 or 480 g DM/d). Each period lasted 17 days with 10 days for the animals to adjust to the diet and 7 days for measurement of feed intake and collection of faeces. The rams were housed in individual metabolism cages (0.7 x 1.2 m). The cages had plastic buckets for water and wooden feeding troughs. The animals were given Ivermectin (0.2 mg kg⁻¹) to eliminate both internal and external parasites before the commencement of the study. At the start of the experiment, the rams were individually weighed, placed in the cages and allowed *ad libitum* access to water. The animals were offered either 360 or 480 g of the supplements *S. saman* and *S. hamata* and 6 % of their body weight of the basal diet (Nerica 1) at 09.00 h throughout the experiment. The rams were fed once a day. The chemical composition of the feeds; i.e. N and NDF were determined. Data for liveweight, intake and digestibility were subjected to analysis of variance for balanced data. During the first 10 days of each period, feed offered and feed refused were weighed daily. Feed samples were randomly collected twice a week for DM analysis using hot air oven (60 °C). During the last 7 days of each period, samples of feed offered were collected every day and divided into two parts; first part was analyzed for DM while the second part was kept in airtight containers and pooled at the end of each period for analyses for N and NDF.

Results The results showed that the feeds used were generally dry (DM > 930g/kg). The CP and ash contents of the three feeds were (Nerica 1; 60.6, 120; *S. saman*; 182.2, 27.5; *S. hamata*; 202.5, 35 g/kg DM). Table 1 shows that intake of supplements increased as offer rate increased and furthermore, total feed intake was higher (P<0.05) in rams offered *S. saman* supplements compared to those offered *S. hamata*. Digestibility of NDF was not affected by *S. saman* supplementation but by *S. hamata*. When *S. hamata* was offered at 360 g DM/d, NDF digestibility was significantly (P<0.05) improved.

Table 1 Level of legume supplementation on rice straw intake and digestibility in sheep

	Supplement offered (g DM/d)				s.e.d	Sig
	<i>Samanea saman</i>		<i>Stylosanthes hamata</i>			
	360	480	360	480		
Intake (gDM/d)						
Rice straw	702.2	737.4	655.5	668.9	9.16	**
<i>S. saman</i>	336.3	447.3			12.85	*
<i>S. hamata</i>			353.9	411.8	12.85	*
Total intake	1038.4	1184.7	1009.4	1080.7	19.18	*
Total intake (g DM/M ^{0.75} .d)	98.9	106.0	106.7	106.4	1.87	*
NDF digestibility	0.66	0.68	0.82	0.68	0.018	*
Final weight, kg	25	28	23	24	0.52	*

Conclusion The results show that supplementation of rice straw with *S. saman* and *S. hamata* improved feed intake by sheep.

Acknowledgement The authors are grateful to Prof. D.B. Okai for suggestions made on the manuscript.

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Effect of Holotrich protozoa on sheep methane emissions

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Introduction Livestock production and activities associated with it (feed production, fuel usage, etc.) represent between 9% and 18% of anthropogenic emissions of greenhouse gases (Steinfeld *et al.*, 2006). Methane represents about half of the greenhouse gases (CO₂ equivalents) emitted from livestock sector and enteric methane production from ruminants is the most important source, representing about 80% of these emissions (Gill *et al.*, 2010). Up to 25% of rumen methanogens are associated with protozoa using the H₂ generated from protozoal metabolism (Newbold *et al.*, 1995). Morgavi *et al.*, (2010) observed a linear decrease of 0.96 litres of methane/kg DM intake per reduction of 10⁵ protozoal cell/ml; and it has been suggested that elimination of rumen protozoa will decrease methane production by circa 10.5%. However, it remains unknown which are the key protozoal species most closely related with the methane emissions. Holotrich protozoa (*Isotricha* and *Dasytricha*) have intracellular methanogens but, differ from the Entodiniomorphs, in that no episymbiotic methanogens have been observed (Vogels *et al.*, 1980). This study was carried out to investigate the effect of the presence of rumen Holotrich protozoa on enteric methane production by sheep.

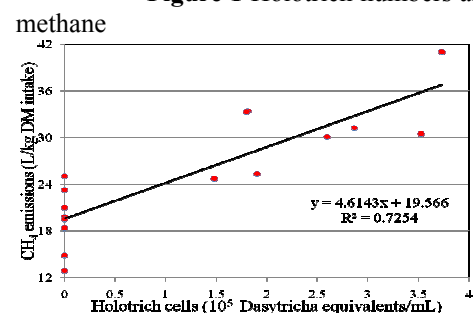
Material and methods Eight crossbreed sheep were used in two consecutive periods. All animals were maintained protozoa-free by separation from the ewes within the first 24h and kept isolated from adult animals. When the animals became adults (4 years old) they were placed in individual pens to control the daily intake of the experimental diet (66% hay and 33% ground barley). The diet was designed to meet maintenance requirements and was distributed in two equal meals per day (09:00 and 18:00h). The experimental protocol was approved by the Aberystwyth University Local Ethical Review Committee. After adaptation to the diet, methane emissions were determined in absence of rumen protozoa. All animals were then inoculated orally with a mixed Holotrich population (*I. protostoma*, *I. intestinalis* and *D. ruminantium*) and sheep were kept for a further two months for adaptation to the new rumen environment; methane emissions were then determined in presence of Holotrich protozoa. Rumen fluid (50 ml/sheep) was extracted by oesophageal tubing at the beginning of both periods to verify the absence/presence of rumen protozoa and to homogenise the rumen microbial population between animals after re-inoculation of the pooled rumen fluid. To determine methane emissions, animals were placed in respiration chambers (1.1 x 1.6 x 4.5m) over a four-day period. Airflow and concentration of methane was measured at the intake and exhaust ducts of each chamber (temperature between 10–20°C). The air stream was subsampled, and methane concentration was measured every 30min using a gas analyser (ADC MGA3000). Calculations of the methane emissions were based on the differences in the methane concentration entering and leaving each chamber and the respective airflows. Mean methane production was calculated for each animal and statistically analysed by ANOVA, blocking by chamber using Genstat. Protozoal concentration was corrected by the differential protozoal volume assuming an equivalence of 15.6 *Dasytricha* per *Isotricha*.

Results Animals remained in good condition throughout the experiment maintaining body weight and DM intake (Table 1). No protozoal cells were detected in protozoa-free animals, while a viable Holotrich population ($5.3 \times 10^4 \pm 1.4 \times 10^4$ cells/ml), composed of 27±9% and 73±9% of *Isotricha* and *Dasytricha* respectively, was observed in Holotrich faunated animals. Methane production increased after inoculation with Holotrich protozoa by between 61% and 65% (Table 1). A linear increase of 4.6 litres of methane/kg DM intake per increase of 10⁵ *Dasytricha* equivalents/ml was observed (Figure 1)

Table 1 Body weight, intake and methane emissions

	Protozoa-free	Holotrich	Response	SEM	P value
Body weight (BW), kg	93.8	92.9	-0.9%	0.36	0.848
DM intake, kg/d	1.57	1.60	1.7%	0.039	0.658
Methane emissions					
Litres/day	30.3	49.5	64%	2.00	<0.001
Litres/kg BW	0.32	0.53	65%	0.022	<0.001
Litres/kg BW ^{-0.75}	0.68	1.13	65%	0.046	<0.001
Litres/kg DM intake	19.3	31.2	61%	1.39	<0.001

Figure 1 Holotrich numbers and



Conclusions Our results show that Holotrich protozoa play a key role in terms of methane emissions and their presence increased such emissions. Holotrich protozoa and methanogens associated with them had a nearly five times higher methanogenic activity than the average protozoal activity reported by Morgavi *et al.*, (2010).

Acknowledgement This experiment has been funded by the Commission of the European Communities FP7, KBB-2007-1.

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Effect of n-3 fatty acids supplementation on semen characteristics in Moghani rams

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Introduction Animals are unable to synthesize polyunsaturated fatty acids (PUFAs) from saturated or monounsaturated fatty acids; therefore, they must acquire them from precursor PUFAs in their diet. Lipids are the main source of energy in metabolism of spermatozoa. In most mammals, docosahexaenoic acid (DHA) is the dominant PUFA, although, in several species docosapentaenoic acid (DPA) is also a major component of the sperm cell membrane (Retterstøl *et al.*, 2001). The high quantity of long chain polyunsaturated fatty acids (LCPUFAs) in the membrane phospholipids of spermatozoa are known to play an importance role in membrane fluidity and flexibility. The importance of polyunsaturated fatty acids in relation to male fertility has been illustrated by studies in humans demonstrating that the amount of DHA in spermatozoa is positively correlated with sperm motility (Zalata *et al.*, 1998). In addition, LCPUFAs which are concentrated in the head and tail membrane regions of spermatozoa have been shown to play an important role in both sperm capacitation (Surai *et al.*, 2000) and the interaction between spermatozoa and uterine surface environment (Vasquez and Roldan, 1997). Hence, the objective of the present study was to investigate the effects of fish oil supplemented in the ram's feed on semen characteristics.

Materials and methods Eight sexually mature ram were randomly allocated into 2 groups and received two different diets: unsupplemented control diet, and supplemented with fish oil (purchased from Ard Mahi Khazar Co., Keyashahr seaport, Anzali, Iran) at 3% dry matter (DM). Both of the diets were isocaloric and isonitrogenous and formulated according to software sheep CNCPS. All rams were trained for semen collection by an artificial vagina (AV). During a training period of two weeks, ram succeeded in serving the AV, and ejaculating. Semen samples were collected at 14 d intervals from Jan 25, 2010 to May 14, 2010 by AV, at week 0, 2, 4, 6, 8, 10 during 12 weeks of feeding the experimental diets. Short time (less than 5 min) after sampling, semen was taken to the laboratory. The semen was maintained at 37°C until evaluation on the farm and during transport to the laboratory for were evaluated the characteristics. After the collected semen was diluted 1:200 with NaCl solution, the concentration of spermatozoa was determined using a haemocytometer and volume of semen was measured with graduated tube. The progressive motility of sperm was also analyzed by placing a sample on a pre-warmed (37°C) microscopic slide covered with a cover slip, and examined under a high power microscope at a magnification $\times 400$. Stained semen smears were prepared by mixing diluted 10 μ l semen with 30 μ l nigrosin–eosin stain for 30s to evaluate sperm viability. The mixed semen and stain were incubated for 2 to 5 min at 37°C before preparing smears on microscope slide and then leaving them to dry. The nigrosin-eosin stained slides were evaluated by examining 200 spermatozoa per slide in duplicate slides. Viable spermatozoa were defined as those that did not take up stain. Data were analyzed by using the PROC MIXED of the SAS program for repeatedly measured data. The data in range 30% \leq and \geq 70% were normalized before analysis. All results are presented as Least squares means \pm standard error. A Tukey-Kramer correction was applied for all pairwise comparisons. Differences are considered significant at $P < 0.05$.

Results The effect of dietary fish oil supplementation as n-3 fatty acid sources on the semen characteristics of the Moghani rams is shown in Table 1. A significant increase observed on semen volume, density, progressive motility, and viability ($P \leq 0.01$). Feeding fish oil also did not affect semen pH significantly.

Table 1 Effect of dietary fish oil supplementation on Moghani ram's semen characteristics

Characteristics	Control	Fish oil
Semen volume (ml)	1.01 \pm 0.3 ^a	1.7 \pm 0.3 ^b
Progressive motility (%)	68.42 \pm 0.44 ^a	73.21 \pm 0.44 ^b
pH	6.67 \pm 0.04 ^a	73.21 \pm 0.44 ^b
Sperm density (10 ⁹ /ml)	3.17 \pm 0.02 ^a	6.64 \pm 0.02 ^b
Viable (%)	79.25 \pm 0.56 ^a	6.64 \pm 0.02 ^b

Mean in a row with a different superscript is significantly different ($P < 0.01$).

Conclusions The present study showed that dietary fish oil supplementation can improve the semen characteristics of Moghani rams without affecting pH values.

Acknowledgements The authors thank the University of Mohaghegh Ardabili for a grant and financial support of M. Fouladi's MSc. thesis research.

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Effect of sesame oil supplementation on fatty acid composition of tail fat and meat of Chaal lambs

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Introduction The nutritional modulation of the fatty acid (FA) profile of ruminant edible fats is currently an important research topic. It is well established that dietary FA composition can affect human metabolism and health. To our knowledge, the effect of sesame oil supplementation on ruminant edible fats composition has not been studied. Therefore, the present work was conducted to study the effect of sesame oil supplementation for fattening lambs on tail fat and meat fatty acid composition.

Materials and methods Eighteen male Chaal lambs were used to evaluate three diets containing 0, 2.5 and 5% sesame oil (SO). Concentrations of main fatty acids in the sesame oil (%) were: C16:0, 8.26; C18:0, 5.28; C18:1, 42.79; C18:2, 39.69 and C18:3, 0.78. Diets and feeding regime were described by Ghafari *et al.*, (2011), and performance data is presented in that paper. At the end of fattening period (84 days) and immediately after slaughtering samples of longissimus dorsi muscle (from the sixth to eighth ribs) and tail fat were removed from the left side of the carcass for fatty acid analysis. According to the method of Folch *et al.* (1957), lipid was extracted. Fatty acid methyl esters (FAME) of intramuscular fat were prepared using a sodium methoxide 0.5M solution in methanol followed by hydrochloric acid in methanol (1:1) and FAME of tail fat were prepared using a sodium methoxide 0.5M solution in methanol (Raes *et al.*, 2001). Analysis of FAME was performed with a Gas Chromatography system (Shimadzu, Model: 14-A, Japan) using a RT-2560 capillary column (Restek, 100m x 0.25mmID x 0.2µm film thickness). Peaks were identified using a 37 FAME and CLA isomers standards. Data were analysed with GLM procedure of SAS (2002) for a completely randomized design.

Results and discussion The significant effects is presented in table 1. With the exception of C10:0, C12:0 and C14:0, inclusion of sesame oil in diet up to 5% caused significant linear decreases in percentages of fatty acids containing less than 18 carbons (C15:0, C16:0, C16:1, C17:0, C17:1) in muscle and tail fat. Also, the percentage of CLA cis-9 trans-11 in both muscle and fat tissue increased linearly with SO supplementation. Tail fat from lambs fed 2.5% SO contained a greater proportion of C18:0 compared with lambs fed 5 % SO (quadratic effect), indicating that low level of oil (2.5%) maybe undergo greater complete biohydrogenation than high level of oil (5%). Total C18:1, C18:2n-6 and C18:3n-3 content of tissues did not ($P > 0.05$) differ among treatments. Overall, about 87% of CLA cis-9, trans-11 present in ruminant tissues results from endogenous desaturation by stearoyl-CoA desaturase (SCD) of *trans*-11 C18:1 (Bessa *et al.*, 2005), and the *trans*-11 C18:1 isomer is a common intermediate in the microbial biohydrogenation of dietary C18:1n-9, C18:2n-6, and C18:3n-3 (Harfoot and Hazelwood, 1997). Thus the increases of CLA cis-9, trans-11 in SO supplemented lambs, a greater supply of *trans*-11 C18:1 would be expected for diets containing SO. Since the C16:0 has been identified as a hypercholesterolemic fatty acid and the CLA cis-9, trans-11 has notable anticarcinogenic proprieties (Shingfield and Griinari, 2007), Lowering of C16:0 and increases in CLA cis-9, trans-11 content of ruminant edible fats is desirable for human health.

Table 1 fatty acid composition of Intramuscular and tail fats (% of total fatty acids)

Fatty acid	Intramuscular fat						Tail fat					
	Diets			SEM	P value		Diets			SEM	P value	
	C	2.5%SO	5%SO		Linear	Quad	C	2.5%SO	5%SO		Linear	Quad
C15:0	0.38	0.30	0.24	0.039	0.023	0.89	1.30	1.07	0.99	0.072	0.0095	0.43
C16:0	19.42	18.54	17.98	0.460	0.043	0.78	23.23	22.29	21.39	0.615	0.052	0.98
C16:1 cis-9	1.34	1.20	0.97	0.101	0.019	0.73	2.52	2.23	2.15	0.103	0.023	0.42
C17:0	1.01	0.81	0.71	0.084	0.026	0.65	2.48	2.16	1.83	0.136	0.0042	0.95
C17:1 cis-9	0.67	0.49	0.39	0.075	0.019	0.72	1.80	1.34	1.26	0.090	0.0006	0.10
C18:0	14.26	14.92	13.96	0.347	0.54	0.075	9.64	10.58	9.19	0.432	0.48	0.045
CLA *	0.78	0.91	1.08	0.070	0.008	0.81	1.06	1.45	1.74	0.092	<.0001	0.67

Effect significant $P < 0.05$. *Conjugated linoleic acid (CLA) = C18:2 cis-9, trans-1

Conclusions Inclusion of sesame oil up to 5% in diet for fattening Chaal lambs improves fatty acid composition and healthful characteristics of tail fat and meat.

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The effects of yeast on the performance and welfare of heifers fed an organic finishing diet

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Introduction Yeast cultures based on *Saccharomyces cerevisiae* are widely used in ruminant diets. Animal responses are diet and animal dependent with a greater response reported in animals in early lactation and in animals fed high concentrate diets (Newbold *et al.*, 1995). However there is little information of the potential benefits of live yeast in high forage rations typical of many organic production systems.

Materials and Methods The effects on production and welfare of live yeast during 12 weeks of a housed finishing period were measured on organically reared Limousin and Charolais cross heifers ($n = 12$). The heifers were placed into two groups of 6 according to age and fed big bale red clover/ryegrass silage ad-libitum, with 2kg/head of 18% protein daily. One of the groups also received 20g/head/day of Biosaf Instant (L13) live yeast (supplied by: S.I. Lesaffre, 137 rue Gabriel Péri, BP 3029 59703, Marcq-en-Baroeuil, France) which was mixed with the 1kg of ground concentrate from the groups daily 12kg of concentrate before being top-dressed. The group fed no yeast also received 1kg of their concentrate in ground form. In week 6 of the trial the animal's were observed in both pens on a group basis for 7 consecutive 24 hour periods, a further 4 hour period observing each individual's behaviour was carried out during week 10 of the trial. The two observation trials observed time spent: standing in the straw area; standing in the scrapped area; lying in the straw area; lying in the scrapped area, while the second trial also observed time spent: ruminating; grooming; scratching; drinking; and feeding.

Results No significant difference was found in production measurements (LWG, 0.73kg/day, 0.84kg/day from control and yeast supplemented animals respectively $p=0.45$). However the observation trial in week 10 showed animals fed yeast spent longer ruminating ($p<0.05$) over the whole 4 hours (Figure 1).

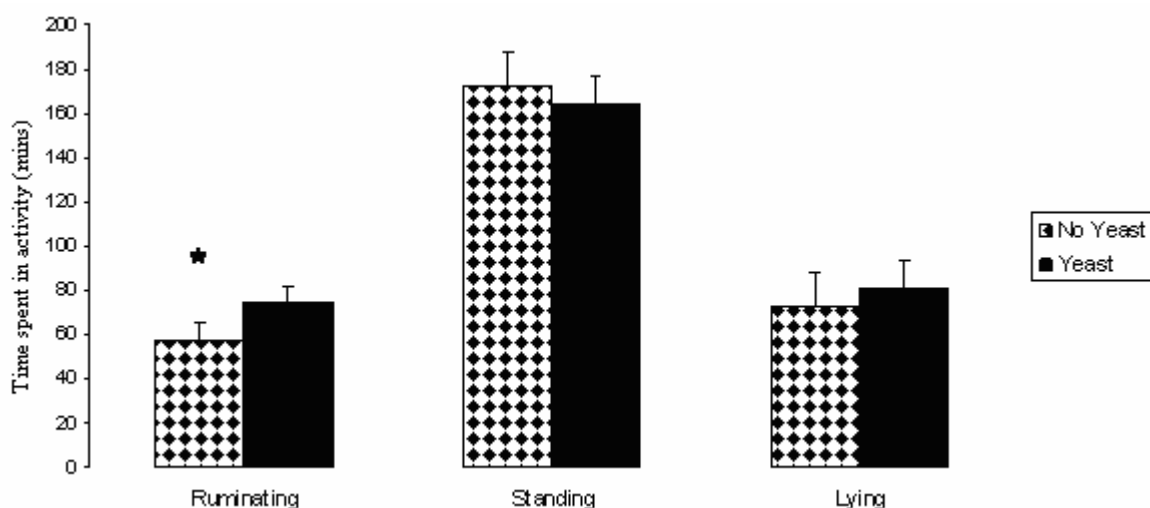


Figure 1 Time spent ruminating, standing or lying during a four hour period

Conclusions No production from yeast supplementation was observed. However it was concluded that there may be a possible welfare benefit to the animals from yeast as rumination is considered an anti-stress function in ruminants.

Acknowledgements Farm staff on Frongoch Farm at IBERS, Aberystwyth University

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The role of Toll-like receptor 2 (TLR2) and dectin-1 in phagocytic and inflammatory response to antigen expressed on yeast surface

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Introduction Yeast species such as *Saccharomyces cerevisiae* (*S. cerevisiae*) are well documented as being potent activators of the immune system (Ardiani, 2010). *S. cerevisiae* activates the innate immune system by engaging pattern recognition receptors such as toll like receptor 2 (TLR2) and dectin-1 (Brown, 2001). In the current project, we express the immunogenic envelope protein E2 from bovine viral diarrhoea virus (BVDV) on the surface of *S. cerevisiae*. We then analyse the innate and adaptive immune response induced by our recombinant yeast *in vitro* to determine if expression in yeast enhances the immunogenicity of the viral protein.

Materials and Methods The coding sequence for the E2 protein of BVDV was cloned into the *S. cerevisiae* expression vector pYD1. Transformation of *S. cerevisiae* with the vector led to induction of the mature E2 protein expressed on the surface of yeast cells. Certain aspects of the bovine innate and adaptive immune responses to our recombinant yeast were measured *in vitro* paying particular attention to processes known to involve TLR2 and dectin-1. TLR2 and dectin-1 were expressed as dye-tagged molecules in HEK293 cells to investigate phagocytic uptake of the recombinant yeast as well as the NF- κ B response. The IL-8 and oxidative burst response of primary bovine macrophages treated with the recombinant yeast was measured. Additionally, the proliferative response of CD4⁺ T-cells was determined in response to macrophages from autologous animals primed with yeast with or without surface E2 expression. This was compared to macrophages primed with the E2 protein alone. Our recombinant yeast was efficiently heat-inactivated without effect to expression of E2 protein at the surface as tested by fluorescent staining. The difference between the immune responses to live and heat-inactivated yeast was determined.

Results *S. cerevisiae* expressing E2 protein was recognised and bound by bovine dectin-1 but not TLR2. The recombinant yeast also stimulated IL-8 production but not oxidative burst in bovine macrophages. Macrophages primed with *S. cerevisiae* expressing the E2 protein induced more CD4⁺ T-cell proliferation than those primed with *S. cerevisiae* without E2 expression or the E2 protein alone (Figure 1). Heat-inactivation of our recombinant yeast increased oxidative burst response but did not affect IL-8 response or NF- κ B activity. Additionally, macrophages primed with heat-inactivated yeast induced more CD4⁺ T-cell proliferation than those primed with live yeast.

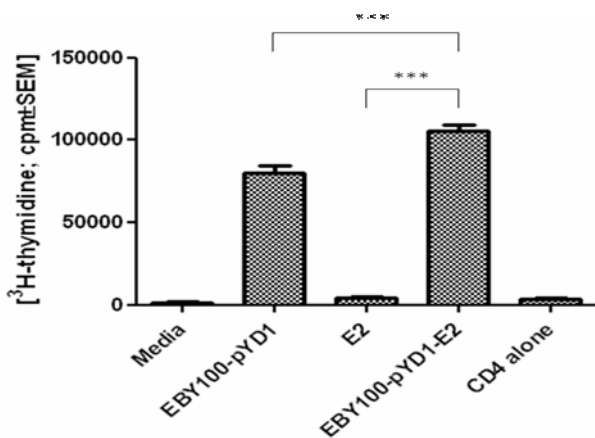


Figure 1 CD4⁺ T-cell proliferation in response to incubation with bovine macrophages primed with EBY100-pYD1, EBY100-pYD1-E2 or E2 protein alone.

Bovine M ϕ were seeded in 96-well plates and primed with either 5 particles per cell (ppc) EBY100-pYD1, 5ppc EBY100-pYD1-E2 or 0.02 μ g ml⁻¹ E2 alone. CD4⁺ T-cells were added to relevant wells and plate was incubated for 5 days before being pulsed with tritium labelled thymidine. Cells were harvested and counts per minute were measured. Asterisks denote a significant difference between groups (***) P < 0.001).

Conclusion Both live and heat-killed *S. cerevisiae* are an attractive vaccine vehicle for delivering the E2 protein of BVDV to antigen presenting cells in order to stimulate cellular immunity directed against this antigen. The yeast not only provides an efficient way to deliver immunogenic protein to antigen processing pathways but also acts as an adjuvant by stimulating inflammatory responses.

Acknowledgements The authors gratefully acknowledge funding from BBSRC, Pfizer and the RCVS trust.

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An investigation of the role of Insulin-Like Growth Factor 1 (IGF-1) in determining adult height of the horse

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Introduction The Insulin-Like Growth Factor-1 (IGF-1) gene is one of a number of determinants of adult height in several animals including the dog (Sutter *et al.*, 2007). However, whilst causative polymorphisms can be tied to height variation between dog breeds, this is not true of other systems, such as humans. Welsh Pony and Cobs (a UK-native breed), are categorised into four distinct sections largely in accordance with their height: Section A, pony type < 122 cm; Section B, pony type < 137 cm; Section C, pony of cob type 122 to 137 cm, Section D, cob type > 137 cm. Using the Welsh Pony and Cob as the example, the aim of this work was to assess whether height of the horse is influenced by single-nucleotide polymorphisms (SNP) of the IGF-1 gene.

Material and method Hair follicle samples were collected from 6 individuals of each section (n = 24) and total DNA was extracted using Qiagen DNA MicroKit (Qiagen, UK). Amplification by PCR was performed using primers (sequences available on request) based upon the published canine, 1 Kb region of the IGF-1 sequence (Sutter *et al.*, 2007) surrounding the primary causative SNP, re-designed for the equine genome and 0.4 μ M of each forward and reverse primer was incubated with 10 μ l (50 ng) template DNA, 20 μ l Sensimix (Kapa Biosystems, UK) made up to a total volume of 40 μ l with water. Reactions were incubated at 95 $^{\circ}$ C for 10 min, followed by 40 cycles of 95 $^{\circ}$ C for 30 sec, 58 $^{\circ}$ C for 45 sec and 72 $^{\circ}$ C for 1 min, with a final extension of 72 $^{\circ}$ C for 10 min. Products were size fractionated by gel electrophoresis, excised, transformed, cloned (TOPO TA Cloning Kit, Invitrogen, UK), extracted (QIAprep Minikit, Qiagen, UK) and sequenced (Geneservice, UK) to produce a comparative set of homologous sequences of the IGF1 gene. In addition, 3 individuals of each section were utilised to determine whether size variation existed between putative microsatellite markers (including those outside the 1 Kb region sequenced) within the IGF-1 gene. Briefly, 7 putative microsatellite primers (sequences available on request) within the IGF-1 gene were designed and 0.4 μ M of each forward and reverse fluorescent markers was incubated with (50 ng) 3 μ l template DNA, 10 μ l Sensimix, made up to a total volume of 20 μ l with water. Reactions were incubated at 95 $^{\circ}$ C for 10 min followed by 35 cycles of 95 $^{\circ}$ C for 30 sec, 55 $^{\circ}$ C for 1 min, 72 $^{\circ}$ C for 2 min extension time, with a final extension of 72 $^{\circ}$ C for 10 min. Amplified PCR products were subjected to capillary sequence analysis (ABI3730 capillary sequencer, Applied Biosystems) and compared using an internal size standard. Together, these data provided the nucleotide sequence for a portion of the IGF-1 gene surrounding the base position homologous to the causative SNP in the canine sequence for each of the four Welsh Pony and Cob sections and provided graphical evidence of minor shifts in the gene size between sections. Consensus sequences were aligned using ClustalW algorithm to identify base pair variation within the amplified portion of the IGF-1 gene across the four sections. Differences in PCR fragment sizes of the microsatellite regions were identified using Gene-Mapper, version 3.7 (Applied Biosystems).

Results There was no section-specific change in nucleotide sequence within the 1 Kb region of IGF-1 surveyed between the four distinct sections and therefore, height classifications of the Welsh Pony and Cob. However, for the 3 animals of each section selected for further putative microsatellite analysis that lay outside the 1 Kb region, differences between size of IGF-1 sequences were apparent; microsatellite SSR2 revealed 2 bp repeat at position 399-401 for Section A and section B individuals that was absent for Section C and D (Fig. 1 a and b); microsatellite SSR7 revealed short 4 bp repeat allele at position 438-442, present in 2 of 3 Section D individuals but were absent in Sections A, B and C (Figure 1, c and d).

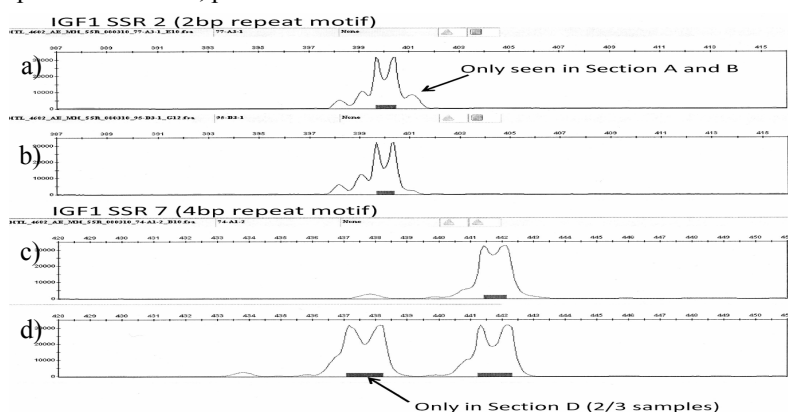


Figure 1 Fluorescent amplified gene sequence fragments from microsatellite region SSR2 from representative individuals from Welsh Pony and Cobs a) Section A and b) Section D and from microsatellite region SSR7 of representative individuals from c) Section A and d) Section D

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Conclusion The lack of section-specific allelic variation around the causative locus of the dog in the Welsh Pony and Cobs suggest that IGF-1 is unlikely to have a major effect upon adult height of this breed of horse. Whilst allelic variation was observed in Section D animals for one microsatellite locus, no IGF-1 variation appeared to correlate with the smaller phenotype of Section A's. However, the study only considered a portion of the IGF-1 gene and therefore, lends justification for further work to extend the investigation of surrounding genes that influence IGF-1 activity.

A preliminary characterisation of the ecology of gut anaerobic fungal populations in horses

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Introduction Understanding the composition and determinants of microbial populations of the equine hindgut is fundamental to our knowledge of feed fermentation, and preventing gut-related disorders. Literature characterising bacterial hindgut populations are numerous. However, other than demonstrating that *Piromyces citronii* and *Caecomyces equi* can be present (Gaillard-Martinie *et al.*, 1995; Gold *et al.*, 1988), studies of anaerobic fungal species in the equine hindgut have been limited. Anaerobic fungi are potent fibre degraders which have been extensively studied in the ruminant gut (Gordon and Phillips, 1998). The aim of this study was therefore to conduct a preliminary characterisation of the structure of anaerobic fungal populations in the equine hindgut, and assess how their size and composition was affected by animal and time.

Material and methods Faecal samples were collected from animals (n = 4) undergoing a concurrent yeast feeding trial. A 2 x 2 randomised latin square design was used. Animals (horse B, H, R and S) were maintained on pasture before and during the experiment. Period 1 (P1) comprised a 3 week feeding period where Group 1 (horses R and S) received a treatment of 20g live Biosaff yeast daily and Group 2 (horses B and H) received a control treatment of 20g killed yeast. Period 2 (P2), also 3 weeks, followed immediately but treatments were reversed for Groups 1 and 2. During the last five days (d1-d5) of both P1 and P2 faecal samples from the first two defecations after 11 am were collected from each horse, and frozen at -20 °C until analysis. Total DNA was extracted using a QiaAmp DNA Stool Kit (Qiagen, UK), with the DNA concentration and integrity verified using a spectrophotometer (Nanodrop, LabTech International, UK) and agarose gel analysis respectively. The presence and quantity of anaerobic fungi was assessed using a Taqman probe based QPCR assay targeting the 5.8S rRNA gene as previously described (Edwards *et al.*, 2008). The population composition of anaerobic fungal positive samples was then assessed using an anaerobic fungal specific Automated Ribosomal Intergenic Spacer Analysis (ARISA) as previously described (Edwards *et al.*, 2008). The ARISA profiles generated were then compared using a curve-based (Pearson) cluster analysis (Fingerprinting software, BioRad, UK).

Results Anaerobic fungi were detected in all four animals. Horses H, R and S were positive for anaerobic fungi in only a few of the P1 samples in contrast to horse B, where anaerobic fungi were routinely detected during both P1 and P2. Consistent with this was the observation that horse B had a larger amount of anaerobic fungi (212 (s.e.m. 47) and 64.0 (s.e.m.24.5) ng anaerobic fungal DNA per g dried faeces (s.e.m. 47) for P1 and P2 respectively) compared to the other three horses (< 3.96 ng anaerobic fungal DNA per g dried faeces). Interestingly the anaerobic fungal population composition of all four horses in P1 generally shared a high similarity (> 95%). Although differences were evident over P1 and P2 with horse B (Figure 1) it is suggested that these population composition changes are more likely to be due to a temporal effect, rather than the loss of the yeast supplement viability. All of the ARISA profiles were dominated by a 391 bp peak (Figure 2), which when sequenced and analysed by BLAST was found to have 99 % identity with an uncultivated anaerobic fungus from cow manure assigned to the *Cyllmayces* genus (Accession No. GQ850302).

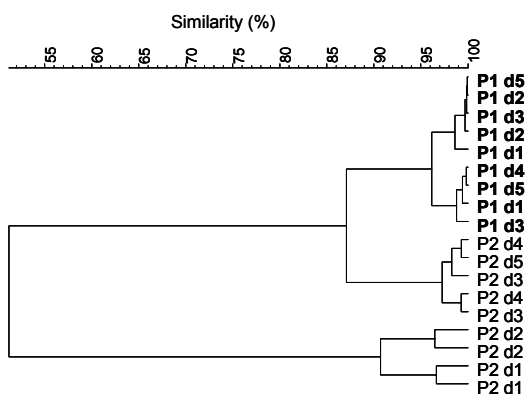


Figure 1 Cluster analysis of anaerobic fungal ARISA profiles generated from horse B faeces sampled on different days (d1-d5) during both feeding periods (P1 and P2).

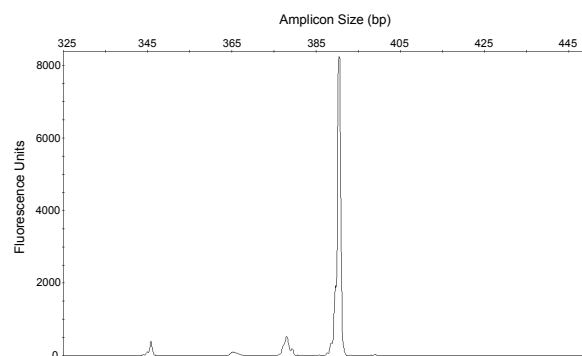


Figure 2. Representative anaerobic fungal ARISA profile of a P1 sample from horse B.

Conclusion Anaerobic fungi were present in the hindgut of all the horses studied. Preliminary evidence indicates that their population ecology is affected by time and host, but not yeast supplement viability. Further studies are required to assess if the occurrence of anaerobic fungi in equids benefits their gut function and health.

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***In vitro* kinetic characterization of inhibition of acetylcholinesterase by organophosphorus and carbamate compounds in food animals**

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Introduction Among pesticides, organophosphorus (OP) and carbamate (CB), compounds, which are acknowledged as anticholinesterases agents, represent the main classes concerned in cases from mild to severe poisoning (Silver, 1974; Gupta *et al.*, 2007). In the current study, we have chosen OP dichlorvos (DDVP) and diazinon (DZN), as well as CB (carbaryl). All of these compounds are routinely used in veterinary medicine. The objectives of this study were to investigate the kinetics of inhibition of acetylcholinesterase (AChE, EC.3.1.1.7) activity by these compounds in liver tissues from animals used for human consumption. A further aim was to study the value of AChE activity as a biomarker of exposure to these pesticides.

Materials and Methods Meat from food animals (5 sheep, 5 cattle and 5 pigs) was obtained from local abattoirs and transported in a cool box to the laboratory. To extract AChE, samples of liver were cut into small pieces (3-5 mm³), homogenised with sodium phosphate buffer, pH 8.0 (ratio 1:9), and centrifuged at 9000 g for 5 min. AChE activity was determined using the Ellman (1961) method, adapted for a plate reader (Pagliosa *et al.*, 2010). For the measurement of half maximal inhibitory concentrations (IC_{50}), AChE was inhibited for 30 min at 20 °C with either 1-8 µM OP compounds or 5-40 µM CB compounds. The decrease in AChE activity with increasing concentration was then plotted with a single exponential decay using SigmaPlot 11 (Systat Inc.). For the measurement of rate constants of inhibition (k_i), AChE was inhibited as above with 8 µM DDVP or DZN, or 40 µM carbaryl. The decrease in AChE activity over different times (0-60 min) was then plotted in the way. Half times ($t_{1/2}$) were calculated using the equation, $t_{1/2} = \ln 2/k_i$.

Results Kinetic parameters were determined in liver for sheep, cattle, and pigs using, DDVP, DZN, and carbaryl as described in the Materials and Methods in Table 1. The order of potency in IC_{50} was decreased according to the rank order of DDVP > DZN > carbaryl. In all inhibitors, sheep had the highest k_i values compared with other animals. In all, inhibitor $t_{1/2}$ in liver was higher in pig compared with other animals. The percentage residual enzyme activity tended to be highest in pig, with the exception of DDVP, where activities were similar in cattle, using all inhibitors (Table 1).

Table 1 Kinetic parameters of AChE by DDVP, DZN, and carbaryl in the liver for sheep, cattle, and pigs

Inhibitor	Animal	IC_{50} (µM)	$t_{1/2}$ (min)	$k_i \times 10^{-3}$ (min ⁻¹)	%Residual AChE activity
DDVP	Sheep	1.0 ± 0.217	4.9 ± 0.301	140.9 ± 9.14	1.6 ± 0.292
	Cattle	1.6 ± 0.017 ^a	13.5 ± 0.159 ^a	51.3 ± 0.599 ^a	3.5 ± 0.25 ^a
	Pig	2.5 ± 0.402 ^{bc}	16.2 ± 0.95 ^{bc}	43.1 ± 2.67 ^b	3.5 ± 0.867 ^b
DZN	Sheep	1.4 ± 0.245	4.6 ± 0.295	152 ± 9.17	1.4 ± 0.057
	Cattle	2.8 ± 0.049 ^a	10.8 ± 0.079 ^a	63.9 ± 0.467 ^a	4.6 ± 0.077 ^a
	Pig	2.6 ± 0.202 ^{bc}	14.5 ± 2.08 ^{bc}	44.5 ± 10.7 ^b	6.1 ± 0.394 ^{bc}
Carbaryl	Sheep	4.8 ± 0.31	4.2 ± 0.25	167.5 ± 9.5	1.8 ± 0.445
	Cattle	7.0 ± 0.217 ^a	9.9 ± 0.014 ^a	68.2 ± 1.44 ^a	4.8 ± 0.799 ^a
	Pig	8.4 ± 0.304 ^{bc}	13.5 ± 0.32 ^{bc}	51.4 ± 1.21 ^b	4.7 ± 0.337 ^b

Values in the table are the mean ± SE obtained from nonlinear regression analysis. ^(a) Significant differences (ANOVA, $P < 0.05$) between cattle and sheep within same inhibitor are in the same column. ^(b) Significant differences (ANOVA, $P < 0.05$) between pig and sheep within same inhibitor are in the same column. ^(c) Significant differences (ANOVA, $P < 0.05$) between pig and cattle within same inhibitor are in the same column.

Conclusions This study provided original data concerning an enzymological characterization of these inhibitors in food animals. In liver, the IC_{50} values in pig were higher than in sheep and cattle for AChE inhibited with OP and CB compounds, with the exception of liver from cattle with DZN where the IC_{50} was higher than for pig and sheep. AChE activity was found to be more sensitive to inhibition by DDVP than by DZN or carbaryl, and activity in liver. Liver k_i values for inhibition of AChE by OP and CB compounds were higher in sheep than cattle and pigs. However, in general for the liver extract and same animal k_i values were similar for all three inhibitors.

Acknowledgments This research was supported by a Ph.D. studentship from the Iraqi Ministry of Higher Education.

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Dogslife, a web-based epidemiological research project for prospective analysis of risk factors affecting the health of domestic dogs

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Introduction Studies of risk factors affecting the health of domestic dogs have largely been based on veterinary clinic populations, and there is little information on the role of daily lifestyle on subsequent risk of disease. In contrast, studies such as the Avon Longitudinal Study of Parents and Children have evaluated the health and development of children from birth and have revealed many unsuspected risks and associations for a wide range of phenotypes in humans, such as obesity (Elks *et al*, 2010) and cognitive functions (Barnett *et al*, 2009). We have developed Dogslife, a web-based data capture system to follow the health and life-style of dogs throughout their lives, in order to gather similar data and analyse risk factors for common canine diseases. Initially the platform is being used for Labrador Retrievers, but it has the capacity to be modified for other breeds of dog and other species.

Material and methods All Labrador Retrievers born after 1 January 2010 and registered with the Kennel Club of the United Kingdom (KCUK) are eligible to enrol in the Dogslife pilot study. KCUK provides a file with daily registration transfers so that animals can be verified by Kennel Club number and date of birth. All individuals registering a Labrador Retriever receive an invitation to join Dogslife with their registration papers, followed up with an e-mail and/or postcard, depending on whether they have given permission for contact. Owners enrol their dog at <http://www.dogslife.ac.uk> and provide base-line data on-line as early in the puppy's life as possible. They then return each month to update information such as weight, height, amount of exercise, and any health problems. If the dog visits the veterinarian, the owners provide the diagnosis and treatment using a form completed by the vet. Once the dog reaches twelve months of age, owners return three-monthly to update the dog's information. Owners are encouraged to return to the site through prize draws, the possibility of having their dog featured on the site and by graphical representations of the dog's progress on its own webpage. Owners who have not returned to the site are sent e-mail reminders and may receive a phone call if they have consented to phone contact. A monthly newsletter is sent to participants and other interested people. To encourage recruitment we are contacting all known breeders of Labrador Retrievers. The data are stored in data files which can be downloaded in suitable formats for analysis.

Results Dogslife was launched on 1 July 2010. There have been over 2500 visits to the site, with interest from as far away as Mongolia, Ethiopia and Argentina. We have averaged about 30 new enrolments per month, representing nearly 2% of all KCUK registrations. The weekly number of new participants has been increasing steadily. The most effective method of recruitment has been the postcard mailed to owners of registered Labrador Retrievers about six weeks after they receive their registration papers. Labrador Retrievers from all over the UK (from Jersey to the Shetland Islands, and including Northern Ireland) have been enrolled. The mean age of dogs at joining is 107 days. The distribution of coat colours and sexes is similar to that of the overall KCUK Labrador Retriever population. To date, the majority of Dogslife dogs are pets (86%) with 5% used for breeding or showing and 9% as working dogs. Over 40% of the dogs already have a second months data, 26% have three months data, 12% have four months data and 3% have five months data. There has been a mean of just under one health problem per dog, with about 42% resulting in a visit to the vet. A number of owners have treated their dogs with alternative therapies such as acupuncture and homeopathy. Owners have shown great interest in having their dog featured on the website and we have been able to feature a different dog every few days. Most people have joined the programme to monitor their dog's progress in the same way that they maintain a health booklet for their children, and for altruistic reasons to improve the health of all dogs. After initial teething problems the web interface is working efficiently and owners have been pleased with the simplicity of the site. 17% of breeders interviewed were already aware of the project and the vast majority of all breeders have agreed to provide new puppy owners with information and encourage them to join.

Conclusions The initial analysis of the pilot study suggests that this web-based interface is an efficient and user-friendly way to acquire data on the life-style and health of dogs. We anticipate that we will have recruited over 2000 Labrador Retrievers in the first year and plan to continue recruitment for a second year at least. The final cohort will have the power to analyse incidence and risks for common diseases as well as identifying potential risk factors for rarer conditions. The Dogslife website is available by negotiation for use with other breeds and species.

Acknowledgements Dogslife is funded by the Kennel Club Charitable Trust. We are also grateful for the continuing support of the Kennel Club and the owners and breeders of Labrador Retrievers in the UK. The authors acknowledge the other members of the Dogslife Team (Professor Kenton Morgan, Professor Bill Ollier, Dr Lorna Kennedy and Professor Jeff Sampson) for their contribution to the project).

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Effect of *Aspilia africana* leaves on the reproductive potentials of rabbit bucks

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Introduction Semen quality is a vital determinant of fertility and reproductive efficiency in livestock production (Oguike *et al.*, 2008). Rabbit has high reproductive potential, fast growth rate and short gestation length of 28 to 30 days (Ojewola *et al.*, 2006). The attributes of fecundity of the doe could be enhanced by the potency of buck semen. In order to boost the nutritional requirements of their animals, many Nigerian smallholder livestock farmers feed forages indiscriminately to farm animals without testing their effects on the performance of the animals. *Aspilia africana* (*A. africana*) known as wild marigold is one of the major forages fed especially to rabbits and thus testing its effect is necessary. The objective of this study was to determine potentials of feeding *A. africana* on the testes and the semen quality of rabbit bucks.

Materials and Methods Eighteen mature Dutch rabbit bucks with average weight of 2.13 kg and average age of 7 months were used in a CRD experiment T1 (control), T2 and T3. Six bucks were assigned to each treatment. Bucks in T1 were fed concentrate without *A. africana*. Bucks in T2 and T3 were fed concentrate in which dried *A. africana* leaves was incorporated at 2.5% and 5% inclusion levels, respectively. The concentrate in all the groups contained 16% CP and 2.7MJ ME/kg dry matter and were supplemented with mixed grass and legumes. Semen was collected twice a week for four consecutive weeks. Artificial vagina (AV) constructed for rabbit buck was dipped into warm water at the temperature of 40°C for at least 15 minutes to simulate the vagina temperature of the doe. The AV was then lubricated with glycerol to make it resemble the vagina and this made it easy for intromission thereby reducing stress. A teaser doe was then introduced into the buck's pen. As the buck mounted the teaser, immediately AV was introduced and ejaculation took place. Semen volume was read off from a calibrated collection tube attached to the AV. Sperm motility was determined by dropping an aliquot of semen on a glass slide and viewed under a microscope. Sperm concentration was assessed by adding 10% formal buffer diluents to another aliquot to immobilize and stop their movement. Thereafter, counting of sperm cells were done with the aid of a Neubauer chamber haemocytometer. Live/dead sperm cells were determined by mixing a drop of semen with a drop of eosin/nergrosin stain. Dead cells picked up the stain. Three bucks from each treatment were slaughtered and the testes carefully incised and testicular weight measured with sensitive scale. Its length and circumference were measured with a thin thread and meter rule. Data generated were analysed using analysis of variance (ANOVA), significant means were separated using LSD.

Results and Discussion The results of the semen quality of the bucks are presented in Table 1. The semen volume, sperm concentration and sperm motility of bucks fed control diet were ($P<0.05$) higher than those of bucks fed T2 and T3 diets. Bucks fed T1 and T2 diets had ($P<0.05$) higher live sperm cells than those fed T3 diet while bucks fed T3 had ($P<0.05$) higher dead sperm cells than those fed T2 and T1 diets. The length of vas deference (5.35 cm), epididymis (4.80 cm) and epididymal weight (1.23 g) of bucks fed T3 diets were ($P<0.05$) lower than the respective vas deference length, epididymis length and epididymal weight of T1 (9.48 cm, 10.00 cm and 3.92 g) and T2 (8.34 cm, 7.63 cm and 3.80 g).

Table 1 Semen characteristics of the bucks

Parameters	Control (T1)	(Tt2)	(T3)	SEM
Volume (ml)	1.917 ^a	0.91 ^c	1.62 ^b	0.30
Motility (%)	70.00 ^a	55.67 ^b	49.67 ^b	1.27
Ph	7.62	7.33	7.67	0.28
Live sperm cells (%)	77.17 ^a	72.83 ^a	63.83 ^b	7.66
Dead sperm cells (%)	22.83 ^b	27.17 ^b	36.17 ^a	1.68
Concentration (x10 ⁶ /ml)	593.00 ^a	350.00 ^b	180.00 ^c	33.31
Reaction time (s)	11.500	11.00	10.60	0.40

^{a,b}. Means on the same row bearing different superscripts are significantly different.

Conclusion The results indicated that inclusion of *A. africana* in the ration of rabbit bucks reduced sperm concentration and motility which are indices of potential fertility of any male animal. *A. Africana*, therefore, has detrimental effects on the potential fertility of the bucks. Based on the findings, there is need for more researches to be done in order to fully ascertain its effect on the physiology of the male reproductive organs of other farm animals in order to properly advice the smallholder farmers.

Acknowledgements The authors are grateful to the College of Animal Science and Animal Production, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria for providing facilities for the study.

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Effects of chicory / perennial ryegrass swards compared with perennial ryegrass swards on the faecal egg counts of grazing beef steers

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Introduction As the intensity of gastro-intestinal parasites is inversely correlated with performance of the grazing animal (Coop *et al.*, 1985), effective control of parasites is fundamental to ensuring both economically and environmentally sustainable livestock farming systems worldwide. Studies have shown that certain forages, including chicory (*Cichorium intybus*) (Marley *et al.*, 2003), can reduce gastro-intestinal parasitic infections in sheep (Marley *et al.*, 2005). However, there has been little research to determine if chicory can also reduce gastro-intestinal parasites in beef cattle. The aim of the present study was to assess the effects of chicory / perennial ryegrass (*Lolium perenne*) swards compared with perennial ryegrass swards on the faecal egg counts of grazing beef steers.

Material and methods Triplicate field plots (2 ha) were established with either a chicory (cv. Puna II) / perennial ryegrass (cv. Premium) mix (7.4 kg ha⁻¹ chicory / 22.2 kg ha⁻¹ ryegrass) or a perennial ryegrass control (cv. Premium), sown at a rate of 29.6 kg ha⁻¹. Forty-eight Belgian Blue - cross steers (approx. 7 months of age at Day 0) were used for the experiment, with 8 animals grazing each replicate plot. All animals were naturally-infected with gastro-intestinal parasites. The experimental approach comprised of a standardisation and a measurement period. During the standardisation period of 28 days (Larsson *et al.*, 2006), steers were placed on a standard ryegrass / white clover permanent pasture as one group. Animals were allocated to their respective treatment, on the basis of live weight, body condition score (BCS) and faecal egg counts (FEC) determined 7 days prior to the measurement period. The measurement period started on 25 May 2010 (day 0) and continued until herbage availability and weather conditions dictated the end of the grazing season on the 28 September 2010. Faecal samples were collected on Day 0, 28, 70, 84 and 126 for faecal egg count (FEC) and parasite culture determinations. Faecal samples for FEC were taken immediately prior to anthelmintic treatment (Eprinex Pour-on, 0.5% eprinomectin (Merial Animal Health, Harlow, Essex, UK) at a rate of 1 ml per 10 kg liveweight, given on Day 28, 84 and 126. FEC were determined using a modified McMaster technique (MAFF, 1997), with one egg representing 50 eggs g⁻¹ of fresh faeces. Faecal cultures of *Trichostrongyle* type eggs to third stage larvae (L3) consisted of a 10 g faecal sample per individual steer, bulked per plot and incubated at 27°C ± 3°C for 7 days. Faecal dry matter (DM) was determined by placing a 15g sample of faeces at 95 °C for 48h. FEC data were adjusted for faecal DM content prior to square root transformation to normalise the data. FEC were compared at each sampling point by analysis of variance with the previous sample value as the co-variate, and the replicate plot of each forage as a block effect, using Genstat® version 11.1.

Results The results of the square root transformed, DM-adjusted, FEC of the steers on Day 0, 28, 70, 84 and 128 of the experiment are presented in Table 1. FEC data, on a wet faeces basis, showed that the animals in this study had a low to medium challenge of parasites at the various time points within this experiment. There were no differences in either the faecal egg counts, the dry matter content of faeces or the DM-adjusted faecal eggs counts of beef steers grazing on either chicory/ryegrass swards or ryegrass only swards. Faecal cultures indicated that *Ostertagia ostertagi* and *Cooperia* species were the main gastro-intestinal parasite species present in the steers, with *Trichostrongylus* species present but in negligible amounts.

Table 1 Square root transformed faecal egg counts (DM adjusted) of grazing beef steers

Day	Chicory / Ryegrass	Ryegrass	SED	Prob
0	51.4	51.7	4.25	ns
28	65.2	65.0	7.40	ns
70	31.2	25.9	2.70	ns
84	45.6	37.2	3.94	ns
128	23.1	20.8	7.64	ns

Conclusions In this study, there was no difference in the faecal egg counts of beef steers grazing chicory / ryegrass swards or ryegrass only swards. Further work is needed using additional measures of nematode parasitism to determine the overall effects of using chicory compared with ryegrass in beef finishing systems.

Acknowledgements The authors gratefully acknowledge funding from EBLEX and Merial Animal Health Ltd., UK. Seed for the swards was kindly donated by Germinal Holdings Ltd, UK.

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The effect of feeding pomegranate seed pulp on dry matter intake and performance of Iranian crossbred goats

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Introduction Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits and belongs to the Punicaceae family. Pomegranate is extensively cultivated in the Mediterranean area and most Near- and Far- East countries, and is indigenous to Iran. The edible part of the pomegranate is called the aril and constitutes about 52% (w/w) of the total fruit and consists of 78% juice and 22% seed pulp. Pomegranate seed pulp (PSP) which is a by-product in the industrial decoction of pomegranate contains large amounts of oil, with some Iranian varieties having total lipid contents on a dry matter basis ranging from 66 to 193 g/kg DM of the fruit. The aim of this study was to investigate the effect of feeding PSP on the dry matter intake (DMI), milk production and composition, and average daily gain (ADG) of crossbred goats.

Materials and methods Twenty-seven southern Khorasan multiparous lactating crossbred goats in the middle of lactation (days in milk 71 ± 12.5), with an average daily milk yield of 1.09 ± 0.13 kg and average body weight of 28 ± 2.5 kg, were housed in individual stall barns for 10-d for barn adjustment and collection of pre-trial data. At the onset of the 45-d trial, goats were grouped according to pre-trial milk yield and then randomly assigned from these subgroups to one of three experimental diets. Goats in each group were fed individually one of three experimental diets as a total mixed ration ad libitum. All diets consisted of 400 g/kg alfalfa and 600 g/kg (DM basis) concentrate but the PSP content was 0, 60 and 120 g/kg (DM basis) for diets 1, 2 and 3, respectively. The diets were formulated to be iso-nitrogenous and iso-energetic and met NRC (1988) recommendations. Does were milked twice daily at 0530 and 1730 h, and milk yield was recorded daily throughout the experimental period. Milk samples were collected at each milking weekly, were combined on an individual doe basis and analysed for fat, protein, lactose and solids not fat (SNF) by MilkoScan S-50 (Foss Electric). Data were analysed by ANOVA and repeated measures using the MIXED procedure of SAS.

Results Dry matter intake of goats was not affected by diets (Table 1). Milk production tended ($P < 0.055$) to decrease with increasing level of PSP in the diet. Feeding PSP had a significant effect on milk fat concentration and increased it but milk fat yield (g/d) was not affected. Milk protein concentration, milk protein yield, milk SNF concentration and milk SNF yield of goats were not affected by diets. Milk lactose concentration increased by feeding PSP, but milk lactose yield was not affected. Fat corrected milk (FCM) yield, ADG, and production efficiency also were not affected.

Table 1 Daily intake, milk yield, milk composition and ADG as affected by diet

Item	Diet ¹			SEM	P-value
	1	2	3		
DMI, g/d	1724	1736	1722	14.3	NS
Milk yield, g/d	1057	988	984	22.6	0.055
Milk yield, 4% FCM, g/d	1138	1065	1055	26.1	NS
Milk fat concentration, g/kg	41.5 ^b	46.6 ^a	47.7 ^a	1.20	0.006
Milk fat yield, g/d	43.2	46.9	46.6	1.77	NS
Milk protein concentration, g/kg	38.0	36.7	36.5	0.50	NS
Milk protein yield, g/d	39.5	36.2	36.2	1.21	NS
Milk SNF concentration, g/kg	96.8	95.7	95.8	1.10	NS
Milk SNF yield, g/d	103	93	95	3.4	NS
Milk lactose concentration, g/kg	41.5 ^b	42.6 ^{ab}	43.2 ^a	0.30	0.005
Milk lactose yield, g/d	45.4	41.3	42.7	1.81	NS
Total ADG, g/d	106	74	96	13.6	NS
Production efficiency ¹	0.66	0.67	0.64	0.169	NS

¹Diets 1, 2 and 3 were containing 0, 60 and 120 g/kg PSP, respectively.

²Production efficiency = average daily 4% FCM (g/d)/ average daily DMI (g/d).

Conclusions The results show that PSP, which is a cost-effective by-product ingredient in many parts of Iran, can be used successfully as a replacement for cereal grains and other energy rich supplements of the diet, presumably because of its relatively high fat content. This feed is rich in punicic acid (9cis, 11trans, 13cis-conjugated linolenic acid; 9c, 11t, 13c-CLNA) but despite the adverse effect of some CLA on milk fat content (e.g. trans-10, cis-12 CLA) feeding PSP did not reduce milk fat concentration and even increased it which might be associated with the higher ether extract concentration of the PSP containing diets.

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Improved extraction method for archaeol in faeces - a potential biomarker for methanogenic Archaea in the ruminant gastro-intestinal tract

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Introduction Ruminant methane production makes a significant contribution to global greenhouse gas production and there is much current work on mitigation technologies. This work has highlighted the difficulty and cost, as well as concerns about the accuracy, of existing techniques for estimating methane production, so Gill *et al.* (2011) investigated a novel approach based on measuring the Archaeal lipid 'archaeol' in faeces. Whilst there were highly significant effects of dietary treatment on faecal archaeol, the relationship between faecal archaeol and methane production was weak when compared within dietary treatment. Archaeol is present in simple lipid (SL), phospholipid (PL) and glycolipid (GL) forms, however only the SLs are detectable by gas chromatography-mass spectrometry (GC-MS). The saponification method used by Gill *et al.* was likely to liberate archaeol from the PLs, but not the GLs, resulting in incomplete extraction. Additionally, the Soxhlet extraction used by Gill *et al.* may have been less effective than the Bligh-Dyer method that is typically used for microbial lipid extraction (Koga and Morii, 2006). An improved method is therefore required.

Materials and Methods Faecal samples obtained from six animals consuming mainly grass silage (GS) and six animals consuming mainly concentrates (CON), which had been analysed by Gill *et al.* (2011) were analysed using a new method. Faecal sample (100mg) was weighed along with an internal standard, and extracted using the Bligh-Dyer method (Bligh and Dyer, 1959) to obtain a total lipid extract (TLE). PL and GL bound archaeol was liberated using acid methanolysis, which was achieved by adding 2ml of 5% hydrochloric acid in methanol to an aliquot of the TLE and heating at 100°C for 3h. The TLE was then separated into "apolar" and "alcohol" fractions using a modified version of procedures described by Bull *et al.* (1999). Briefly, two eluents were obtained, where dichloromethane (DCM) and DCM:methanol afforded the "apolar" and "alcohol" fractions respectively. The alcohol fraction was derivatised by adding 50µl of *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% triethylchlorosilane and heating at 70°C for 1 hour. The samples were dissolved in ethyl acetate prior to analysis by gas chromatography (GC) and GC-MS. Archaeol was identified on the basis of its characteristic mass spectrum (Teixidor and Grimalt, 1992) and quantified by comparison to the internal standard 1,2-Di-*O*-hexadecyl-*rac*-glycerol. A paired t-test was used to compare concentrations of archaeol using the two methods and the effect of dietary treatments on faecal archaeol concentrations was tested using a one-way analysis of variance.

Results The new method extracted significantly more archaeol than the old method (55.9 versus 17.9 µg/g DM, SED=10.33, $P=0.004$). The effects of dietary treatments on the faecal concentration of archaeol for both methods are presented in Table 1. Figure 1 illustrates the relationship between faecal archaeol concentration and methane production expressed per kg DM intake; there was no significant relationship between the two when comparing within treatment groups.

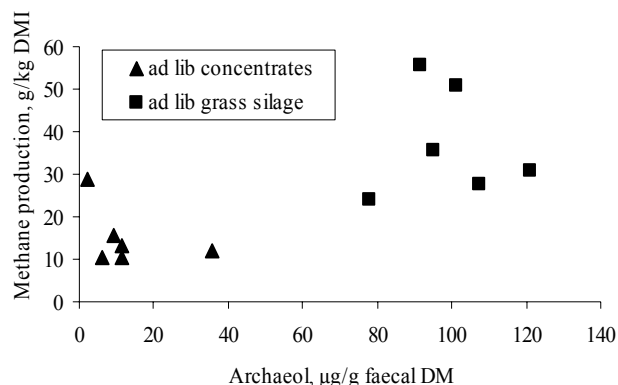


Table 1 Effects of dietary treatment on faecal concentration of archaeol (µg/g DM) - old and new methods

	Treatment:		SED	F pr.
	GS	CON		
Archaeol (old)	30.6	5.1	5.51	<0.001
Archaeol (new)	99.1	12.6	7.68	<0.001

Figure 1 Relationship between methane production (g/kg DMI) and the concentration of archaeol in faeces (µg/g DM)

Conclusions The new method extracted up to three times as much archaeol from samples and provides for both increased precision and reduced sample requirements. This improvement probably reflects extraction of archaeol from glycolipids, as well as improvements in extraction efficiency and quantification. The continued weak relationship between methane production and faecal archaeol (Figure 1) may be attributed to difficulties with the SF₆ technique rather than the incomplete extraction of archaeol.

Acknowledgements Financial support from the Teagasc Walsh Fellowship Scheme is gratefully acknowledged.

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Relationships between stage of growth and chemical composition of grass

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Introduction Fresh grass is the most important forage for ruminant animals across the world, but it is widely recognised that the nutritive value of fresh grass varies considerably with stage of growth. There is little information in the literature on the relationship between stage of growth and nutritive value within the same grass sward. A project was thus undertaken to examine the effects of grass maturity on nutritive value of grass. The effects on nutrient digestibility and metabolisable energy concentration were reported previously (Yan *et al.*, 2009). The objectives of the present study were to evaluate relationships between grass maturity and chemical composition and between concentrations of fibre and other nutrients in the same ryegrass.

Materials and methods Fresh herbage was harvested daily at 13.00 h for a 7 week period from the primary growth and the first regrowth of perennial ryegrass swards. Harvesting commenced on 13 April 2007 for the primary growth and on 4 June 2007 for the first regrowth. The initial sward height was approximately 5 cm. Prior to harvesting, all swards were fertilised with 56.8 kg/ha of nitrogen (N) for primary growth and with 8.1 kg N/ha for the first regrowth. Immediately after harvesting, five samples of each sward were taken from 4 directions and from the centre of the grass mass. They were completely mixed and then divided into 2 portions. One portion was used for an oven DM determination (at 85°C) while the other portion was dried at 60°C and then retained for analysis of N, ash, gross energy (GE), neutral detergent fibre (NDF) and acid detergent fibre (ADF), lipid, and water soluble carbohydrates (WSC). Linear regression was used to examine relationships between harvesting day and chemical composition and between fibre and other nutrient concentrations.

Results and discussion The results are presented in Table 1. All variables were similar between the primary and first regrowth swards, except for WSC concentration which was higher in the first regrowth sward. Therefore, the two datasets were combined for evaluating the effects of harvesting day on chemical composition and evaluating, also, relationships between fibre and other nutrient concentrations. As expected, increasing stage of growth significantly increased DM, ADF, NDF and WSC concentrations ($P < 0.001$), but harvesting day was negatively related to ash, GE, CP and lipid concentrations ($P < 0.001$). The weak relationship between harvesting day and DM concentration might be attributable to changes in weather conditions during the harvesting period. The negative relationship between harvesting day and GE concentration was probably due to the decrease in lipid and CP concentrations with increasing the stage of growth. With regard to fibre fractions, increasing ADF concentration significantly reduced GE ($P < 0.05$), ash, CP and lipid contents ($P < 0.001$), while it was positively related to DM ($P < 0.05$) and NDF ($P < 0.001$) concentrations. Similar relationships were also found for NDF concentrations. A number of equations with ADF were developed, including (all units = g/kg DM): ash = $-0.236 \text{ ADF} + 124$ ($R^2 = 0.48$); lipid = $-0.179 \text{ ADF} + 70$ ($R^2 = 0.59$); NDF = $1.326 \text{ ADF} + 166$ ($R^2 = 0.85$); CP = $-0.757 \text{ ADF} + 294$ ($R^2 = 0.59$). The last of these equations is for reference purposes only, because grass CP concentration is influenced considerably by application rates of N fertilisers.

Table 1. Chemical composition data and R values in linear relationships with cutting days and fibre concentration (n = 95)

	Primary growth sward			First regrowth sward			R values in linear relationships ¹		
	Mean	s.d.	Range	Mean	s.d.	Range	Cutting days	ADF (g/kgDM)	NDF (g/kgDM)
DM (g/kg)	212	26.6	152-261	200	32	103-265	0.41***	0.20*	0.05
Ash (g/kg DM)	66	11.0	48-86	65	11	46-91	-0.83***	-0.69***	-0.54***
GE (g/kg DM)	18.2	0.23	17.8-18.8	18.2	0.28	17.3-18.8	-0.56***	-0.32*	-0.26*
CP (g/kg DM)	109	35.7	60-187	103	28	65-163	-0.90***	-0.77***	-0.64***
ADF (g/kg DM)	251	40.8	177-322	247	23	204-298	0.84***	--	0.92***
NDF (g/kg DM)	503	56.9	382-601	488	34	415-550	0.73***	0.92***	--
Lipid (g/kg DM)	26	8.5	15-43	26	7	12-39	-0.87***	-0.77***	-0.62***
WSC (g/kg DM)	207	28.0	141-255	231	36	146-311	0.36***	0.01	-0.11

¹ ***, ** or * indicate that the relationship was significant at $P < 0.001$, $P < 0.01$ or $P < 0.05$

Conclusions Increasing stage of growth of perennial ryegrass enhances DM, ADF, NDF and WSC contents but reduces GE, CP and lipid concentrations. Perennial ryegrass ADF content can be used to predict ash, lipid and NDF concentrations.

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Histochemical localization of acetylcholinesterase in liver of food animals

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Introduction Acetylcholinesterase (AChE, acetylcholine acetylhydrolase, E.C.3.1.1.7) is a serine esterase with catalysis resembling that of the serine proteases such as lipases, and trypsin, belonging to the large family of serine hydrolases. Determination histochemically of AChE in tissues is the appropriate tool for the diagnosis of organophosphorus and carbamate exposures and intoxication (Silver, 1974). The objective of this study was to determine the histochemical localisation of AChE in liver of sheep used for human consumption. A further aim was to study the localisation of AChE activity as a biomarker of exposure to pesticides.

Materials and methods Liver from sheep was obtained from local abattoirs and transported in a cool box to the laboratory. Tissue sectioning was prepared by using unfixed cryostat sections as described, according to Cornelis *et al.*, (1992), as follows: (i) one piece of liver tissue is required that measures at least 0.5 cm³, separate using a scalpel blade and rinsed until the blood was fully removed, (ii) the liver was then wrapped in aluminium foil and carefully immersed into the liquid nitrogen -196 °C at least 5 min., (iii) the liver was placed in block to metal chunk in the cryostat cabinet using cryoprotectant at an ambient temperature between -20 °C and -30 °C, without allowing the liver block to be warmed up by the liquid cryoprotectant, (iv) sectioning started when block was trimmed to the desired level in the tissue block, and (v) cut into blocks up to 12-µm thick sections and placed on a polysine slide. Since the section is cut correctly, it remains flat on the microtome knife under the anti-roll plate. Following this it was then placed on the slides. It was important during sectioning to ensure that (i) tissue block is adjusted to the cryostat cabinet temperature, (ii) knife and/or knife holder is firmly fixed, (iii) anti roll plate is properly adjusted, and (iv) the speed of sectioning should be constant (Cornelis *et al.*, 1992). At present, the most widely used method for the histochemical localisation of AChE activity is the direct method of Gomori (Gomori, 1948). This is a simple and direct method of localisation AChE activity in tissues. It is based on the reaction between thiocholine, which is one of the products of the enzymatic hydrolysis of the synthetic substrates acetylthiocholine iodide (ATCI) or butyrylthiocholine iodide (BTCI), with the medium containing (copper sulphate, glycine, maleic acid, magnesium chloride, sodium hydroxide, and sodium sulphate). The formation of the shadow yellow colour in the tissues indicates AChE activity.

Results Modified Gomori method showed the most AChE in the cytoplasm of the cell lining the sinusoids, with a decreasing concentration gradient from central vein to the periphery of the lobule (Figure 1).

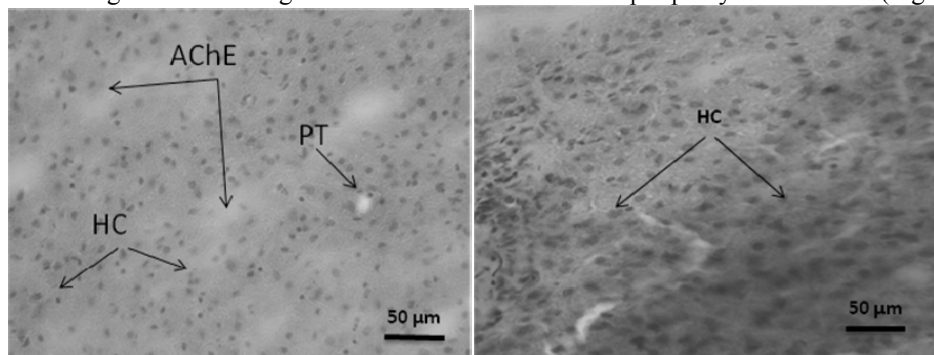


Figure 1 The figures above show histochemical Gomori method used to characterize the location of AChE in liver sections from sheep showing in cytoplasm and sinusoids of liver (left) and normal liver without substrate acetylthiocholine iodide (right) (scale bar = x 40). PT = portal tract and HC = hepatic cells.

Conclusions Our study reported the evidence of localisation of AChE in sheep liver. Although further experiments are needed to explore precise actions of AChE in liver, the present results also have provided strong evidence to suggest that AChE is involved in the cells of liver. Finally, the histochemical procedure for showing the presence of AChE is used widely in diagnosing neurodegenerative disease, and in most laboratories dealing with AChE tissues are therefore already familiar with its application. Furthermore, our results also pointed at the importance of estimating localisation of AChE prior to using in animals as biomarker tools of environmental exposure to Anti-AChE pesticides.

Acknowledgments This research was supported by a Ph.D. studentship from the Iraqi Ministry of Higher Education.

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A kinetic characterization of acetylcholinesterase and butyrylcholinesterase in the tissues of food animals

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Introduction Cholinesterases (ChE) are specialized carboxylic ester hydrolases that break down esters of choline. In general, there are two types of ChE activity have been identified in mammalian tissues; these are distinguished according to their substrate specificity and sensitivity to the selective inhibitors. The first is acetylcholinesterase (AChE, EC.3.1.1.7), which is systematically called acetylcholine acetylhydrolase. The second is butyrylcholinesterase (BChE, EC.3.1.1.8), referred to systemically as acylcholine acylhydrolase (Gholivand *et al.*, 2010). The preferred substrate for AChE enzymes is acetylcholine; BChE enzymes prefer butyrylcholine and/or propionylcholine, depending on the species (Wilson, 2010). The aim of this study was to investigate the kinetics of characterization of ChE activities in the tissues from food animals used for human consumption. A further aim was to study the value of ChE activities as a biomarker of exposure to Anti-ChE.

Materials and methods Meat from food animals (5 sheep, 5 cattle and 5 pigs) was obtained from local abattoirs and transported in a cool box to the laboratory. To extract ChE, samples (liver, muscle, and kidney) were cut into small pieces (3-5 mm³), homogenised with sodium phosphate buffer, pH 8.0 (ratio 1:9), and centrifuged at 9000 g for 5 min, 4 °C. Enzyme activity was determined using the Ellman (1961) method, adapted for a plate reader (Pagliosa *et al.*, 2010). For the determination of maximum reaction velocity (V_{max}), ChE was monitored for 5 min at 410 nm with either 0.05-3 mM acetylthiocholine iodide (ATCI) substrate or 0.05-10 mM butyrylthiocholine iodide (BTCl) substrate for AChE and BChE, respectively. The increase in enzyme activity with increasing substrate concentration was then plotted with a single rectangular hyperbolic equation decay using SigmaPlot 11 (Systat Inc.). The Michaelis-Menten constant (K_m) was experimentally calculated as the concentration at which the rate of the enzyme reaction is half V_{max} .

Results Kinetic parameters were determined in tissues from food animals as described in the Materials and Methods in Table 1. The V_{max} ATCI in animals decreased according to the rank order of pig > cattle > sheep for liver and muscle, and kidney. Nevertheless, V_{max} for BTCl increased as follows: pig > cattle > sheep for liver and pig > sheep > cattle for muscle, while it increased pig > cattle > sheep for kidney. The K_m ATCI in animals was increased according to the rank order of sheep > pig > cattle for liver and cattle > sheep > pig for muscle and kidney. However, K_m for BTCl was increased as follows: pig > sheep > cattle for liver and sheep > cattle > pig for muscle, while it increased as follows: sheep > pig > cattle for kidney.

Table 1 Substrate affinity constant (K_m , expressed in mM) and maximum velocity (V_{max} , expressed in nmol min⁻¹ g⁻¹)

Animal	Substrate	Kinetics	Liver	Muscle	Kidney
Sheep	ATCI	V_{max}	167.6 ± 2.43	49.2 ± 2.03	48.14 ± 1.91
		K_m	0.297 ± 0.016	0.317 ± 0.047	0.309 ± 0.044
	BTCl	V_{max}	234.8 ± 3.49	43.1 ± 2.54	46.61 ± 1.34
		K_m	0.178 ± 0.015	0.254 ± 0.077	0.271 ± 0.041
Cattle	ATCI	V_{max}	218.1 ± 11.17	50.54 ± 2.02	49.17 ± 2.59
		K_m	0.166 ± 0.035	0.348 ± 0.049	0.334 ± 0.062
	BTCl	V_{max}	352.6 ± 21.3	33.58 ± 2.57	132.1 ± 9.44
		K_m	0.176 ± 0.058	0.188 ± 0.078	0.144 ± 0.047
Pig	ATCI	V_{max}	270.7 ± 14.4	79.29 ± 2.14	360.2 ± 0.179
		K_m	0.261 ± 0.052	0.159 ± 0.018	0.018 ± 8.92
	BTCl	V_{max}	386.5 ± 14.48	36.61 ± 2.0873	261.4 ± 18.45
		K_m	0.218 ± 0.043	0.069 ± 0.0241	0.514 ± 0.157

Values in the table are mean ± SE obtains from nonlinear regression analysis.

Conclusions This study provided original data concerning an enzymological characterization in food animals. There was significantly higher V_{max} in liver and kidney was used as the BTCl substrate compared to when ATCl substrate in all cases with the exception of sheep where kidney ATCl substrate activity was higher than that seen in BTCl substrate. In all cases (sheep, cattle, and pig, using both substrate concentrations) ATCl activity was higher in muscle than BTCl activity. K_m value for sheep, cattle, and pig using ATCl substrate were higher than BTCl substrate, with the exception of cattle liver and pig kidney where BTCl activity was higher than that seen in ATCl, and much higher.

Acknowledgments The authors gratefully acknowledge funding from Iraqi Ministry of Higher Education.

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Silage fermentation end products and microbial populations and their relationships to silage quality of orange pulp

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Introduction Increasingly higher costs of typical feedstuffs in many parts of the world have resulted in increased attention to citrus by-products as specific feeds for ruminants (Bampidas and Robinson, 2006). This by-product contains high amount of pectin and soluble carbohydrates that are high in energy and low in crude protein (CP) and neutral detergent fibre (NDF) (Fegeros *et al.*, 1995). The orange pulp is one of citrus by products used in animal diets after dehydration or ensiling processes. In order to reach an appropriate dry matter for ensiling, citrus pulp may be co-ensiled with high dry matter feeds such as chopped wheat straw. This experiment was carried out to assess the microbiological and qualitative characteristics of orange pulp co-ensiled with straw and different protein supplements.

Material and method In this experiment fresh but post-juicing orange pulp was used. The orange pulp was chopped to 5 cm pieces and mixed with straw. The chemical composition of the raw material was determined by AOAC (1990). The compositions of silages were as follows: 1) 73% orange pulp + 27% straw (control), 2) 74% orange pulp + 12% straw + 14% poultry by-product meal (OSP) and 3) 63% orange pulp + 25% straw + urea solution (3%) (OSU). Blends were ensiled for 90 days in triplicate. Total volatile fatty acids and ammonia N concentrations were determined as described by Markham (1942). Extracts needed for measurement of pH and enumeration of microorganisms were provided from fresh silage as described by Zahiroddini *et al.* (2004). A semi-selective lactobacilli medium (MRS) and the nutrient agar (NA) were used for the isolation of lactic acid bacteria (LAB) and total bacteria, respectively. Sabouraud's dextrose agar (SDA; Difco) was used for the isolation of yeasts and molds. Experimental data were analyzed in completely randomized design using GLM procedure of SAS (1991). The means were compared using Duncan's multiple range comparison tests.

Result The chemical composition and microbial counts (\log_{10} cfu/g fresh silage) of silages were presented in Table 1. The pH values, $\text{NH}_3\text{-N}$ and total VFA concentrations were affected by treatments ($P < 0.05$). Highest mean of total bacterial population was observed in OSU ($P < 0.05$) whereas LAB population of the same treatment was lower ($P < 0.05$). In the present study counts of yeasts and molds were not affected by introducing different protein supplements. It is noticeable that no yeasts and molds were observed in OSU cultures.

Table 1 Chemical and microbial composition of silages. Within a row, means followed by different letters differ ($P < 0.05$).

	control	OSP	OSU	SEM
DM (g/kg)	276.5	311.2	270	5.45
pH	4.14 ^c	4.29 ^b	8.43 ^a	0.70
Analysis (g/kg DM)				
Organic matter	806.29 ^a	904.42 ^a	844.38 ^a	27.27
Crude protein	6.3 ^c	19.64 ^a	14.8 ^b	1.97
$\text{NH}_3\text{-N}$ (mg/ml)	84.00 ^b	100.33 ^b	1015.00 ^a	154.07
Total VFA (mmol/ml)	15.66 ^b	25.00 ^a	5.00 ^c	2.94
Enumerations (log cfu/g silage)				
Total bacteria	3.48 ^b	3.50 ^b	3.65 ^a	0.02
LAB	3.45 ^a	3.47 ^a	3.38 ^b	0.01
Yeasts	2.33 ^a	2.39 ^a	-	0.21
Molds	4.00 ^a	2.30 ^a	-	0.51

Conclusions Results of this study showed that various microorganisms present in silage may affect the nutritive value of silages. Fermentation by LAB was more efficient than fermentation by other microorganisms and the silages with lower numbers of total bacteria had higher pH values. LAB was probably mainly responsible for lowering the pH during ensiling, while the same bacteria did not exhibit antifungal activity. Fermentative products and microbiological assessments of silage can help us describe the type of fermentation that occurred in the silo.

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Effects of supplementation of rice straw with readily digestible fiber on populations of fiber-associated ruminal microbes by real-time quantitative PCR *in vitro*

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Introduction Supplementation is a usual feeding technology to manipulate rumen fermentation. Chemically treated straw was considered as a source of readily digestible fiber. Supplementation with readily digestible fiber may be beneficial to digestion and utilization of basal diets (Liu *et al.*, 2002). Intake and digestion of straw diet were increased by supplementation of untreated rice straw with chemical treated straw in Holstein heifers. Limited work has been conducted on supplementation of straw-based diets with readily digestible fiber sources. The objective of the present study is to determine the effects of supplementary readily digestible fiber on populations of fiber-associated ruminal microbes in untreated rice straw using real-time quantitative PCR *in vitro*.

Material and methods One gram of the untreated rice straw, NaOH treated rice straw and untreated rice straw supplemented with NaOH treated rice straw by 50 % (w/w) were incubated *in vitro* in triplicate with 10 ml rumen fluid and 90 ml buffer medium prepared as described by Menke and Steingass (1988), respectively. At 24 h of incubation time, the gas production were recorded, the fermentations were stopped and fermentation contents were filtered through nylon bags (40 µm pore size) and the residues were used to extract total fiber-associated ruminal microbial DNA for quantification of populations of *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens* and total fungi. Species-specific real-time quantitative PCR was performed using the ABI 7500 real time PCR system (Applied Biosystems) with fluorescence detection of SYBR Green dye. Specificity of amplified products was confirmed by melting temperatures and dissociation curves after each amplification. Amplification efficiencies for each primer pairs were investigated by examining dilution series of total rumen microbial DNA template on the same plate in triplicate.

Statistical analyses The effects of supplementation of easy digestible fiber on microbial population were analyzed by the general linear model (GLM) procedure of SAS (1997). The difference of means for the treatments was tested by using Duncan's new multiple range test.

Results Table 1 shows the results of populations of ruminal microbes expressed as a proportion of total rumen bacterial 16S rDNA and percentage difference between the observed value of the mixtures of untreated and NaOH treated straws and that calculated values from microbial populations of straws fermented individually. NaOH treatment could significantly increased microbial populations of *R.albus*, *R.flavefaciens*, *B.fibrisolvens* and total fungi. Positive associative effect was observed on gas production and microbial populations when untreated rice straw was supplemented with NaOH treated straw. The differences were significant in ruminococcus and *B. fibrisolvens* between the predicted and observed values.

Table 1 Effects of supplementation of easy digestible fiber on populations of rumen microbes *in vitro* and difference (%) of observed value of the mixtures of untreated and NaOH treated straws and that predicted from straws separately

% of total bacterial 16S rDNA	Rice straw			SEM	
	Untreated	NaOH treated	50%Untreated + 50%NaOH treated		
			Observed value		Difference ^d
<i>R.albus</i>	0.137 ^c	2.111 ^a	1.269 ^b	12.9 ^c	0.0167
<i>R.flavefaciens</i>	0.764 ^c	1.063 ^a	0.954 ^b	4.4 ^c	0.0097
<i>F.succinogenes</i>	12.093 ^a	2.611 ^c	7.747 ^b	5.4	0.1722
<i>B.fibrisolvens</i>	0.010 ^c	0.034 ^a	0.024 ^b	7.9 ^c	0.0002
Total fungi	1.573 ^c	3.688 ^a	2.641 ^b	0.4	0.0519

^{a, b, c} means within a row with different superscripts are significantly different ($P < 0.05$).

^d Difference (%) = [(Observed value – predicted value)/predicted value] × 100.

^e Significant differences ($P < 0.05$).

Conclusions Supplementation of rice straw with readily digestible fiber could affect the population of fiber-associated ruminal microbes by real-time quantitative PCR *in vitro*. It inferred that the improved digestibility of rice straw may result from the increase in microbial population.

Acknowledgements The authors gratefully acknowledge funding from National Natural Science Foundation of China.

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Effect of chemical processing using alum solution on *in vitro* gas production parameters of barley grain

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Introduction With some exceptions (e.g. maize and sorghum), hydrolysis of starch from grains in the ruminant digestive tract occurs primarily in the rumen (Huntington, 1997). However, rapid ruminal fermentation of starch to produce volatile fatty acids is a less efficient means of metabolising these carbohydrates than is intestinal digestion to glucose (Owens *et al.* 1986). Chemical manipulation of starch fermentation in the rumen, therefore, has become an important research topic in animal nutrition with some studies focusing on reducing the susceptibility of starch sources to microbial degradation, so as to increase the amount of starch digested in the small intestine. The aim of the present study was to evaluate the potential of alum (potassium aluminum sulphate) to process barley grain and its effect on *in vitro* gas production parameters.

Material and methods A sample of 100g barley grain DM was combined with 25ml distilled water or 25ml aqueous alum (200 g/l) to yield final alum concentrations of zero (control) and 50g/kg barley DM. Five treatments were: control (unprocessed), soaked with water for 24h and 48 h and soaked with alum solution for 24h and 48h. The barley grains were then dried in a forced-air oven at 65 °C for 48h. The dried barley grains were milled to pass through a 1mm mesh. Three ruminally-fistulated sheep (body weight= 49.5±2.5kg) were used as rumen liquor donors. The animals were fed 0.8kg DM alfalfa hay and 0.5kg DM concentrate consisting of barley grain, sugar beet pulp, soybean meal, wheat bran and minerals (165g CP/kg of DM). Rumen fluid was collected before the morning feed and strained through four layers of cheesecloth into a CO₂-filled flask. Incubations of the samples carried out using approximately, 200mg of each sample in a 120ml serum bottle (n=4). The bottles were pre-warmed at 39°C before the injection of 30ml rumen fluid-buffer mixture, followed by incubation in a water bath at 39°C. Gas production was recorded after 2, 4, 8, 12, 24, 36, 48, 72 and 96h of incubation, using a manual pressure transducer technique. Cumulative gas production data were fitted to a model of $Y = b(1 - e^{-ct})$; where: Y= potential of gas production at time t; b= gas produced from the soluble and insoluble fraction (ml); c= gas production rate constant (/h); t= incubation time (h). Data of 24 h gas production were also used to estimate the organic matter digestibility and metabolisable energy of the samples. Data were statistically analysed using SAS (1999) software.

Results Gas production parameters of untreated and treated barley grains are shown in Table 1. The results indicated that the values of 'b' and 'c' from processed barley grain, except those treated with alum for 48h, were lower ($P < 0.05$) than those of the control. The processing of the barley grain with alum decreased the rate of gas production in both 24 and 48h treatments, but did not reduce the amount of cumulative gas production. The impact of processing time (24 and 48h) on gas production kinetics was depended on the type of processing. In processing with only water, time of processing had no effect on cumulative gas production and gas production rate constant, but in processing with alum the amount of cumulative gas production was significantly decreased ($P < 0.05$).

Table 1 Gas production parameters, organic matter digestibility (OMD) and metabolisable energy (ME) content of barley grain processed with aqueous alum solution.

Parameter	Treatment				s.e.d	P	
	A ₀ W ₀	W ₂₄	W ₄₈	A ₂₄			A ₄₈
b (ml)	70.7 ^b	68.0 ^{b,c}	68.1 ^{b,c}	60.9 ^c	78.7 ^a	1.01	<0.01
c (/h)	0.089 ^a	0.074 ^{b,c}	0.084 ^{a,b}	0.074 ^{b,c}	0.071 ^c	0.0015	<0.05
*OMD	0.81 ^a	0.75 ^b	0.78 ^{a,b}	0.70 ^c	0.79 ^a	0.006	<0.01
**ME (MJ/kg)	12.1 ^a	11.2 ^b	11.7 ^{ab}	10.5 ^c	11.9 ^a	0.09	<0.01

A₀W₀: control; W₂₄ and W₄₈: barley grain soaked with water (25ml/100g barley) for 24h and 48h, respectively; A₂₄ and A₄₈: barley grain soaked with alum (50g/kg DM) for 24h and 48h, respectively

*OMD = 0.9991 Gas + 0.0595 CP + 0.0181 CA + 9; **ME = 0.157 Gas + 0.0084 CP + 0.022 EE - 0.0081 CA + 1.06 (Menke and Steingass, 1988)

^{abc} Values in the same row with no common superscripts differ significantly ($P < 0.05$).

Conclusions The present study suggests that alum could be a valuable as an agent to decrease ruminal fermentation and increase the by-pass value of barley grain for ruminants. In contrast to many other chemicals proposed for manipulating ruminal fermentation, alum is readily available, inexpensive and safe to use, therefore be judged more acceptable by livestock producers. Given the rapid hydrolysis of cereal meal in the rumen, however, strategies which effectively slow its ruminal disappearance over the initial 12h would be of interest from a practical point of view. More research on the effect of treated barley grain with alum on animal performance is in progress.

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The effect of milking season on milk yield and composition of Khuzestan water buffalo

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Introduction Buffalo milk has second ranking in the world after cow milk, as more than 12% of the world's milk production is comes from these animals. Compared to milk of cows, buffalo milk has more fat, lactose, protein, total solids, vitamins and minerals, such as calcium, magnesium and inorganic phosphate (Ahmad *et al.*, 2008). The variation in milk, milk yield and composition depends on many factors, some of which are breed, species, genetics, stage of lactation, health condition of the animals, daily variation, parity, type of diet, age, udder health, manner of milking (manual or automatic) and season (Kilic and Kilic, 1994; Wolfson and Sumner, 1993). As Khuzestan is a subtropical region and has good forage during whole of year. Calving time is distributed in whole of year. There is less research about the effect of season on buffalo milk yield and composition compared to dairy cattle. Therefore, the aim of this study was to evaluate the effect of season on the milk yield and composition of waters buffalo's milk in Khuzestan province of Iran.

Materials and methods In order to study the effect of season on milk yield and production in water buffalo, we used information that was collected from 1992 to 2009 (17 years) in animal science centre (Khuzestan province, Iran). This includes number of animals, milk yield, fat percentage and protein percentage. Data were analyzed statistically by complete randomized design using SAS program, and Duncan's multiple range tests was used to comparison treatment means at $P < 0.05$.

Results Effect of milking season on milk yield and composition of buffalo are shown in Table 1. The season of milking had a significant effect ($P < 0.05$) on milk yield and compositions of buffalo milk. Animals milking in autumn and winter produced the most milk and those milking in spring the least. The season of milking had significant effect ($P < 0.05$) on fat percentage of buffalo milk being highest in spring lowest in autumn/winter. Milk protein was highest in spring and lowest in autumn. Animals milking in winter had the highest and those milking in autumn had the lowest protein percentage. However, there was no significant difference in milk protein between winter and summer milk.

Table 1 Effect of milking season on milk yield and composition of Khuzestan Waters buffalo

Season	Winter	Spring	Summer	Autumn	SEM	P-value
Milk yield (kg)	9.87 ^a	8.55 ^c	8.96 ^b	9.82 ^a	0.011	0.0001
Milk fat (g/kg)	64.8 ^c	69.3 ^a	67.4 ^b	62.9 ^c	0.007	0.0001
Milk protein (g/kg)	39.2 ^b	40.1 ^a	39.4 ^b	38.8 ^c	0.004	0.0001
Fat yield (g)	636 ^a	591 ^d	602 ^c	619 ^b	1.021	0.0001
Protein yield (g)	369 ^a	342 ^b	312 ^c	367 ^a	1.005	0.0001

Conclusions Milk yield of buffalo was significantly higher in winter and autumn than summer and spring. However, higher milk compositions (g/kg) found in spring. Therefore, according to the results of the present study, season does influence the milk yield and compositions of Khuzestan buffalo.

Acknowledgment The authors are grateful to animal science centre of Khuzestan.

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The effect of the low temperature steam (142, 130 and 90 °C) and sulphuric acid on *in vitro* gas production parameters of sugarcane pith

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Introduction Sugarcane bagasse and sugarcane pith, the residue after rind removal, annually much amount of them are produced in Iran and world. Sugarcane pith is highly lignified by products of the sugar and paper industries. Disrupting the plant cell walls as to allow complete access to nutrients caused to increase the nutritional value of lignocellulosic materials. Under conditions $t > 180$ °C, acetyl groups are released from the hemicellulose matrix and suitable levels of cell wall disruption are achieved, also resulted in formation of furfural by secondary dehydration reactions of hemicellulosic pentoses that inhibit the activity of rumen microbes and cell-free enzymes (Brownell *et al.*, 1986). The researchers reported using lower temperatures with an acid can achieve comparable cell wall disruption to steam treatment at high temperatures, and results lower amounts of toxic compounds (Clausen and Gaddy, 1983). Steam and pressure treatments alone or allied with chemical treatments are known to disrupt lignocellulosics in a way which allows improved utilization of cell wall polysaccharides by cell-free enzymes and rumen microbes (Grohmann *et al.*, 1985). The objective of this experiment was to estimate the effects of low temperature steam with sulphuric acid on *in vitro* gas production parameters of sugarcane pith.

Material and methods Sulphuric acid (H₂SO₄) solution was added to ground sugarcane pith by 1.5 % (15 g acid/kg DM). Then, these acidified samples were autoclaved at 90, 130 and 142 °C for 120 min. The samples were oven-drying overnight at 55 °C. Rumen fluid was supplied from fistulated Holstein steers (400±12 Kg, body weight) fed twice daily a diet containing 5.72 kg lucerne hay and 3.08 kg concentrate mixture in prior to the morning meal, homogenized in a laboratory blender, filtered through three layers of cheese-cloth and purged with CO₂, and was added to the anaerobic mineral buffer solution (1:2 v/v). Gas production of samples were assessed by incubating approximately 200 mg experimental sample (1.0 mm screen, triplicate) with 30 ml of rumen buffer mixture in 100 ml glass syringes based on Menke and Steingass (1988) procedures. Gas production (ml) were recorded at 2, 4, 6, 8, 10, 12, 16, 24, 48, 72 and 96 h. Total gas values were corrected for blank with a known gas production. Cumulative gas production data were fitted to the exponential equation $Y=B(1-e^{-Ct})$, where B is the gas production from the fermentable fraction (ml), C; the gas production rate constant (/h), t; the incubation time (h) and Y is the gas produced at time t. The values of organic matter digestibility (OMD, g/kg OM) and metabolisable energy (ME, MJ/kg DM) of experimental samples were calculated by the equation of Menke and Steingass (1988). Data of *in vitro* gas production, ME, and OMD were subjected to analysis as a completely randomized design using the General Linear Model (GLM). Duncan's multiple range test was used to compare treatment means at $P < 0.05$.

Results The low temperature steam (142 °C, 120 min, 2.2 bar) associated H₂SO₄ (15 g acid/kg DM) increased gas production (GP) parameters, OMD and ME. Amount of GP in this condition is comparable with GP of the sugarcane pith treated in high temperature steam (210 °C, 3 min, 19 bar) (Chaji and Naserian, 2006).

Table 1 Gas production parameters of sugarcane pith treated with low temperature steam and sulphuric acid

Item	B	C	OMD	ME
0.0 T, 0.0M, 0.0 A	75.6±2.70	0.018±0.002	124a	1.23a
90 T, 120M, 1.5A	79.2±1.51	0.02±0.004	103b	1.12b
130T, 120M, 1.5A	84.2±1.50	0.04±0.004	95c	1.07c
142T, 120M, 1.5A	115.5±1.60	0.05±0.001	53d	0.71d
s.e.m	3.12	0.001	0.4	0.011

T, temperature; M, minute; A, acid; B, Gas production from the fermentable fraction (ml), C: Gas production rate constant (/h), OMD: organic matter digestibility; ME: metabolisable energy, s.e.m: standard errors of mean, means within each column with different letters are significantly different ($P < 0.05$).

Conclusions The results of present experiment showed the low temperature steam (142 °C, 120 min, 2.2 bar) associated H₂SO₄ (15 g acid/kg DM) increased gas production parameters, OMD and ME, and therefore improved nutritional values of raw sugarcane pith.

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Loss of activity of the putative protein protectant polyphenol oxidase (PPO) occurs as a result of preservation of red clover forage samples by freeze-drying

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Introduction The enzyme PPO occurs naturally within red clover and in the presence of oxygen causes browning reactions due to complexing of oxidised phenols with proteins. PPO has been suggested to have a role in protein protection in ruminant feeds (Winters and Minchin, 2001), particularly during cell damage caused by mastication of ingested fresh forage. As part of a larger study into PPO-mediated protein complexing this study investigated the effect of forage sample storage method on PPO quantification in wild-type red clover (*Trifolium pratense* L.) and a low-PPO red clover mutant. Techniques to preserve plant material to allow for subsequent analysis and representative sub-sampling are routinely needed for forage analysis. In these experiments the activity of PPO following freeze drying and grinding was compared with that obtained after freezing alone. Freezing at -80°C is a standard method for preservation of enzyme activity in samples for PPO analysis (Winters *et al.*, 2008) but large samples of fibrous material pose technical problems for subsequent extraction or representative sub-sampling. Freeze-drying and grinding of plant material is a common technique allowing uniformity of sub-samples and processing. The freeze drying process keeps the material at minus 40°C throughout, and water vapour is removed by sublimation under vacuum.

Materials and methods Fresh, field-grown wild-type and mutant red clover samples were harvested approximately 5cm above soil level and immediately flash frozen in liquid nitrogen to be stored in -80°C until use. Half of the samples were then freeze dried. Samples were ground with a Cyclotec mill to produce a uniform powder and stored in labelled screw cap plastic pots. Sub-samples of 0.05g of the freeze dried powders were removed and extracted in McIlvaine buffer, and desalted using P6 column and centrifuged according to Winters *et al.*, 2008. PPO activity was assessed in reaction mixtures containing 1.1ml of McIlvaine buffer, 15ul of 0.25% CuSO₄, 20ul aliquot of PPO extract and 375ul of methylcatechol substrate were added to determine the initial rate of the reaction (units) from change in absorbance at 420 nm. Replicate reactions were supplemented with 20% SDS which has been shown to activate PPO *in vitro* (Winters and Minchin, 2001). An analysis of variance was conducted with storage treatment as the main factor for each genotype using GenStat (13th edition) statistical software.

Results The PPO activity of the frozen wild-type red clover extracts was approximately double that observed in the extracts from the mutant in both the presence and absence of SDS. Freeze-drying significantly decreased the recoverable PPO activity in all samples compared with equivalent frozen samples for both wild type and mutant plants, with each genotype showing over ten-fold less activity as a result of freeze drying. Notably, after freeze-drying the presence of SDS in the reaction mixture did not result in enhanced PPO activity compared with that in the absence of SDS.

Table 1 PPO activity of wild type and mutant red clover extracts prepared from frozen or freeze dried and ground forage samples

Plant genotype	+SDS U/g DM		-SDS U/g DM		s.e.d.	P
	Frozen	Freeze-dried	Frozen	Freeze-dried		
Wild type (PPO+) red clover	1103.7	291.8	681.2	202.4	81.75	<0.001
Mutant (low PPO) red clover	554.5	39.8	324.0	23.1	13.62	<0.001

Conclusions Significant loss of PPO activity occurred as a result of the freeze drying process when compared with frozen material in both wild type and mutant red clover, meaning that PPO activity measurements made after freeze drying would be inaccurate for forage assessment. Whether this loss in activity is due to inactivation of the enzyme during the freeze dry and grinding process or due to degradation of the enzyme itself during this process is currently unknown but it appears from these data that the enzyme is stable at very low temperatures but becomes unstable during the freeze-drying process. This could be associated with the non-freezing temperatures associated with sublimation or a consequence of physical changes to the protein structure following loss of cellular water. Freeze dried and ground material may still be appropriate for PPO quantification if methodology can be developed to recover activity. Although SDS was ineffective here, other candidate treatments include change in assay pH or inclusion of potential co-factors, such as reductants (e.g. ascorbate, dithiothreitol).

Acknowledgements This work was supported by EBLEX, HCC and QMS.

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Effect of various Iranian native essential oils on *in vitro* ruminal methane emission and feed fermentation efficiency

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Introduction Rumen fermentation includes some disadvantages such as methane emission and ammonia extraction. Methane represents 8 to 12% loss of intake energy (Johnson and Johnson, 1995) in ruminants and it is a greenhouse gas having a global warming potential 21 times that of CO₂ (Crutzen *et al.* 1995). More recently, extracts and/or essential oils of various natural plants have been proposed for use in ruminant diets as growth promoters (safe to animal and the consumer) place of antibiotics (ionophores). The objective of the present study was to investigate, *in vitro*, the effect of various Iranian native plant essential oils on potential ruminal methane emission and feed fermentation efficiency.

Material and methods A mixture of lucerne hay: concentrate (50:50, DM basis) was used as a substrate feed (NDF 319, Non-fibre carbohydrate 43 and CP 177 g/kg of DM). It was then ground to pass through a 1 mm screen. Approximately 500 mg of the substrate alone (as control) or substrate plus cinnamon, dill, oregano or peppermint essential oils (1 µl of each essential oil per ml of medium) were placed into a 125 ml serum bottles (n= 6) containing 50 ml of buffered rumen fluid (buffer: rumen fluid ratio, 2:1 v/v), and the bottles were placed in a water bath for 24 h at 38.5 °C. Rumen fluid was obtained from three adult ruminally fistulated sheep (49.5 ± 2.5 kg, body weight), before the morning feeding, and immediately strained through four layers of cheesecloth. The gas produced was recorded using a pressure transducer (Theodorou *et al.*, 1994) at 2, 4, 6, 8, 10, 12, 16 and 24 h of the incubation, with the gas being released after each measurement time. A sample of the gas was collected into a 10 ml vacuum tube at 6, 8, 10 and 24 h. After 24 h of the incubation, the bottle contents were filtered (48 µm pore size) and residues dried (60 °C for 48 h). The *in vitro* dry matter disappearance (IDMD) was calculated. Feed fermentation efficiency (FFE) was estimated as FFE= IDMD (g/kg) per cumulative gas produced (ml) at 24 h post incubation. Gas pressure was converted into volume using an experimentally calibrated curve. The methane content of the gas was determined by gas chromatography (GC), using a 6% cyanopropylphenyl, 94% dimethylpolysiloxane column. Data were statistically analysed using SAS (V. 9/1) and Dunnett's test was used to compare the means with that of the control (P< 0.05).

Results The results indicate that the essential oils evaluated under the present experimental conditions, caused a significant (P< 0.05) decrease of methane and total gas produced compared with those of the control (Table 1). In the present study dill essential oil caused an approximate 74% increase (P< 0.05) in FFE compared with that of the control.

Table 1 Effect of various Iranian native plant essential oils *in vitro* on total gas produced, methane emission and feed fermentation efficiency, when added to a lucerne hay:concentrate substrate.

Items	Control	Cinnamon	Dill	Oregano	Peppermint	s.e.m
Total gas (ml/g IDMD)	276.1	115.4 *	253.1 *	186.5 *	187.8 *	0.61
Methane (ml/g IDMD)	41.3	13.9 *	36.9*	24.3*	23.6 *	4.8
FFE	6.1	10.6 *	5.9	6.0	6.5	0.5

Within a row, means with an asterisk differ significantly from the control (P< 0.05)

Conclusions This work demonstrated the potential of essential oils to alter the methane production of a forage-rich diet without any negative effect on FFE. The present results confirmed previous work indicating the ability of essential oils from medicinal plants and spices to decrease methane production (Garcia-Gonzalez *et al.*, 2008), which may help to improve the efficiency of energy used in the rumen. These essential oils might thus be of interest in the development of new additives as alternatives to growth promoter antibiotics. However, there is a need to evaluate these natural additives using wider range of doses and under *in vivo* conditions.

Acknowledgements The authors gratefully acknowledge funding from Excellence Centre for Animal Science, Ferdowsi University of Mashhad.

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Seasonal variation of milk production, fat and protein percentage in Iranian Holstein dairy cows

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Introduction Milk production of dairy cows and its components are influenced by various factors such as feeding, management, weather conditions and region. In order to increase the quality of the milk, it is necessary to identify factors effect on milk composition. The aim of this paper was to study the effect of calving season on milk production, fat and protein percentage of Iranian Holstein dairy cows.

Materials and methods Records from the animal breeding centre of Iran that were collected from April 2005 until March 2006 were used to determine how calving season impact lactation, fat percentage and protein percentage. The analysis was based on 32828 milk production and fat percentage records and 24039 protein percentage records of first lactation dairy cows. Before analysis, records were adjusted to 305d twice milking mature equivalents. To determine the presence of seasonal variation in milk production, fat and protein percentage the month of calving was tested as a fixed effect in a general linear model using SPSS, 15th edition. The analysis model for all 3 traits was: $Y_{ijk} = \mu + M_i + H_j + e_{ijk}$ where Y_{ijk} =record of trait, μ =overall population mean, M_i =fixed effect of calving month, H_j =fixed effect of herd and e_{ijk} = random residual effects.

Results Means \pm standard deviations for all traits were 8623 ± 1375 kg of milk and $3.266 \pm 0.425\%$ of fat percentage and $3.099 \pm 0.215\%$ of protein percentage. Coefficients of variation (CV) of milk production, fat and protein percentage were 15.94, 13.02 and 6.95% respectively. The values of CV show that protein percentage is rather constant throughout the season. There was a highly significant effect of the month of calving and herd ($P < 0.001$) on average all traits. The monthly variation in lactation, fat and protein percentages is shown in Figure 1. In general, lactation had a minimum value in the spring (-192 kg in May) and a maximum value in the autumn (+222 kg in November). Fat percentage increased from a minimum value in the winter (-0.037% in March) to a maximum value in the summer (0.051% in September). Protein percentage was less responsive to season, with the lowest value in the autumn (-0.035% in December) and the highest value in the summer (0.037% in August).

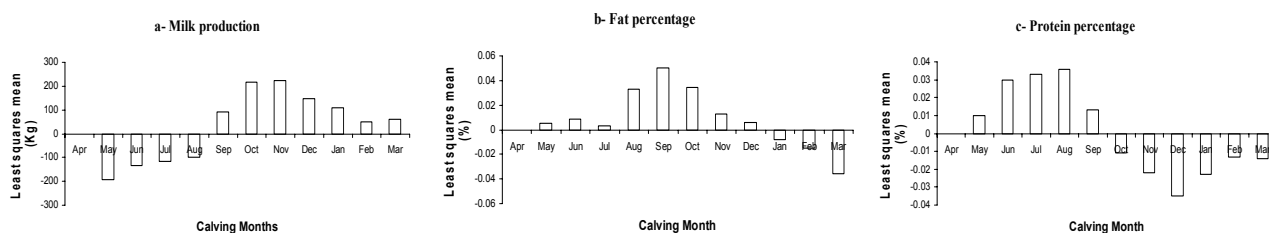


Figure 1 Seasonal variation of milk production (a), fat percentage (b) and protein percentage (c) in first lactation Iranian Holstein dairy cows in year 2006.

Conclusion In this work, we investigated the seasonal variation of lactation, fat and protein percentages in Iranian Holstein dairy cows. The results show, large seasonal variation exists in milk production and its components. The results of this study can provide a standard of the quality of cow's milk in Iran.

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The characteristics of extended lactations in the UK dairy herd

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Introduction Hopkins and Lobley (2009) suggested that UK greenhouse gas emissions could be reduced by adopting extended lactations in dairy systems. In order to test this assertion it was necessary to define the characteristics of extended lactations. This paper describes an analysis of UK Holstein cow performance to investigate the parameters of lactations with different lengths.

Materials and methods Lactations were selected from the database of Holstein records obtained from NMR for the study reported by Albarran-Portillo and Pollott (2008). Three groups of completed lactations were selected to represent different lactation lengths; 305d (traditional annual calving interval), 370d (the current national average) and 440d (representing an extended lactation with an 18-mo calving interval). The biological model of Pollott (2000) was fitted to all 1st, 2nd and 3rd lactations selected. A range of lactation curve traits were analysed by REML with a mixed model (ASReml; Gilmour *et al.*, 2009). Factors in the model included lactation number, lactation length group, their interaction as fixed effects, and herd-year-season (HYS) and a residual term as random effects.

Table 1 ANOVA summary of 7 lactation curve traits

Effect Trait	Lact. No.	Lact. length	Inter- action	HYS	Residual
Max. secr. Potential (kg/d)	***	NS	NS	***	28.83
Relative cell death rate	***	***	***	***	0.8375-07
Day of peak yield	***	***	NS	***	56.83
Peak yield (kg/d)	***	***	NS	***	24.948
Milk increase (g/d)	***	***	***	***	2,047
Persistency (g/d)	***	***	***	***	514
Total yield (kg)	***	***	***	***	2,106,870

Table 2 Least-squares means for key lactation curve parameters for both lactation number and lactation length group

	Max.secret -ion potal. (kg/d)	Relative cell death rate	Day of peak yield	Peak yield (kg/d)	Milk increase rate (g/d)	Persist- ency (g/d)	Total yield (kg)
Lactation number							
1	29.1	0.00104	35.2	27.8	144	49	8.189
2	37.5	0.00131	34.3 ^a	35.4	207	76	9.659
3+	41.1	0.00137	34.4 ^a	38.7	231	86	10.340
Lactation length group							
305d	35.8 ^a	0.00137	34.1 ^a	33.7	203	76	7.969
370d	36.0 ^a	0.00127	34.3 ^a	34.0	197	72	9.238
440d	36.0 ^a	0.00107	35.5	34.2	182	63	10.979

Means within a column with the same superscript were not significantly different ($P < 0.05$)

lactation length but there were no significant differences between maximum secretion potential, and peak yield differences were significant but very small, with longer lactations having slightly higher peak yields. Longer lactations were more persistent than shorter ones and had a slower rate of cell death in later lactation but the difference between the 305-d and 370-d groups were smaller than between the 370-d and 440-d groups. These results raise some interesting questions about extended lactations. Modern Holstein cows are said to have poorer fertility and so extended lactations occur because farmers can't get cows back into calf early enough to achieve an annual calving interval. This is not completely reflected in Table 2. Cows with longer lactations had a slower rate of increase in milk yield and all lactation lengths had similar maximum secretion potentials. Lactations of 440 d had higher peak yields but the shorter two lengths were indistinguishable from each other. The second scenario is that farmers manage the modern higher yielding cow to maximise milk output without regard to annual calving intervals.

Conclusions Cows with longer lactations tend to be more persistent and have slightly higher peak yields than cows with shorter ones. Longer lactations are not always the result of poor fertility and some farmers appear to manage their herds for longer lactations.

Acknowledgements NMR for providing the records used in these analyses. Funding from Defra for Project AC0223.

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Effect of dietary starch source and alfalfa hay particle size on chewing time and ruminal pH in mid-lactation Holstein dairy cows

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Introduction Alfalfa hay and barley grain are the major respective fibre and starch sources used in almost all commercial dairy farms in Iran, but the particle size of alfalfa hay fed varies greatly between farms. The NRC (2001) recommend a geometric mean particle size of 3 mm. Many studies on particle size using alfalfa hay have used extreme chop lengths that may not be relevant to what actually occurs on farms. Small changes in alfalfa particle size and dietary starch source can influence rumen pH and the occurrence of sub-acute ruminal acidosis. Therefore, the objectives of this study were to investigate the effects of, and interactions between, alfalfa hay particle size and source of starch in the diet on chewing time and rumen pH at a constant level of dietary forage neutral detergent fibre (FNDF).

Material and methods Eight cows (175 days in milk) were used in two 4 x 4 Latin squares with four treatments in a 2 x 2 factorial design. Treatments included fine alfalfa (FA) and long alfalfa (LA) that were combined with concentrates based on either only ground barley (GB; FAGB and LAGB) or 50g/kg ground barley + 50g/kg ground maize (GBC; FAGBC and LAGBC). Diets were fed *ad libitum* as total mixed rations with a concentrate to forage ratio of 60:40. Diets averaged 6.61MJ/kg predicted net energy of lactation (NEL), 164g/kg CP, 216g/kg FNDF and 36g/kg NFC on a DM basis. Average NDF and ADF were 372g/kg and 168g/kg for GB- based diets and 365g/kg and 161g/kg for GBC-based diets, respectively. Geometric mean particle size for FA and LA, FAGB, LAGB, FAGBC, and LAGBC were 3.43, 4.33, 3.64, 3.78, 3.39, and 3.68mm, respectively. Experimental periods lasted 21d (14d adaptation and 7d of data collection). On day 19 of each period, eating, rumination and resting time were monitored visually for each cow over a 24-h period. Eating and ruminating activities were noted at 5-min intervals, and each activity was assumed to persist for the entire 5-min interval. Samples of rumen fluid were taken on days 20 and 21 of each experimental period at 3 and 6h after feeding, respectively, using a stomach probe. Ruminal pH measurements were made immediately after sampling. Data on all variables were analysed using the mixed procedure of SAS (SAS, 1998); period, source of grain, particle size of alfalfa hay, and the interaction of grain and alfalfa hay were the fixed effects in the model, and period was used as a repeated measurement with the first-order auto-regressive covariance structure. The random statement included square and cow within square.

Results Data from chewing time and rumen pH are showed in Table 1. Increasing hay particle size tended to increase eating and rumination time which resulted in greater total chewing time. Ruminal pH at 3h post-feeding increased as hay particle size increased. Ruminal pH at 6h post-feeding and time spent eating were higher for GBC than for GB. The increased rumen pH observed 3h after morning feeding in animals fed the larger alfalfa particles indicates that buffering of fermentation acids by increased saliva production occurred as a result of the increased chewing activity in these animals. Also, the positive effect of GBC on rumen pH 6h after the morning feeding can be attributed to the inhibiting effect of maize starch in the diet on the accumulation of large amounts of fermentation acids in the rumen digesta because of its slow rate of digestion and VFA production (Allen, 1997).

Table 1 Effects of alfalfa hay particle size and source of grain on eating, ruminating and chewing time (min/d) and rumen pH

	Treatments				SEM	Statistical significance (P-value)		
	FAGB	LAGB	FAGBC	LAGBC		particle size	grain	particle size × grain
Eating	203	225	232	232	10.0	0.08	<0.01	0.10
Rumination	351	372	352	364	28.8	0.08	0.69	0.65
Total chewing	557	589	583	601	38.2	0.02	0.30	0.21
Ruminal pH (h post-feeding)								
3	6.3	6.5	6.3	6.5	0.09	<0.01	0.94	0.75
6	6.4	6.4	6.5	6.6	0.08	0.42	0.02	0.71

Conclusions This study shows that both grain source and forage particle length have the potential to improve chewing activity and rumen pH of mid-lactation dairy cows.

Acknowledgements The authors gratefully acknowledge funding from Isfahan University of Technology.

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Effect of N underfeeding and energy source on milk production and N partition in dairy cows

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Introduction Improving dietary N utilization by dairy cows is a way to reduce N output in manure and is desirable due to global concerns about contribution of agricultural N to environmental pollution (Calsamiglia *et al.*, 2010). However, this strategy should not impair animal performance. It is hypothesised that the nature of digested energy may interact with protein digestion and metabolism. The objective of this study was to determine the consequences of a large decrease in N dietary supply in dairy cows and its interaction with the nature of energy, on milk production and N partition.

Material and methods Four Holstein cows weighing on average 662 ± 62 kg and at 71 ± 10 d of lactation at the beginning of the experiment, fitted with rumen, proximal duodenum and terminal ileum cannulae, were used in 4x4 Latin square design. Treatments were two N levels (low and high level) combined with two energy sources rich in starch (S) or fibre (F). On a dry matter (DM) basis, the four diets had the same forage proportion (0.405 maize silage, 0.10 natural grassland hay, first cut; 0.09 dehydrated lucerne). The high level of N (H) met 110% of N cow requirements expressed in the French protein digestible in the intestine (PDI) system (INRA, 1989) with an adequate supply in ruminal degradable N, whereas the low level (L) covered 80% of PDI requirements with a shortage in ruminal degradable N. In the H diet, the main N sources were soybean meal and urea. The four diets were iso-energetic and the difference between the two energy sources was based on the composition of energetic concentrate used. In the S diets, the starchy concentrate was made with mixture of a proportion of 0.39 of barley, 0.46 of wheat and 0.15 of maize. In the F diet, the fibrous concentrate was made with a mixture of soybean hulls and dehydrated beet pulp. The DM intake was the same among diets so that any effect of intake level on digestion was avoided. Diet was distributed twice daily at 0900 and 1700h. Each experimental period lasted 28d and consisted of 22d of adaptation to the diet and 6d of measurements. N partition was determined by total milk, faeces and urine collection. Milk N was calculated by dividing milk protein by 6.38. Statistical analysis was performed using GLM procedure of SAS with N level, energy source, interaction between N level and energy source, and animal as effects.

Results The DM intake was similar for both treatments (Table 1), as defined by the experimental design. Both milk production ($P < 0.01$) and urea content of the milk were higher ($P < 0.01$) with H than with L diets. Neither milk fat content nor milk protein content was affected by the N level of the diet. Milk protein was higher ($P < 0.05$) for S diets. There was no interaction between the N level and the energy source for any parameter. Daily output of N in urine and milk were 0.52 and 0.11 times lower with L diets than with H diets, respectively. When expressed as percentage of N intake, urinary N excretion was 1.6 times higher ($P < 0.01$) with H than with L diets, whereas the faecal N excretion and the milk N excretion was 0.18 and 0.14 times lower ($P < 0.05$ and $P < 0.01$, respectively) with H diets than with L diets.

Table 1 Performances and N balance in dairy cows receiving diets containing starch or fibre concentrates, at low or high N level

	L		H		SEM	Statistical analysis
	S	F	S	F		
Dry matter intake (kg/d)	20.0	20.3	19.9	20.4	0.39	ns
Milk yield (kg/d)	22.5	22.7	24.1	25.7	0.55	N**
Milk fat (g/kg)	36.0	37.8	35.1	38.6	2.72	ns
Milk protein (g/kg)	30.0	28.4	30.7	28.2	0.67	E*
Milk urea (g/kg)	0.113	0.166	0.222	0.266	0.0271	N**
N intake (g/d)	352.1	359.9	452.6	470.9	7.18	N**
Faecal N (g/d)	149.1	156.6	155.2	168.1	7.77	ns
Urinary N (g/d)	52.8	61.3	115.0	120.6	7.49	N**
Milk N (g/d)	105.3	100.8	116.6	113.6	3.88	N*

N: effect of N level; E: effect of energy source; ns: non significant; *: $P < 0.05$ - **: $P < 0.01$

Conclusions A strong reduction of dietary N significantly decreases both milk yield and N excretion in the environment. When dietary N decreases, the percentage of transfer of N into milk increases, and there is a strong decrease in urinary losses, in percentage of N intake as in daily output. In this study, only milk fat and protein were changed by the energy source, whatever the N level was.

Acknowledgment This study was granted by the Commission of the European Communities; project FP7-KBBE-2007-1 "Rednex"

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Effect of N underfeeding and energy source on ruminal digestion and protein metabolism in dairy cows

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Introduction Optimising the efficiency of N utilisation in the rumen of dairy cows is a way to improve the use of dietary N by animals, and thus to limit N output in manure (urine and faeces). In the rumen, efficiency of N utilisation is affected by modification of some key factors including protein degradation, N uptake and efficiency of microbial synthesis (MS) but there is little information with very low N diets. At the same time, the MS efficiency may depend on energy source. This study aimed to determine the effect of a strong decrease in dietary N on protein ruminal digestion and metabolism in cows, when concentrates were based on starch or fibre.

Material and methods Four Holstein cows in mid-lactation fitted with rumen, proximal duodenum and terminal ileum cannulae were used in a 4x4 Latin square design. Treatments were two levels of N (low and high level) and two energy sources (starch and fibre). On a DM basis, the four diets had the same forage content based on maize silage, hay, and dehydrated lucerne. The high N level (H) was sufficient in rumen degradable N, the low N level (L) was deficient in rumen degradable N. Energy sources differed by their nature, starch (S) from barley, maize and wheat, or fibre (F) from soybean hulls and dehydrated beet pulp. The CP content of the diets was 142, 144, 109, and 111 g/kg DM for treatments HS, HF, LS and LF, respectively. Differences in degradable N supply were obtained mainly by soybean meal and urea. Diet was distributed twice daily at 0900 and 1700 h. Each experimental period lasted 28 d. Total tract digestibility was determined by total faeces collection. Duodenal digesta flow was determined using YbCl₃ as marker. Microbial N duodenal flow was determined using purine and pyrimidic bases as marker, from a mixed bacteria sample. The efficiency of microbial N synthesis was calculated as the ratio between microbial N duodenal flow and OM fermented in the rumen. In ruminal liquid, kinetics of ammonia and volatile fatty acids in the rumen were determined before feeding and 1, 2.5, 5 and 8 h after feeding, and protozoa were counted before and 2.5 h after feeding. Statistical analysis was performed using GLM procedure of SAS.

Results Organic matter (OM) total tract digestibility, non ammoniacal N (NAN) duodenal flow, N intestinal digestibility, rumen ammonia, rumen ammonia mean and post-prandial peak were higher with H diets than with L diets (Table 1). Rumen ammonia post-prandial peak was higher with fibre diets, especially at low N level (significant interaction). Despite important numerical differences, no significant difference was observed among diets for OM apparent ruminal digestibility, microbial duodenal flow and efficiency of microbial synthesis. Total volatile fatty acids and rumen protozoa concentrations did not vary among diets.

Table 1 Ruminal digestion and protein metabolism in dairy cows receiving concentrates rich in starch or fibre at low or high N level

	L		H		SEM	Statistical analysis
	S	F	S	F		
OM intake (kg/d)	18.8	19.0	18.7	19.0	0.37	ns
OM total tract digestibility (g/kg)	664	658	705	677	8.4	N**
OM apparent ruminal digestibility (g/kg)	432	507	432	467	38.9	ns
NAN duodenal flow (g/d)	390	347	499	437	23.6	N** E*
NAN duodenal flow (% N intake)	111	96	110	93	5.4	E*
Microbial N duodenal flow (g/d)	317	277	374	306	32.2	ns
Microbial synthesis efficiency (g N/kg OM fermented)	26.0	20.6	28.1	23.9	2.68	ns
N intestinal digestibility (% duodenal N)	62.2	55.7	69.8	62.0	2.64	N* E*
Rumen ammonia, post-prandial peak (mg/l)	71.0	245.4	224.5	288.4	24.15	N** E** N×E*
Ammonia, average (mg/l)	23.8	123.1	126.8	150.6	13.49	N** E**
Total volatile fatty acids, average (mM)	95.6	101.8	101.2	104.8	2.71	ns
Acetate / propionate (mol/mol)	4.02	3.77	3.79	3.90	0.142	ns
Total protozoa (× 10 ³ /ml) prefeeding	163.3	247.4	283.2	244.4	88.67	ns
Total protozoa (× 10 ³ /ml) 2.5 h postfeeding	56.8	96.5	81.8	87.5	24.15	ns

N: effect of N level; E: effect of energy source; ns: non significant; *: P < 0.05 - **: P < 0.01

Conclusions A substantial shortage in fermentable N in the rumen resulted in a decrease in OM digestibility, and in a very low ammonia concentration, as previously shown in low N diet (Doreau *et al.*, 1990), especially when concentrate was rich in starch. Low N diets decreased NAN duodenal flow but microbial N duodenal was not significantly decreased, and microbial synthesis efficiency was not modified. Low dietary N does not impair N ruminal metabolism, with diets differing in fibre/starch ratio.

Acknowledgment This study was granted by the Commission of the European Communities; project FP7-KBBE-2007-1 "Rednex"

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Effects of dry period length on the subsequent performance of lactating Holstein cows

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Introduction A dry period is necessary for involution of the mammary gland and to maximize milk yield in the subsequent lactation in cattle (Rastani, *et al.*, 2005). A shorter dry period reduces the frequency of diet change, thus reducing the animal stress. Also, reduced dietary changes in the dry period may improve the survival of desirable population of rumen microbial flora (Pezeshki *et al.*, 2007). Therefore, the optimum length of the dry period might be shorter than previously considered. The objective of this study was to determine whether shortening the dry period would affect performance and energy status of dairy cows.

Materials and methods A total of 24 multiparous Holstein dairy cows were used in a completely randomized experimental design with 40 (LOD), and 20 day (SHD) dry period lengths. Actual dry period lengths for respective treatments were 45 ± 6.2 and 22 ± 4.1 days. All cows were fed a TMR formulated for production level and gestation stage. Dry matter intake (DMI), milk yield, and weekly milk composition were determined. Milk samples were collected weekly at 3 consecutive milkings until the eighth week of lactation. Fat, protein, lactose and solid non fat (SNF) concentrations of milk were measured by Milkoscan Analyzer (Foss Electric, Conveyor 4000). Data were analyzed using mixed procedure of SAS (2004) as repeat measures in time. Statistical model was $Y_{ijk} = \mu + T_i + A_{ij} + D_k + (T \times D)_{ik} + \epsilon_{ijk}$; where Y_{ijk} = Dependent variable, μ = The overall mean, T_i = Treatment effect, A_{ij} = Cow in treatment, D_k = Time effect, $(T \times D)_{ik}$ = Treatment and time interaction, ϵ_{ijk} = Error.

Results Feed intake, milk production, feed efficiency (milk yield/feed intake) and milk composition of early lactating Holstein cows in SHD or LOD treatments are shown in Table 1. There was a significant difference between treatments ($P < 0.05$) when postpartum DMI was considered and was significantly greater in SHD than LOD cows. Although, milk yield was not affected by the dry period length, milk composition was significantly affected by treatments ($P < 0.05$).

Table 1 Feed intake, milk production, milk composition and feed efficiency of lactating Holstein cows with different dry period length

Item	Treatments ¹		Treatment effect		Time effect	
	SHD	LOD	s.e.m	P	s.e.m	P
Dry matter intake (kg/d) (postpartum)	24.3	22.9	0.57	<0.05	0.62	<0.05
Milk yield (kg/d)	38.8	39.6	1.27	0.641	1.38	<0.05
Feed efficiency (milk/feed intake)	1.6	1.7	0.66	0.279	0.07	<0.05
Milk fat (g/kg)	36.2	32.9	0.77	<0.05	0.94	<0.05
Milk protein (g/kg)	30.4	31.2	0.28	0.057	0.46	<0.05
Milk lactose (g/kg)	48.2	47.3	0.34	0.078	0.46	<0.05
Milk SNF(g/kg)	90.7	89.4	0.35	<0.05	0.6	<0.05

SHD: Short dry period (22d), LOD: Long dry period (45d)

Conclusions Postpartum DMI was significantly increased in SHD cows. This result could be due to reduce in dietary changes in the dry period that may improve the survival of desirable population of rumen microbial flora (Pezeshki, *et al.*, 2007). Cows assigned to the LOD had 3 diet changes whereas cows in the SHD treatment experienced only 2 diet changes. Many authors reported a significant decrease in milk yield with shortening of dry period (Bachman and Schairer., 2003), But in the present study milk yield was similar for both SHD and LOD cows. The present study suggested that a 22 day dry period length, might have a better effect on the feed intake of multiparous dairy cows, compared with 45d dry length.

Acknowledgments Financial support of Ferdowsi University of Mashhad is gratefully acknowledged.

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Evaluation of the inclusion of intact or physically broken flaxseed in place of extruded soya seed on Holstein lactating dairy cow performances

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Introduction One way of improving energy status, and thereby reproductive performance in dairy cows, is to increase the energy density of the diet with fat supplementation. Flaxseed is an excellent source of n-3 fatty acids (FA). Methods to decrease ruminal bio hydrogenation would increase the transfer of PUFA from flaxseed into milk. However processing of seed would increase availability of oil in the rumen and as a result, greater bio hydrogenation of flaxseed PUFA by rumen microbes may occur, thus increasing milk conjugated linoleic acid (CLA) concentration (da Silva, *et al.*, 2007). Experiments carried out with flaxseed used ground (Gonthier, *et al.*, 2005) or whole (Petit, 2002) seeds. The aim of the present experiment was to determine the effect of physical processing of flaxseed on production responses of Holstein lactating dairy cows.

Materials and methods A total of 9 primiparous Holstein cows averaging 495±34.5 kg body weight (BW) and 70 ± 5 days in milk (DIM) were assigned to a 3×3 Latin square design to determine the effects physically processing of flaxseed on feed intake, milk production and milk composition. Three isonitrogenous and isoenergetic diets (crude protein (CP):180 g/kg DM, ME (metabolizable energy): 12.6 MJ/kg DM] containing different oilseed sources (extruded soya seed (ESS): 110, ground flaxseed (GFS): 90 or whole flaxseed (WFS): 90 g/kg of DM) were provided. The diets were offered *ad libitum* as a total mixed ration for twice daily. Each experimental period consisted of 21 d adaptation to the diets and 7d for collection of milk yield and feed intake. Fat, protein, lactose and solid not fat (SNF) concentrations of milk were measured by Milkoscan Analyzer (Foss Electric, Conveyor 4000). Data were analyzed using mixed procedure of SAS (2004).

Results Feed intake, milk production, feed efficiency (milk yield/feed intake) and milk composition of Holstein lactating dairy cows fed ESS, GFS or WFS diets are shown in Table 1. There was no significant difference between diets when dry matter intake and milk yield were considered. Milk fat and lactose concentration were similar for all treatments, but milk protein concentration was significantly increased in GFS compared to other treatments.

Table 1 Feed intake, milk production, milk composition, and feed efficiency of Holstein lactating dairy cows fed diets containing different oil seed sources.

Item	Treatments ¹			s.e.m	P
	ESS	GFS	WFS		
Dry matter intake (kg/d)	25.9	25.2	24.8	0.71	0.554
Milk yield (kg/d)	30.5	30.6	30.9	0.76	0.923
Feed efficiency (milk/feed intake)	1.2	1.2	1.2	0.04	0.408
Milk fat (g/kg)	30.7	31.4	32.8	1.13	0.645
Milk protein (g/kg)	28.8	30.2	28	0.89	<0.05
Milk lactose (g/kg)	45.2	45.8	46.2	0.6	0.441
Milk SNF (g/kg)	87.8	89.3	88.4	1.15	<0.05
Body condition Score(BCS)	2.7	2.8	2.7	0.13	0.859

ESS: extruded soy seed, GFS: ground flaxseed and WFS: whole flaxseed

Conclusions Present data indicate that physical processing of flaxseed did not affect milk yield, however milk protein concentration was significantly (P <0.05) increased when physically processed flaxseed was included in the diet. It has been previously indicated that the physically breakdown of the oil seed might cause increase the crude protein availability in the rumen and enhance the microbial production (Stern, 1994). Consequently, this source of protein leads to the higher milk protein yield.

Acknowledgments Financial support of Ferdowsi University of Mashhad is gratefully acknowledged.

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A study of secondary follicle development in Iranian Raeini Cashmere goat kids

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Introduction Iran has 5 million cashmere Raeini goats. These goats have two types of follicle. Hair (coarse outer-coat) is produced by primary (P) follicles and cashmere (fine inner-coat) by secondary (S) follicles (Sumner and Bigham, 1993). The greater the ratio S to P the finer the cashmere (Koul *et al.* 1987). Almost all P follicles are mature at birth, while S follicles continue to mature between birth and, depending on breed, 30 to 120 days of age (Henderson *et al.* 1991). A higher population of S follicles result in higher production of fine fibres (Koul *et al.* 1987). The aim of this study was to determine S to P follicle ratio, the development of S follicles and the age at which all these follicles have reached maturity (contain cashmere fibre) in Raeini cashmere goat kid from birth to 105 days of age.

Material and methods The experiment was conducted from birth to 105 days of age. A total of 70 Raeini kids (35 male and 35 female) were used in the experiment. A 10 mm diameter skin biopsy was collected from the mid-side of each kid at each time using a circular trephine under local anaesthetic (Lidocane). Skin specimens were processed and embedded in paraffin wax. After cutting and mounting, sections were stained using the Saccip method (Ryder and Stephenson, 1968). Samples were taken from each kid at 1 day of age and then every two weeks (a total of 8 biopsies /kid). Twelve sections from each individual skin biopsy were examined per specimen to measure P and S follicle numbers in follicular groups (magnification x 200). From each section at least 10 follicular groups were examined. The majority of groups were of the trio form (three primary follicles and several associated secondary follicles normally 12-16 in Raeini goats). Mature S follicles were defined as follicles containing a definite cashmere fibre. Data about the mean S:P ratios of male and female goat kids were compared with t test. Times, sex effects and interactions on S:P ratio trait were analysed and these differences were not significant with the exception of 1 day and second week after birth.

Results Raeini kids achieved maximum S follicle development (by numbers) at about 60 days after birth. Follicular observations showed that 60% of S were mature at 15 days of age, that is, able to produce cashmere and 98.5% were mature at day 45 of age. Thus, overall, the first 45 days after birth are more important for S follicle development and cashmere production in Raeini kids. There were no significant differences between sexes in S:P ratio, although, S:P ratios were numerically higher in females than males. The S:P ratios were lower at birth and 15 days of age compared to older ages (Table 1). The mean total S:P ratios of female and male Raeini kids were of 12.46 and 11.77 respectively. Difference was not significant for this trait.

Table 1 Mean S:P ratios for male and female Raeini kids from 1 to 105 days of age

Day	Male	Female	s.e.d.
1	7.11 ^a	7.44 ^a	1.11
15	10.77 ^b	10.93 ^b	1.93
30	12.00 ^c	12.74 ^c	1.84
45	12.90 ^c	13.88 ^c	1.54
60	12.98 ^c	13.75 ^c	1.84
75	13.00 ^c	13.40 ^c	1.60
90	13.06 ^c	13.84 ^c	1.44
105	12.37 ^c	13.17 ^c	1.58

Means in same column with different superscript are significantly different ($P < 0.05$)

Conclusions Since S follicles are initiated in the uterus (day 90 of pregnancy) and development continues after birth in Raeini kids, it is possible that pre and post natal nutrition of the doe (for more milk production) and its foetus (kid) may affect follicle development and the productive potential of kids for cashmere growth. The results suggest nutrition before 45 days of age will be important. Hence, nutrition improvement may increase S follicle development, density and S:P ratio (Galbraith *et al.* 2000). As a result, kids will be able to produce more cashmere in future. The high S:P ratio can be also used to select best goat kids to replace per herd per year.

Acknowledgements The authors gratefully acknowledge funding from National Animal Science Research Institute

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Effects of probiotic and prebiotic on performance and plasma IgG1 concentration of dairy calves

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Introduction Interest in the effects of feeding probiotics and prebiotics on animal health and performance has increased due to concern regarding the use of antibiotics in the animal feed industry. Probiotics and prebiotics have been shown to have different functions such as increasing feed efficiency and weight gain and improve immune system (Heinrichs *et al.*, 2003; Timmerman *et al.*, 2005). The aim of the current experiment was to study the effects of feeding milk containing probiotic, prebiotic and probiotic + prebiotic (synbiotic) on performance and plasma immunoglobulin G1 concentration.

Material and methods Thirty two Holstein calves (initial Body weight (BW) = 40±3.0 kg) were grouped based on sex and BW. Calves were removed from their dams and housed in individual pen hutches bed led with straw during the study. Calves fed 1.5 L of fresh colostrum by nipple bottle at birth, and again after 4h, and every 12h thereafter. Calves were fed colostrums for 3d, then switched to whole milk. Calves were assigned randomly at birth to one of four treatments. Treatments included: whole milk with no additives(control), whole milk containing probiotic at 1g Protexin (multi-strain probiotic contains 7 bacteria strains and 2 yeast strains with 2×10⁹ cfu/g) per day, whole milk containing prebiotic at 4g Tipax (polysaccharides of *saccharomyces cerevisiae* cell wall) per day, whole milk containing 0.5g probiotic and 2g prebiotic (synbiotic) per day. Calves received whole milk twice daily at 0700 and 1600 h. calf starter and water were offered *ad lib* throughout the trial of 60 days. Composition of calf starter on as-fed basis was: corn, 35%; barley, 20%; cotton seed meal, 5%; soybean meal, 25%;fish meal, 1%; bran, 9.5 %; salt, 0.5%; vitamin premix, 1%; mineral premix, 1%; calcium carbonate, 1%; sodium bicarbonate, 1%. Starter intake was recorded daily throughout the trial. BW were measured at birth and thereafter weekly up to 60 d of age. Blood samples were collected from jugular veins at 3, 30 and 60 days, approximately 3h after the morning feeding and transported to the laboratory. Plasma samples were extracted and stored frozen for ELISA analysis of IgG1. The ELISA analysis was described previously by Rivera *et al* (2002). The experimental design was a complete randomized design. Data were analyzed by General Liner Model procedure of SAS program (SAS1996) and means were compared by Duncan test.

Results Performance and plasma IgG1 concentration of calves fed with or without additives are shown in Table1. Comparison of dry matter intake (DMI) illustrated that there was no significant difference among treatments till the end of trial. Data on average daily gain (ADG) indicate that calves fed synbiotic and calves fed prebiotic had higher ADG than probiotic and control treatments. Feed efficiency was greater in calves fed probiotic, prebiotic and synbiotic than control calves (P<0.05). No treatment differences in plasma IgG1 concentration were detected during the trial (P>0.05).

Table 1 Probiotic, prebiotic and synbiotic effects on performance and plasma IgG1 concentration of dairy calves

	Treatment				SEM	P-value
	control	probiotic	prebiotic	synbiotic		
DMI(g/d)	521	514	459	501	31.7	0.26
ADG(g/d)	489 ^c	514 ^{bc}	530 ^b	557 ^a	22.2	0.02
Feed Efficiency(g/g)	0.94 ^c	1 ^b	1.16 ^a	1.11 ^a	0.05	0.03
IgG1(mg/dl)						
d 3	922	1158	1456	1098	264.5	0.33
d 30	875	897	994	921	157.7	0.38
d 60	842	982	1072	1013	101.6	0.46

Conclusions In the present study, beneficial effects were seen in performance when probiotic, prebiotic and especially synbiotic were added to milk of dairy calves compared with control group.

Acknowledgements The authors gratefully acknowledge Hossini Company for preparing calves and other facilities.

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Incremental effects of a novel calcium salt of *cis*-monounsaturated fatty acids product on milk fatty acid composition

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Introduction Research has demonstrated that cardiovascular disease risk factors can be improved by the isoenergetic replacement of saturated fatty acids (SFA) with *cis*-monounsaturated fatty acids (MUFA) in the human diet (Mensink *et al.*, 2003). Studies at the University of Reading have demonstrated that including milled rapeseed in the dairy cow diet can decrease SFA and enhance *cis*-MUFA concentration in milk fat to 55 and 30 g/100 g total fatty acids, respectively (Givens *et al.*, 2009). However, this was accompanied by a significant increase in *trans* fatty acid concentrations, which are the consequence of rumen biohydrogenation of dietary unsaturated fatty acids. Feeding rumen-protected sources of *cis*-MUFA such as calcium salts could minimise the appearance of *trans*-monoenes in milk fat, but results of previous research have been inconsistent, possibly due to dissociation of calcium salts in the rumen (Chouinard *et al.*, 1998). The objective of the present study was to measure the effect of feeding incremental amounts of a novel calcium salt (CS) of *cis*-MUFA on feed intake, milk yield and milk fatty acid composition of dairy cows.

Material and methods Four multiparous, late-lactation Holstein-Friesian cows (mean yield 29 litres/day, mean 244 days in lactation) were randomly allocated to one of four dietary treatments in a 4 x 4 Latin Square design with 21 day experimental periods. Treatments were a control diet (Control) containing no supplemental fat, or the same basal diet with CS of *cis*-MUFA (Volac International Ltd., Royston, UK) fed at incremental rates of 20, 40 and 60 g/kg diet dry matter (DM; CS2, CS4 and CS6, respectively), thereby diluting other dietary ingredients. The diet was a total mixed ration consisting of 50:50 forage:concentrate (DM basis), and the forage proportion consisting of 75:25 maize silage:grass silage. DM intakes and milk yields were recorded daily throughout the experiment. Milk composition was analysed during the last 3 days of each experimental period for fat, protein, lactose and full fatty acid profile according to the methods of Givens *et al.*, (2009). Data were analysed using Mixed Model procedures of SAS, with a model that included fixed effects of treatment and period, and random effects of cow. Orthogonal contrasts were used to test for linear effects of CS inclusion level.

Results With increasing inclusion of CS of *cis*-MUFA, diet nutrient content tended to decrease due to the dilution effect of the supplement. Ca-salt supplementation resulted in a linear ($P=0.05$) reduction in DM intake (Table 1), but no effect was observed ($P=0.546$) on calculated metabolisable energy intake. Milk yield and milk constituent yields were not affected ($P>0.05$) by CS supplementation, but milk fat and protein concentrations decreased linearly ($P<0.02$ and $P<0.001$, respectively) with increasing supplement level. Milk fat concentration of all short and medium chain SFA apart from 4:0 were linearly reduced ($P<0.01$) by CS inclusion, and total SFA concentration was reduced to 51.6 g/100 g fatty acids at the highest inclusion level (CS6). This was mainly due to a decrease ($P<0.001$) in 16:0 concentration from 32.3 to 20.9 g/100 g fatty acids for the control and CS6 diets, respectively. Feeding CS also resulted in linear increases ($P<0.02$) in milk fat 18:0, total conjugated linoleic acids and total non-conjugated 18:2 concentrations. The concentration of total *cis*-MUFA in milk fat also linearly increased with CS feeding ($P<0.001$; Table 1), mainly due to *cis*-9 18:1. Linear increases ($P<0.01$) in all *trans*-18:1 isomers contributed towards an overall linear enhancement of milk fat total *trans* MUFA when CS were fed.

Table 1 Effect of calcium salts of *cis*-MUFA supplementation on intake, milk yield and milk fatty acid profile

	Control	CS2	CS4	CS6	SEM	<i>P</i> (LIN)
Dry matter intake (kg/d)	21.2	20.3	20.0	19.8	1.76	0.050
Ca-salt of <i>cis</i> -MUFA intake (g/d)	0.0	405	802	1186	73.9	<0.001
Milk yield (kg/d)	25.5	25.6	24.8	26.3	3.86	0.709
Total SFA (g/100 g fatty acids)	71.0	63.1	57.1	51.6	1.66	<0.001
Total <i>cis</i> -MUFA (g/100 g fatty acids)	19.9	24.4	27.7	30.8	0.91	<0.001
Total <i>trans</i> -MUFA (g/100 g fatty acids)	4.7	7.4	9.6	11.7	0.80	<0.001

Conclusions The CS preparation used in this experiment was effective at reducing milk SFA concentration and increasing *cis*-MUFA concentration, without incurring any negative effects on milk and milk component yields. However, reduced milk fat and protein concentrations together with increases in milk *trans* fatty acid and other biohydrogenation intermediate concentrations suggest at least partial dissociation of the CS within the rumen.

Acknowledgements The authors gratefully acknowledge the BBSRC DRINC research funding scheme and Volac International Ltd., for funding this study.

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Resumption of cyclicity: its associations with metabolic profiles during the early postpartum period, body condition score and first service conception rate in a spring calving dairy herd

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Introduction The poor reproductive performance of dairy cows is widely acknowledged. Delayed resumption of cyclicity after calving combined with low conception rates have contributed to extended calving intervals and increased empty rates causing major costs to the individual producers and the industry. Recently studies Lynch *et al.*, (2008) and Patton *et al.*, (2007) have recorded positive associations between plasma concentrations of metabolic hormones and metabolites during the early postpartum period on subsequent conception rate. The objective of this study was to examine the association between metabolic hormones and metabolites during the early postpartum period and the interval from calving to resumption of cyclicity and in turn the association between this interval and conception rate to first service.

Material and methods Fifty spring calved multiparous Holstein Friesian dairy cows from a single herd were used in the study. Blood samples were collected via the coccygeal vein once weekly for the first 4 weeks postpartum. Plasma was extracted and stored at -20°C until assayed. Total plasma IGF-1 was determined using a non extracted two site immunoradiometric assay. Insulin was measured by a solid phase time resolved fluoroimmunoassay. Plasma glucose, non-esterified fatty acids (NEFA), β -hydroxybutyrate (BHB) and urea was measured by enzymatic colorimetry using an ABX Mira auto analyser. Body condition score (BCS) was assessed on weeks 0, 4 and 6 postpartum using the 6 point scale (Lowman *et al.*, 1976). Commencement of luteal activity (CLA) was determined from milk progesterone samples which were collected three times per week commencing 8 to 11 days *post partum* and continued until 68 to 71 days *post partum*. Milk progesterone concentration was determined by enzyme immunoassay, using a commercial EIA kit. All cows were artificially inseminated at detected oestrus and all services were recorded. Pregnancy diagnosis was determined using ultrasound scanning initially once 30 days and again once 100 days post AI. Associations between metabolic hormones/metabolites and CLA was analysed using regression analysis (SAS, 2003), whilst associations between individual metabolic hormones and metabolites and conception rate to first service was analysed using logistic regression of (SAS, 2003).

Results Conception rate to first service was 49% with 17.7% of cows not pregnant at the end of the breeding season. There was evidence ($P=0.06$) for a negative relationship between CLA and first service conception rate, whereby a long CLA was associated with a reduced likelihood of conceiving to first service (odds ratio= 0.975, CI 0.949 – 1.001). There was no association between BCS at any of the time points measured or change in BCS and CLA or first service conception rate ($P<0.05$). CLA was associated with concentrations IGF-1 in weeks 2, 3 and 4 ($P<0.05$). CLA was not associated with any of the other hormones or metabolites measured. First service conception rate was positively associated with plasma concentrations of IGF-1 and urea on individual weeks as shown in Table 1.

Table 1 Associations between plasma concentrations of IGF-1 and urea and first service conception rate

Variable	Odds Ratio	95 % CI	P-value
IGF-1 week 2	1.015	0.999 — 1.031	$P=0.05$
IGF-1 week 3	1.018	1.002 — 1.034	$P=0.02$
IGF-1 week 4	1.014	0.998 — 1.031	$P=0.06$
Mean IGF-1 week 1 - 4	1.021	1.001 — 1.043	$P=0.03$
Urea week 3	2.822	1.099 — 7.243	$P=0.02$

There was no significant association between concentrations of IGF-1 on week 1, urea in weeks 1, 2, and 4 and first service conception rate ($P<0.05$). There was no association between plasma concentrations of insulin, glucose, NEFA or BHB on any of the weeks measured and first service conception rate ($P<0.05$). There was no association between BCS at any of the time points or the change in BCS between these and first service conception rate ($P<0.05$).

Conclusion This study has shown low concentrations of IGF-1 during the early postpartum period are associated with both delayed resumption of cyclicity and low conception rate in dairy cows. Conception rate was negatively associated with delayed resumption of cyclicity. The effects of IGF-1 on conception rate may at least in part be a mediated through its positive association with resumption luteal activity. The association of plasma urea with conception rate may be reflective of animals more rapidly mobilising body reserves to meet their energy demands. This study has shown that metabolic changes during the early postpartum period have direct effects on subsequent fertility.

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Effect of replacing barley grain with starch processing wastage on production performance in Holstein dairy cow

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Introduction Wheat flour is one of the main sources used for the production of starch. During the process of starch making, gluten and starch content of wheat flour are separated and the other nutrients including carbohydrate, protein, fat and minerals are waste which may cause environmental pollution. Starch processing waste was dried and mixed with wheat bran at a ratio of 40:60 by a commercial name of Powerfeed. Previous studies have shown the possibility of using Powerfeed in the diet of Holstein bulls (Pirzadeh Naeiny *et al.*, 2010a) and Shall lambs. (Pirzadeh Naeiny *et al.* 2010b). The aim of this experiment was to study the effects of Powerfeed when used as a replacement for barley grain in the diet on feed intake, milk yield and composition in lactating Holstein cows.

Material and methods Eighteen Holstein dairy cows (at 19 ± 2 weeks of lactation) were assigned into two groups (control and treatment) based on milk yield, calving date and parity. Both groups fed *ad libitum* a TMR diet containing (g/kg) of dried lucerne 150, maize silage 328 and concentrate 522 on a dry matter basis. The diet was based on NRC (2001) and offered in three equal portions daily immediately after milking. The concentrate contained (g/kg) ground barley 334, maize 98, wheat bran 169, soya bean meal 159, canola meal 166, whole cotton seed 41, and mineral/vitamin supplement 14, sodium bicarbonate, 9, limestone, 7 and salt 3. For treatment groups 20% of concentrate was replaced with Powerfeed (provided from Shahdineh Aran Co, Esfahan, Iran) at the expense of barley flour and salt was excluded. Cows were milked three times per day at 06.00, 14.00 and 22.00 h and milk volume was recorded at each milking time. Milk samples were collected from the morning milking for each cow once weekly for analysis of fat and protein. Chemical composition of diet ingredients was determined and feed intake was also measured for each group daily. Data were analysed by analysis of variance using Minitab.

Table 1 Chemical composition of diet ingredients and overall composition of concentrates

Ingredients	DM	g/kg dry matter		
	g/kg	CP	NDF	ADF
Lucerne hay	920	155	435	345
Corn silage	334	72	652	388
Barley grain	915	92	235	135
Corn grain	884	105	115	25
Canola meal	928	387	305	195
Whole cotton seed	910	225	392	294
Powerfeed	885	142	315	115
Soybean meal	885	437	120	80
Wheat bran	920	155	455	135
Control concentrate	913	210	252	127
Powerfeed concentrate	907	220	268	123

Table 2 Effects of Powerfeed on feed intake, milk yield, and composition in lactating Holstein cows.

	Powerfeed	Control	s.e.m	P	Sig.
DM intake (kg/d)	19.8	19.5	0.23	0.371	NS
Milk yield (kg/d)	25.8	26.1	1.22	0.847	NS
3.5% FCM (kg/d)	24.9	23.6	1.18	0.448	NS
Milk composition (g/kg)					
Fat	32.9	29.4	0.97	0.019	*
Protein	30.7	31.8	0.69	0.139	NS
Milk component yield (g/d)					
Fat	849	767	43.7	0.176	NS
Protein	792	830	42.1	0.574	NS

* $P < 0.05$ FCM = fat-corrected milk

Results Chemical composition of diet ingredients are shown in Table 1. Feed intake and milk production data are in Table 2. Dry matter intakes expressed as kilogram per day and milk yield were not affected by treatments but milk fat content in control group was significantly lower than those receiving Powerfeed. However, when milk yield expressed on 3.5% fat-corrected milk (FCM) the differences in this parameter was not significant. Milk protein was also unaffected by treatments. The yield of milk fat and protein between the groups were also not statistically significant.

Conclusions The results of this study indicate that the wastage of starch factory can be used in dairy cattle feed and nutritionally may be comparable with barley grain as a part of concentrate in the diet. This will result in a higher amount of milk fat without any effect on feed intake and milk yield in lactating Holstein cows.

Acknowledgement The financial support of Shahdineh Aran Co. and the help of the staff of Aminabad research institute of veterinary faculty are kindly acknowledged.

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The effect of silage and concentrate type on milk fatty acids and the occurrence of subacute ruminal acidosis in dairy cows

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Introduction Subacute ruminal acidosis (SARA) in dairy cows can be caused by feeding diets high in non-structural carbohydrates (NSC) and/or low in effective fibre (EF). Biomarkers diagnosing this metabolic disorder are of major interest. As diet changes the rumen fermentation and milk fatty acids reflect these changes, the aim of this study was to determine the effect of diets differing in NSC and EF on the milk fatty acid profile and the occurrence of SARA.

Material and methods Two Latin square experiments were conducted to evaluate the effect of 5 dietary treatments using 5 fistulated dairy cows during early, mid and late lactation. Each period lasted 3 wk, with the first 2 wk for adaptation to the diet. All dietary treatments consisted of TMR with 55% roughage and 45% concentrate. Roughage was either corn silage (CS), grass silage (GS) or a mixture of both (CSGS). Concentrates were either rich in structural carbohydrates (SC), rich in non-structural carbohydrates (NSC), mainly starch, or a mixture of both (SCNSC). Details on dietary treatments and sampling are described in Abrahamse *et al.* (2008). pH was measured manually in rumen samples collected relative to individual meals during 12 hrs/d using an electronic pH meter (pH electrode HI 1230, Hanna Instruments B.V., IJsselstein, the Netherlands). During the last 2 d of each measurement period, milk samples were taken during 4 consecutive milkings, stored and milk FA analysis was performed by GLC after extraction and methylation as described by Vlaeminck *et al.* (2005). All samples were injected on a GC CP-Sil88 column for FAME analyses. Statistical analyses were performed with SPSS 15.0 (SPSS 15.0, SPSS, Inc., Chicago, IL). Milk FA were compared using linear mixed models: $Y_{ijk} = \mu + A_i + B_j + C_k + D_l + AB_{ij} + AC_{jk} + BC_{ik} + \epsilon_{ijkl}$, with μ = mean, A_i = fixed effect of lactation stage, B_j = fixed effect of treatment group, C_k = fixed period effect within Latin square, D_l = random animal effect, AB_{ij} - AC_{jk} - BC_{ik} = the interaction terms and ϵ_{ijkl} = the residual error term. SARA cases are defined based on the threshold value time pH < 5.8 = 475 min/d (Alzahal *et al.*, 2007).

Results The CS/SCNSC compared to the GSCS/NSC treatment showed the longest time of rumen pH below 5.8 (5.9 vs 2.5 h/d) and 4 out of 5 acidotic cases occur during the period with the CS/SCNSC treatment (1 acidotic case with the CSGC/NSC treatment). The CS treatment has the lowest FA proportions for iso FA and highest of C15:0, C17:0 and C18:1 trans10+trans 11 (Table 1). These milk FA changes are related to SARA (Colman *et al.*, 2010).

Table 1 The effect of treatment and lactation stage (LS) on the time with a rumen pH < 5.8 (min/d) and the proportion in milk fat (g/100 g milk FA) of odd and branched chain FA and the sum of the predominant trans C18:1 FA

	Treatment					SEM	LS		SEM	P-value	
	GS	CSGS	CSGS	CSGS	CS		Early	Late		Treat	LS
Roughage											
Concentrate	SCNSC	SC	SCNSC	NSC	SCNSC						
Time pH <5.8	76.6 ^a	51.8 ^a	116.6 ^a	148.4 ^a	356.6 ^b	42.24	112.1	187.9	26.7	0.001	0.062
Anteiso C13:0	0.017 ^{bc}	0.017 ^c	0.015 ^{ab}	0.014 ^a	0.015 ^{abc}	0.001	0.014	0.017	0.001	0.032	0.001
Anteiso C15:0	0.482 ^a	0.493 ^a	0.475 ^{ab}	0.443 ^b	0.450 ^b	0.011	0.457	0.480	0.007	0.021	0.034
Iso C13:0	0.026 ^a	0.028 ^a	0.025 ^a	0.025 ^a	0.018 ^b	0.001	0.026	0.023	0.001	0.000	0.008
Iso C14:0	0.084 ^a	0.077 ^b	0.069 ^c	0.065 ^c	0.050 ^d	0.002	0.070	0.068	0.001	0.000	n.s.
Iso C15:0	0.234 ^a	0.229 ^a	0.211 ^b	0.202 ^b	0.155 ^c	0.005	0.213	0.199	0.003	0.000	0.008
C15:0	0.987 ^a	0.987 ^a	1.015 ^a	1.008 ^a	1.171 ^b	0.024	1.027	1.041	0.015	0.000	n.s.
C17:0	0.453 ^a	0.458 ^{ab}	0.478 ^{bc}	0.491 ^{cd}	0.510 ^d	0.008	0.504	0.452	0.005	0.001	0.000
C18:1 t10+t11	1.159 ^a	1.263 ^a	1.157 ^a	1.567 ^a	3.841 ^b	0.214	1.794	1.801	0.135	0.000	n.s.

Conclusion In the current study SARA mainly seemed to be associated with the exclusive supply of CS as roughage rather than with high amounts of NSC in the concentrate. Occurrence of SARA was associated with increases in milk fat of C15:0, C17:0 and C18:1trans10+11 and decreases of iso C14:0. This shows that milk FA have potential as a marker for SARA.

Acknowledgements PhD research of Ellen Colman is financed by the IWT-Institute and the Commission of the European Communities, FP7, KBB-2007-1 (Rednex). Bruno Vlaeminck is a Postdoctoral Fellow of the Fund for Scientific Research-Flanders (Belgium). The financial support of the Product Board Animal Feed and the Dutch Dairy Board is greatly acknowledged.

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Effect of dietary copper sulphate or organically complexed copper (Bioplex® Cu) fed either without or with dietary Cu antagonists on the intake, performance and mineral status of early lactation dairy cows

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Introduction Dietary copper (Cu) is an essential micro mineral for dairy cattle that is required in a number of enzymes, proteins and as a catalytic agent (McDowell, 1992). The presence of high levels of molybdenum (Mo) and sulphur (S) has the potential to reduce the availability of dietary Cu due to the formation in the rumen of thiomolybdates that bind available Cu (Suttle, 1991). If thiomolybdates are in excess of available Cu they may pass into the blood stream and also bind to Cu making it unavailable to the animal. The aim of this study was to examine the effect of Cu source and presence/absence of additional dietary Mo and S on performance and Cu status of lactating dairy cows.

Material and methods Fifty six Holstein-Friesian dairy cows that were approximately 35 d into lactation were used. The cows were blocked according to parity, milk yield and fat content, body condition score, days in milk and live weight and randomly allocated to one of four dietary treatments in a 2 x 2 factorial design examining the effect of Cu source and the presence/absence of Cu antagonists. The basal ration was predicted to contain 6.99 mg Cu/kg DM, 1.67 mg Mo/kg DM and 1.68 g S/kg DM. In order to reduce the availability of the dietary Cu by 50% (NRC, 2001) an antagonist mix was formulated containing sodium molybdate and ammonium sulphate in order to supply 7.5 mg Mo/kg DM and 3.15 g S/kg DM. Therefore, the four dietary treatments were: 10 mg Cu/kg DM as CuSO₄ fed either without (C-) or with (C+) the antagonist mix and 10 mg Cu/kg DM as organically complexed Cu (Bioplex®, Alltech UK) fed either without (B-) or with (B+) the antagonist mix. All cows received the same basal diet as a total mixed ration (TMR) that contained 0.61 kg/kg forage. The TMR was fed through computerised roughage bins that automatically recorded intake. The diets were mixed once daily and fed at 1.05 x previous recorded intake. Liver biopsy samples were taken from all cows during weeks 0 and 16 of the study and stored at -80°C prior to subsequent analysis. Blood samples were collected via jugular venepuncture during weeks 0, 1, 2, 4, 8, 12 and 16 and sub-samples of sera and plasma were stored at -20°C prior to subsequent analysis. Cows were milked twice daily at 06:00 and 16:00 h. Milk yield was automatically recorded at each milking with samples taken fortnightly for subsequent analysis of fat, protein and lactose. Cows remained on study for 16 weeks. Weekly samples of the TMR were stored at -20°C prior to subsequent analysis. Following nitric acid digestion the TMRs and liver biopsies were analysed for Cu and Mo using ICP-MS along with the plasma samples. Data were analysed as repeated measures ANOVA using Genstat with performance parameters recorded in the week prior to allocation as a covariate where appropriate. Liver minerals were analysed using ANOVA using week 0 as a covariate where appropriate. Significant differences were declared as $P \leq 0.05$.

Results There was an interaction ($P=0.025$) observed for DMI; feeding the antagonist decreased intake in cows receiving C but had no effect on those receiving B. Milk fat content was increased ($P=0.042$) in cows offered B diets. There were no ($P > 0.05$) other effects on animal performance. Feeding + diets resulted in a decreased ($P<0.001$) hepatic Cu concentration, an increased ($P=0.035$) hepatic Mo and an increased ($P<0.001$) plasma Mo concentration, but there was no effect ($P>0.05$) on plasma Cu concentrations.

Table 1 Effect of diet on performance, milk composition, hepatic minerals and blood mineral of cows

	Diet				s.e.d.	Significance (P)		
	C-	C+	B-	B+		Cu	Ant	Int
Intake, kg DM/d	22.6	20.8	21.0	21.4	0.76	0.271	0.134	0.025
Milk yield ¹ , kg/d	33.0	32.9	31.5	33.5	1.55	0.825	0.254	0.511
Milk fat, g/kg	35.8	35.5	36.9	38.7	1.46	0.042	0.481	0.342
Week 16 ² hepatic Cu, mg/kg DM	419	280	375	285	31.3	0.649	<0.001	0.261
Hepatic Cu change, mg/kg DM	+12	-100	-3	-78	35.5	0.894	<0.001	0.464
Week 16 ² hepatic Mo, mg/kg DM	2.9	2.9	2.9	3.1	0.12	0.791	0.035	0.837
Plasma Cu ² , µmol/l	13.5	12.9	13.2	12.8	0.46	0.526	0.143	0.965
Plasma Mo ² , µmol/l	0.16	0.36	0.16	0.36	0.017	0.600	<0.001	0.914

¹ adjusted to 38 g fat/kg, ² week 0 used as covariate (data not shown)

Conclusions Feeding cows diets containing Bioplex® Cu improved milk fat content (g/kg) but had no further effect on animal performance. Additional Mo and S did not affect performance, but hepatic Cu had been reduced through mobilisation over the 16 week study.

Acknowledgements This work was funded by Alltech (UK) Ltd.

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Effect of level of inclusion of copper sulphate and organically complexed copper (Bioplex® Cu) on indicators of Cu status, performance and milk fatty acid profile in dairy cows

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Introduction Dietary copper (Cu) is an essential micro mineral for dairy cattle that is utilised in enzymes, proteins and as a catalytic agent (McDowell, 1992). Dietary Cu has traditionally been supplied as Cu sulphate (CuSO₄) but Ward *et al.* (1996) reported that supplying Cu in an organic form increased Cu bioavailability. High dietary levels of Cu (40 mg Cu/kg DM) have also been shown to alter the milk fatty acid profile of milk, although the effects at lower levels is less clear (Engle *et al.*, 2001). The aim of this study was to examine the effect of replacement of CuSO₄ with an organic source (Bioplex® Cu, Alltech UK) on the indicators of Cu status, performance and milk fatty acid composition of lactating dairy cows.

Material and methods Fifty six Holstein-Friesian dairy cows that were post peak production were selected for this study. Cows were blocked according to parity, milk fat, milk yield, condition score, days in milk and live weight and randomly allocated to one of four dietary treatments: either no supplemental Cu (B-0), Bioplex® Cu supplying 5 (B-5) or 10 (B-10) mg Cu/kg DM or CuSO₄ supplying 10 (C-10) mg Cu/kg DM. All cows received a basal total mixed ration (TMR) that contained 0.57 kg/kg forage. The diets were fed through computerised roughage bins that automatically recorded intake and were mixed once daily and fed at 1.05 x previous recorded intake. The basal ration contained 5.66 mg Cu/kg DM, 1.47 mg Mo/kg DM and 2.59 g S/kg DM. Blood samples were collected via jugular venepuncture during weeks 0, 4, 8 and 12 and sub-samples of sera and plasma were stored at -20°C prior to subsequent analysis. Cows were milked twice daily at 06:00 and 16:00 h. Milk yield was automatically recorded at each milking with samples taken weekly for subsequent analysis of fat, protein and lactose. During week 12 an additional milk sample was collected for subsequent determination of milk fatty acids. Cows were weighed and condition scored weekly following the Wed pm milking and remained on study for 12 weeks. Weekly samples of the TMR were collected and stored at -20°C prior to subsequent analysis. Mineral concentration of the basal TMR and plasma samples were determined using ICP-MS. Serum samples were analysed for ceruloplasmin (Cp) by colourimetry using p-phenylenediamine oxidase as a substrate. Milk samples were analysed for fat, protein and lactose using FTIR. Milk fatty acids were determined by gas chromatography Data were analysed as repeated measures ANOVA using Genstat with performance parameters recorded in the week prior to allocation as a co-variate where appropriate. Milk fatty acids were analysed using ANOVA. Significant differences were declared as P≤0.05.

Results There was no effect of diet on intake, milk yield, milk composition or plasma mineral concentration. There was a tendency (P=0.087) for cows offered B-5 to have a higher Cp concentration compared to those offered C-10. However, cows offered B-10 had a higher (P<0.05) Cp:Cu ratio than those offered C-10. There was no effect (P>0.05) of dietary treatment on milk fatty acid concentration.

Table 1 Effect of diet on performance, milk composition and blood parameters of cows

	Diet				s.e.d.	P
	B-0	B-5	B-10	C-10		
Intake, kg DM/d	22.1	22.6	22.3	21.8	0.68	0.727
Milk yield, kg/d	36.4	36.1	36.0	35.6	1.45	0.954
Milk fat, g/kg	39.0	39.9	39.8	39.3	0.16	0.939
Milk protein, g/kg	30.8	30.8	32.0	31.3	0.80	0.407
Plasma Cu, mmol/l	12.6	12.8	13.0	12.5	0.50	0.751
Ceruloplasmin (Cp), mg/dl	17.4	18.3	17.0	15.4	1.14	0.087
Cp:plasma Cu ratio	1.37 ^{ab}	1.45 ^b	1.30 ^{ab}	1.23 ^a	0.073	0.022
C18:2 c9 t11, g/100 g fatty acids	0.83	0.77	0.77	0.79	0.033	0.319
C18:2 t10 c12, g/100 g fatty acids	0.30	0.27	0.30	0.29	0.016	0.356

Conclusions These results show that level and source of Cu had no effect on intake and performance of lactating dairy cows. The increased Cp:plasma Cu ratio observed in cows offered B-5 is indicative of increased Cu bioavailability. This suggests that a dietary supply of 11.4 mg Cu/kg DM is optimal in accordance with NRC (2001). At moderate levels of dietary Cu inclusion there is no effect on the milk fatty acid profile.

Acknowledgements This work was funded by Alltech (UK) Ltd.

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Calving ease and the subsequent occurrence of mastitis and lameness in dairy cows: retrospective analysis from a UK farm

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Introduction Mastitis and lameness are the two most costly diseases to the UK dairy industry. Following difficulty at calving, dairy cows have a depressed immune system and are at more risk of contracting diseases. Moreover, calving and the associated subsequent external stresses play a major role in the development of lameness. Therefore, it is possible that dystocial cows might be more vulnerable to mastitis and to developing lameness. The objective of the study was to determine if cows that experienced difficulty at calving have a higher prevalence of mastitis and lameness over their subsequent lactation.

Material and methods Calving ease scores and subsequent treatment records for mastitis and lameness across lactations of Holstein cattle were extracted from the SAC experimental farm database (UK) between 1990 and 2000 inclusive (Edinburgh herd, EDI, n=2430) and from 2003 to Sept 2009 (Crichton herd, CR, n=1413). Calving ease was scored as: no assistance (N), Farm assistance without/with malpresentation (FN/FM), Veterinarian assistance without/with malpresentation (VN/VM) and caesarean section (VC). Lameness was divided into two types depending if it was due to skin disease (SD lameness) or claw horn disease (CHD lameness). The occurrence of at least one episode (binary data) and the total number of episodes (count data) of mastitis, lameness, SD lameness and CHD lameness during the duration of the lactation was calculated. Similarly, occurrences of mastitis at up to 30DIM and all types of lameness at up to 120DIM were considered. All vet assisted scores were grouped together (V) for the purpose of the study. Only descriptive statistics were performed on the CR herd because of the limited size of this dataset. In the Edinburgh herd, prevalence of mastitis and lameness was analysed using generalized linear mixed models with a binomial distribution using a logit link function. The number of episodes were analysed using a Poisson distribution with a logarithm link function after having restricted appropriately to lactations with at least one episode of either mastitis or lameness.

Results The prevalence of mastitis, lameness, SD lameness and CHD lameness were 15.0, 35.1, 23.4, 22.2 in the EDI herd, and 20.7, 41.3, 24.9, 28.6% in the CR herd. In the EDI cows, there was no evidence that calving difficulty resulted in higher prevalence of mastitis at up to 30DIM and over the lactation (Figure 1, $P>0.05$). Similarly, there was no effect on lameness, SD lameness and CHD lameness at up to 120DIM (Figure 2, $P>0.05$) and over the lactating period ($P>0.05$). Descriptive results for lameness at up to 120DIM in the CR herd are shown in Figure 2. There was no evidence that dystocial cows had more episodes of mastitis at 30DIM and over the lactation, than cows who calved naturally ($P>0.05$). Similarly, there was no effect of dystocia on the number of episodes for lameness, SD lameness and CHD lameness at up to 120DIM and over the lactation.

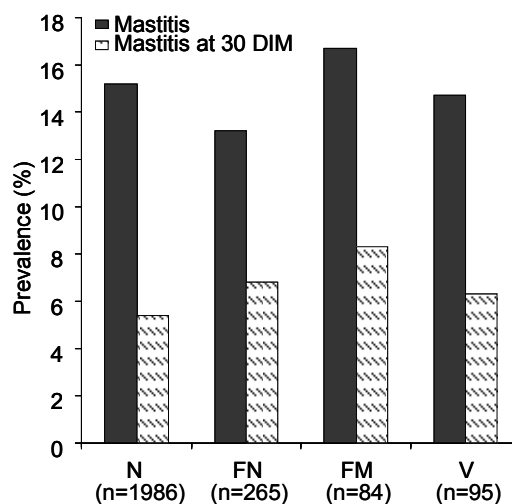


Figure 1 Calving difficulty and prevalence of mastitis (%). Raw data presented

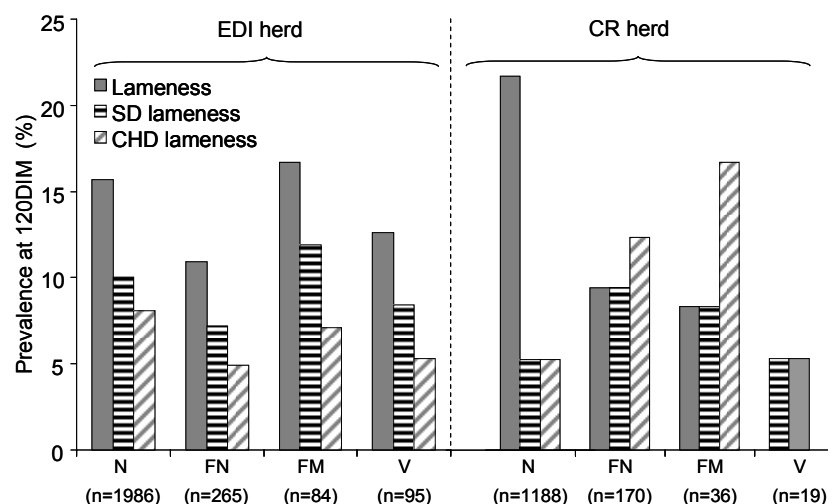


Figure 2 Calving difficulty and prevalence of lameness at up to 120 DIM for EDI and CR herd. Raw data presented

Conclusion In this study, there was no evidence that cows that had experienced a difficult calving had a higher prevalence of mastitis and lameness in their subsequent lactation. It could be that dystocial cows get culled early and therefore do not have the opportunity to express such health problems or that farm management compensated any effect. However, it was felt that the size of the EDI dataset limited the exploration of such relationships and that this topic would merit further investigation.

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

Use of farmer recorded lameness data to improve the genetic evaluation of lameness in UK dairy cattle

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Introduction Lameness is one of the most important causes of economic losses and can be a source of welfare problems, which the dairy industry is keen to address. The inclusion of health traits in UK selection programs has been limited due to a lack of reliable data on disease events. The UK national profit index (£PLI) currently utilises locomotion (LOC) and feet and leg (F&L) traits to select against lameness. The use of on-farm computer software and an upsurge in farm assurance schemes has brought greater focus on active monitoring and prevention of lameness at a herd level through the use of herd health plans which require record-keeping of lameness (Whay, 2002). Health data could then be made available to national databases and genetic evaluation centres by milk recording organisations (MROs). The objective of this study was to assess the suitability of farmer-recorded lameness events for UK dairy genetic evaluations and to estimate genetic parameters of lameness events and the relationship with LOC and F&L type traits.

Material and methods Lameness events were included that took place 0 to 305 days from calving. Lameness was treated as a binary trait (lame = 1, not lame = 0 within lactation). Lameness data on the first three lactations were used from Holstein Friesian dairy cattle in the UK. Edits included; 1) calving ages for first, second and third lactation were within the ranges of 18-42 months, 30-62 months, and 42 to 70 months, respectively; 2) sires were born from 1990 onwards; 3) sires had at least 10 eligible daughters, and of these up to the first 300 daughters born were selected; 4) at least three animals per herd-year; 5) at least one animal with a lameness record per herd-year; and 6) at least one percent of animals with a lameness record per herd-year. The final dataset consisted of 77,786 cows with 112,165 lactations for cows which calved in years 1995 to 2009, from 1,100 herds, and sired by 2,379 bulls. Overall 9.8% and 12.6% of lactations and animals had at least one lameness event recorded across the first three lactations, respectively. (Co)variance components and genetic parameters were estimated using ASReml (Gilmour *et al.*, 2006), with a sire model for both lameness and type traits. Fixed effects for lameness were month of calving, herd-year, heterosis, recombination, and age in months at calving as a covariate.

Results Recording of health events by farmers in the UK have rapidly increased for several diseases. Before edits, the number of herds recording lameness events rose from 192 herds in 1995 to 1,178 herds in 2008, which led to an increase in recorded lameness events from 651 to 17,762 in the same years. First recorded lameness events were highest at the start of lactation and were greatest in March and fewest in August. Lameness incidence increased with increasing parity. The heritability estimates for lameness were low, but significant ($p < 0.05$) and ranged from 0.01 to 0.03 for lactations one to three, increasing with lactation number. The heritability estimated from a repeatability model was 0.02. Genetic correlations between lameness with F&L and LOC were significant ($p < 0.05$) with values of -0.58 and -0.59 for lactation one and -0.48 as a repeated trait (Table 1).

Table 1 Variance components and heritability estimates of lameness and genetic correlations with F&L and LOC

Lactation	Count	Mean	σ_s^2	σ_e^2	h^2	σ_p^2	r_G with F&L	r_G with LOC
1	44150	0.09	0.0002	0.0735	0.013 (0.006)	0.074 (0.001)	-0.58 (0.205)	-0.59 (0.199)
2	38404	0.09	0.0004	0.0751	0.023 (0.008)	0.076 (0.001)		
3	29611	0.12	0.0007	0.0898	0.030 (0.010)	0.091 (0.001)		
Repeated	112165	0.10	0.0004	0.0726	0.019 (0.003)	0.080 (0.001)	-0.48 (0.145)	-0.48 (0.146)

σ_s^2 = sire genetic variance; σ_e^2 = error variance; h^2 = heritability; σ_p^2 = phenotypic variance; r_G = genetic correlation

Conclusions Using farmer-recorded lameness data for genetic evaluations appears promising, however standardised protocols for recording lameness would need to be established and farmers need to be further encouraged to record. At present, it is likely that data is not recorded absolutely consistently, therefore it is most appropriate to analyse data as simply as possible. However, with the improvement in future data recording, in terms of continuity, quantity and quality, alternative methods of analysis may be suitable. A trait that accounts for the duration of lameness or the number of lameness cases within lactations is likely to produce higher heritability estimates, since there should be more variation between animals. The heritability estimates of lameness were low, therefore genetic gain through selection upon lameness alone would be slow, yet still positive and cumulative. Although current selection for LOC and F&L are valuable in reducing lameness, direct selection for lameness in addition to current selection through type traits should aid response for lameness resistance.

Acknowledgements Many thanks to the farmers for recording the data in MRO's as well as funding from Defra under the Sustainable Livestock Production LINK Programme, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR.

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Estimated genetic parameters of calving performance in UK Holstein-Friesian cattle, using a multitrait animal model

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Introduction Calving traits are important to the dairy cattle industry being linked to impaired performance, loss of animals, compromised animal welfare and high additional costs. A genetic evaluation of calving ease (CE) and stillbirth (SB; defined as calf mortality at, or within 48 hours after, birth) is therefore essential to allow genetic selection on these traits. The indicator trait of gestation length (GL) is relevant to the dairy cattle industry and may improve predictive ability of the model when included (Norman *et al.* 2008). CE, SB and GL are affected by direct and maternal effects, leading to several statistical issues, in particular model choice and estimation of the direct-maternal genetic correlation. The objective of this study was to make inferences about direct and maternal heritabilities of GL, SB and CE at first and second parity, using an animal model, while taking statistical complications into consideration

Material and methods CE and SB data was provided by two milk recording organizations (MRO's) in the UK. GL was derived from insemination and calving dates and restricted to 265-295 days. Multiple births were deleted plus herds and sires with less than two observations. Calving date was checked for validity and age at calving was restricted to 18-48 months for heifers and 30-70 months for cows. Final first and later parity datasets consisted of 30,640 and 54,744 records originating from 2,098 and 2,050 herds, respectively. Scoring of CE differed between MRO's (Eaglen *et al.*, 2010). To harmonise both scoring scales, categories 2 and 3 of the MRO B scale were merged. CE scores were then transformed to average liability values within data source and parity. The frequency of stillborn calves in both datasets was 11.6% and 4.3% respectively. Linear univariate, bivariate and multivariate mixed models in ASREML were used for statistical analyses, building up to a full 12x12 variance covariance matrix. Fixed effects fitted were sex of the calf, age of dam (months), parity*age of dam (multiple parity), herd, data source, sire breed (only for GL) and year*month of calving. Random effects fitted were calf, dam and herd-year. To avoid potential bias in the estimate of the genetic direct maternal correlation due to the presence of an environmental dam-offspring covariance, individuals appearing both as calf and dam were deleted from the data (Eaglen *et al.*, 2009).

Results The estimated heritabilities for CE, SB and GL, (Table 1) agree with previously published estimates (Norman *et al.*, 2008, ; Wall *et al.*, 2010). Heritabilities differ between parities, being higher for heifers, again consistent with literature. Estimated SB heritabilities suggest that calf and dam have different contributions to stillbirth across parities. The estimated direct heritability in GL is larger than the maternal heritability, which relates to the onset of parturition by the fetus (Norman *et al.*, 2008). The direct-maternal genetic correlation within traits was only significant for GL, although it differed highly between parities. This difference will be enlightened by future bivariate analyses across parities, also expected to show if heifer calving traits differ to later parity calving traits. High positive correlations between direct and maternal SB and CE suggest a close genetic relationship between these traits. Direct GL was found to be genetically associated with maternal CE and SB.

Conclusions

Estimated heritabilities of CE, SB, and GL in UK Holstein heifers are consistent with literature, including UK data (Wall *et al.*, 2010). Direct-maternal genetic correlations within calving traits were not significantly different from zero. Heritabilities for GL were high and genetic relationships between calving traits and GL were found. Improvement of the model by inclusion of GL therefore seems plausible. Future analyses, filling Table 1, will give insight into the genetic relations between the traits across parities.

Estimated heritabilities of CE, SB, and GL in UK Holstein heifers are consistent with literature, including UK data (Wall *et al.*, 2010). Direct-maternal genetic correlations within calving traits were not significantly different from zero. Heritabilities for GL were high and genetic relationships between calving traits and GL were found. Improvement of the model by inclusion of GL therefore seems plausible. Future analyses, filling Table 1, will give insight into the genetic relations between the traits across parities.

Table 1 Genetic parameters of calving ease and fertility traits. The '1' and '2+' represent first and later parities respectively, dir and mat stand for direct and maternal effects. Heritabilities on the diagonal and genetic correlations on off diagonal.

		CE 1		SB 1		GL 1		CE 2+		SB 2+		GL 2+	
		Dir	Mat	Dir	Mat	Dir	Mat	Dir	Mat	Dir	Mat	Dir	Mat
CE 1	Dir	0.12*											
	Mat	0.30	0.04*										
SB 1	Dir	0.84*	-	0.02									
	Mat	0.28	0.85*	0.55	0.03*								
GL 1	Dir	0.19	0.27*	0.08	0.40*	0.45*							
	Mat	0.20	0.15	-	0.03	0.77*	0.013*						
CE 2+	Dir							0.03*					
	Mat							-0.25	0.02*				
SB 2+	Dir									0.007*			
	Mat									0.00	0.004		
GL 2+	Dir											0.41*	
	Mat											-0.16*	0.06*

*P<0.05

Acknowledgements The authors gratefully acknowledge funding from Defra under the Sustainable Livestock Production LINK Programme, the Scottish Government, CIS, Cogent, DairyCo, Genus, Holstein UK and NMR

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The genetics of wool shedding in a composite breed of sheep

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Introduction In many countries the income from wool is a minor part of returns from a sheep flock, and in some instances shearing has been shown to have a negative effect on farm profitability (Vipond, 2008). As part of a two-part process to increase profitability many breeders are concentrating on reducing costs as well as increasing output. Developing a type of sheep which requires no shearing contributes to the first part of this process. Historically, primitive sheep shed their wool annually in the spring but one effect of domestication has been to breed a type of sheep which keeps its fleece unmoulted (Ryder, 1983). A number of breeds are known to possess the ability to shed wool, over and above the response found in most sheep to lose their fleece in times of nutritional stress, poor health and other environmental influences. This paper reports a genetic analysis of one sheep breeding group in the UK which set out to breed a type of sheep that sheds its wool and proposes a mode of inheritance for wool shedding in these sheep.

Material and methods A number of 'modern' sheep breeds are known to naturally shed their wool e.g. Wiltshire Horn, Dorper, Katahdin. New or composite breeds such as the Easycare have also been bred to have wool shedding characteristics. Crosses involving these breeds with non-shedding breeds were investigated in a flock developing a new composite and two key characteristics relating to wool shedding were analysed; the ability to shed and the speed/extent of shedding through the summer. Wool shedding was scored by a single observer on a 1 to 5 scale where 1 was no evidence of shedding and 5 was complete shedding; intermediate scores largely related to different proportions of the fleece shed. Initial matings involved using shedding rams (Easycare, Dorper, Wiltshire Horn and Katahdin) with non-shedding ewes (Friesland, Lley, Suffolk, Texel). Scoring occurred in May/June for adult animals and in August/September for lambs. First-cross ewes (F₁) were backcrossed to the shedding ram breeds and the resulting ewes (BC₁) further backcrossed to shedding rams (BC₂). Shedding status was defined for each recorded animal as follows: shedder – at least one recorded instance of shedding (score >1); non-shedder – no recorded instance of shedding as an adult animal; unknown status – animal not shedding as a lamb but not having a chance to express its shedding status as an adult (e.g. castrates). The initial analyses of wool shedding concentrated on defining the mode of inheritance of the 'ability to shed' (shedders v non-shedders). The four common modes of Mendelian inheritance (all combinations of autosomal and sex-linked with dominant and recessive) were fitted to the F₁ and BC₁ data using Fisher's exact test in SAS (2009). The speed/extent of shedding was considered a polygenic trait within the shedding animals. Mixed-model analyses of shedding score were undertaken on these animals using ASReml (Gilmour *et al.*, 2009) with an animal model. Two datasets were analysed; all wool scoring records of lambs (lamb analysis) and records from all animals scored as adults (adult analysis). In these analyses the fixed effects of age at scoring (d; lamb analysis, effect fitted as a 2nd-order polynomial; y for adult analysis), year of record, sex and birth type were fitted as well as the additive genetic effect. The effect of breed/cross was investigated by running both models with and without this effect included in the model.

Results A total of 2,527 wool scores were available from 1,467 animals recorded between 2007 and 2010; of these 261 failed to shed as a lamb (18%) and 15 were classed as non-shedders. Analysis of the F₁ data (261:13, shedders : non-shedders) eliminated sex-linked dominant, sex-linked recessive and autosomal recessive as the mode of inheritance for the ability to shed ($P < 0.001$) and the most likely mode was autosomal dominant. This was confirmed in the BC₁ data (683:1, shedders : non-shedders; $P > 0.05$). The quantitative genetic analysis of speed/extent of shedding amongst shedders was based on a pedigree file of 2,018 animals. In the lamb analysis all fitted effects were significant ($P < 0.01$) with females having a higher score than males (2.08 v 1.73), singles > twins/triplets (2.12 v 1.70), and older animals > younger animals. The heritability of wool scoring was 0.44±0.079 when breed type was fitted in the model and 0.55±0.070 when it was excluded. From the adult analysis the heritability of wool scoring was 0.09±0.052 when breed type was fitted and 0.22±0.055 when it was excluded from the model. Once again all fitted fixed effects were significant ($P < 0.05$) with similar differences within effect as for the lamb analyses.

Conclusions Wool shedding in sheep from 4 known wool-shedding breeds appears to be under the control of a dominant gene. Within the wool shedding population there is genetic variation for the speed/extent of wool shedding which is greater as a lamb than as an adult. However, not all shedders express the trait as a lamb and so a genetic test for carrier status may be desirable. A selection programme to increase the speed of shedding would be possible given the heritability of the trait.

Acknowledgements Mr. Peter Baber and the Sheep Improved Genetics group for access to their records and unstinting cooperation in supplying both ideas and data for these analyses. Bioscience KTN for a SPARK award to fund the data collection and analyses.

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Known mutations with large effects on ovulation rate not involved in the prolificacy of Finnish Landrace sheep

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Introduction Mutations with a major effect on ovulation rate (OR) have been invoked to explain the exceptional prolificacy observed in many sheep populations and in some of these cases the causative mutations have been identified (Table 1). The Finnish Landrace is a well known high prolificacy sheep breed and the heritability of OR in the breed has been shown to be of the order of 0.5 (Hanrahan, 1987). While Finnsheep have been used in many countries to increase fecundity of local breeds (Maijala, 1996) no evidence has been adduced to suggest a role for mutations with a large effect on ovulation rate in the exceptional prolificacy of Finnsheep. The objective of this study was to ascertain if any of the seven established mutations with large effects on ovulation rate in sheep are responsible for the high prolificacy of the Finnish Landrace breed using material from lines developed by divergent selection on ovulation rate.

Material and Methods Three lines of the Finnish Landrace sheep were developed over the period 1976 to 1997 by divergent selection on ovulation rate at 18 months of age (Hanrahan, 2002). The mean (s.e.) ovulation rate at 18 months of age was 4.6 (0.05), 2.1 (0.06) and 2.6 (0.05) for High, Low and Control lines, respectively, for animals born in 1994 to 1997. DNA, extracted from the whole blood samples using a modified detergent based method (Hanrahan *et al.*, 2004), was available for animals born in 1994 and 1995 and represented 37 High, 38 Low and 49 Control females. Genotyping for six of the seven mutations (*FecX^G*, *FecX^B*, *FecG^H*, *FecB*, *FecX^L* and *FecX^H*) was initially carried out via PCR-RFLP analysis (Galloway *et al.*, 2000, Hanrahan *et al.*, 2004, Davis, 2005) and confirmed using the Sequenom MassArray® iPLEX Gold assay. The remaining mutation (*FecX^L*) was genotyped using the Sequenom MassArray® iPLEX Gold assay.

Results None of the seven mutations listed in Table 1 was detected in the set of sheep tested.

Table 1 Known mutations with large effects on ovulation rate in sheep

Gene	Breeds involved	Alleles	Chromosome	Reference
<i>BMP15</i>	Romney, Lacaune, Cambridge, Belclare	<i>FecX^L</i> , <i>FecX^H</i> , <i>FecX^L</i> , <i>FecX^G</i> , <i>FecX^B</i>	X	(Galloway <i>et al.</i> , 2000, Hanrahan <i>et al.</i> , 2004, Bodin <i>et al.</i> , 2007)
<i>GDF9</i>	Cambridge, Belclare	<i>FecG^H</i>	5	(Hanrahan <i>et al.</i> , 2004)
<i>BMPR-1B</i>	Merino	<i>FecB^B</i>	6	(Wilson <i>et al.</i> , 2001)

Conclusion The large divergence in OR generated between the High and Low lines by selection was not due to any of the known mutations listed in Table 1. Thus none of these mutations can account for the exceptional prolificacy of Finnsheep. The genes contributing to the response to selection are most likely not involved in the *BMP15/GDF9* pathways due to the crucial role these growth factors play in normal reproductive development. Although the possibility exists that unknown mutations with large effects on OR are segregating in Finnsheep statistical analysis of the variation within the lines used in the present study did not yield any evidence that a single gene with large effect was segregating but rather was consistent with cumulative effects of many genes (Hanrahan, 2002). With the advent of dense genome-wide SNP markers, the ovine 50K SNP chip (and bovine 777K HD SNP chip), and the rapidly developing next generation sequencing technology, identifying genomic loci directly affecting complex production traits in ruminants including the prolificacy of the Finn breed is becoming increasingly feasible.

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Estimation of (Co)variance components for economical traits in Moghani sheep

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Introduction Lamb weight is an important component of marketing in lamb production. Growth, carcass and some other traits are not only influenced by the direct genetic composition of the individual and the environment under which the animal is raised, but also were affected by the maternal genetic composition and maternal environment. When maternal genetic effects are important, but not accounted for in the evaluation, heritability estimates are biased upwards and realised selection-efficiency would reduce. Development of effective genetic evaluation and improvement programmes requires knowledge of the genetic parameters for these economically important production traits. The aim of this study was estimation of (co)variance components for some growth traits in Moghani sheep.

Material and methods Pedigree of 4876 Moghani lambs from 242 sires and 1394 dams were used in this study. Data were collected from year 2000 to 2010 at Jafar Abad sheep breeding station in Ardebil province of Iran. Five traits were considered in the analyses, i.e. Birth weight (BW); Weaning weight (WW); 6-month weight (W6); 9-month weight (W9) and Yearling weight (YW). The data analysed by REML, and Animal model was used in eight models of DFREML for the estimations. The best model was chosen based on the Log Likelihood ratio test. The following model (Maria *et al.*, 1993) was fitted to the data:

$$Y = Xb + Z_a a + Z_m m + Z_p pe + e$$

Where Y is vector of observation for traits; b, vector of fixed effects; a, vector of direct random effects; m, vector of maternal random effects; pe, vector of permanent environmental effects; e, vector of random residual effects; X, Z_a, Z_m and Z_p are incidence matrices for fixed, direct, maternal and permanent environmental effects, respectively.

Results The result of the univariate analysis under eight models is shown in table 1. The direct heritability estimates of BW and WW were similar to the estimates reported for other sheep breeds. Regarding to the direct heritability values for weights after weaning, the estimates obtained for W6, W9 and W12 were also similar to ones reported for other sheep breeds. Direct heritability values for body weights showed a tendency to increase with age. This tendency has also been reported for other sheep breeds (Safari *et al.*, 2005; Snyman *et al.*, 1995).

Table 1 Estimates of (co)variance components, genetic and phenotypic parameters for growth traits.

Traits	δ_a^2	δ_m^2	δ_{pe}^2	δ_p^2	δ_e^2	δ_{am}	$h^2(\pm SE)$	$m^2(\pm SE)$	$pe^2(\pm SE)$	r_{am}
BW	0.12	0.09	0.05	0.5	0.23	-0.1	0.24(±0.01)	0.18(±0.02)	0.1 (±0.01)	-0.97
WW	4.49	3.64	1.92	21.4	11.5	-4.46	0.21(±0.02)	0.17(±0.01)	0.09(±0.03)	-0.95
W6	7.17	3.86	2.2	27.6	15.4	-4.48	0.26(±0.07)	0.14(±0.01)	0.08(±0.02)	-0.9
W9	4.12	1.14	0.57	14.2	8.33	-3.73	0.29(±0.07)	0.08(±0.02)	0.04(±0.01)	-0.88
YW	4.14	0.45	0.3	14.8	9.85	-1.09	0.28(±0.1)	0.03(±0.005)	0.02(±0.008)	-0.8

Discussion Genetic progress is achievable for growth traits in Moghani sheep breed. However, the inclusion of any selection programme would need to take into account the maternal and genetic correlations between growth traits especially for preweaning traits. This study showed how the maternal is affecting the growth traits, and therefore must be considered in any genetic evaluation and selection programme for these traits. The direct and maternal heritability estimates obtained in this study indicate that it would be possible to improve growth traits through genetic selection at any of these ages.

Acknowledgements The authors thank Moghani Sheep Breeding Station for providing the pedigree data and records.

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Genetic and environmental parameters for ewe productivity in Moghani sheep

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Introduction Development of effective genetic evaluation and genetic improvement programmes require knowledge of the genetic parameters of economically important traits (safari *et al.*, 2005). The heritability of a trait indicates whether there is the possibility of obtaining genetic gain through its selection (Lobo *et al.*, 2009). The objective of this study was to estimate heritability, repeatability and genetic correlations of reproductive traits in *Moghani* sheep.

Material and methods Data and pedigree information used in this study collected from 1994 to 2008 in breeding station of *Moghani* sheep, located in Jafarabad of Iran. Studied traits are included age at first lambing (AFL), total number of lambs born per season (TLB), total number of lambs weaned (TLW), total birth weight of lamb (TWB) and total weight of lambs weaned (TWW) per ewe. Genetic parameters and correlations were estimated by REML procedure using the DFREML program (Meyer, 2000) with 1761 records of ewes for age at first lambing, 4967 records of ewes for number and weight of born lambs and 4695 records of number and weight of weaned lambs. AFL was fitted by an animal model with direct genetic effect. For number and weight of born and weaned lambs, repeatability models were fitted. The fixed effects considered in the analytical model after testing of their significance, included year and season of lambing and age of dam. Fitting a multi-trait animal model as follows: $y_i = X_i b_i + Z_i a_i + W_i p e_i + e_i$ where, y_i , b_i , a_i , $p e_i$ and e_i are the vector of observations, fixed effects, direct additive genetic effects, permanent environment effects and residual effects, respectively. Incidences matrices X, Z and W relating the observations of the i^{th} trait of the respective fixed effects, additive genetic effects and permanent environment effects, respectively.

Results Age of dam, year and season of lambing had significant effect on all studied traits ($p < 0.01$) except for age at first lambing. The estimates of (co)variance components, heritability (h^2), fraction of variance due to permanent environmental effect (pe^2) and repeatability (r) for considered traits are shown in Table 1. Estimates of genetic and phenotypic correlations among reproductive traits are shown in Table 2. Estimates of heritability for AFL, TLB, TLW, TWB and TWW were 0.344, 0.038, 0.051, 0.066 and 0.056, respectively; and estimates of repeatability for these traits except AFL were 0.133, 0.132, 0.134 and 0.085, respectively.

Table 1 Estimates of (co)variance components, heritability, fraction of variance due to permanent environmental effect and repeatability for AFL, TLB, TLW, TWB and TWW.

Traits	σ_a^2	σ_{pe}^2	σ_e^2	σ_p^2	$h^2 \pm S.E.$	$pe^2 \pm S.E.$	r
AFL	0.242	-	0.461	0.702	0.344(0.050)	-	-
TLB	0.007	0.017	0.154	0.178	0.038(0.016)	0.095(0.019)	0.133
TLW	0.009	0.015	0.159	0.184	0.051(0.018)	0.081(0.021)	0.132
TWB	0.051	0.053	0.672	0.776	0.066(0.021)	0.068(0.023)	0.134
TWW	2.172	1.132	35.548	38.852	0.056(0.021)	0.029(0.021)	0.085

Table 2 Estimates of genetic (above diagonal) and Phenotypic (below diagonal) correlations among reproductive traits

Traits	AFL	TLB	TLW	TWB	TWW
AFL	-	0.16	0.33	0.34	-0.01
TLB	0.08	-	0.98	0.82	0.93
TLW	0.05	0.29	-	0.96	0.95
TWB	0.09	0.28	0.30	-	0.61
TWW	0.04	0.23	0.21	0.11	-

Conclusions The low estimates of heritability and repeatability of ewe production traits obtained in the current study indicate that selection based on the ewe's own performance may result in slow genetic improvement. Therefore, the improvement of non-genetic factors such as nutrition levels can lead to the improvement for these characteristics. The high genetic correlation between traits showing that selection for each of these traits due to high correlated response is desired.

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Estimation of variance components for reproductive traits of Zandi sheep

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Introduction The main aim of sheep breeding is meat production in Iran. Genetic improvement in reproduction and growth traits is major goals in sheep breeding. Development of effective genetic evaluation and improvement programmes require knowledge of the genetic parameters for these economically important traits. Zandi sheep are a dual purpose (meat and wool) breed in the central of Iran. In sheep production, reproductive traits such as fertility, litter size and lamb survival are undoubtedly the most important traits in all systems of sheep production and in all environments (Matika *et al.* 2003). The objective of this study was to estimate heritability of reproductive traits for Zandi sheep, which are necessary to develop efficient selection programmes for improvement of reproduction.

Material and methods The data used in the study, were collected at Zandi sheep Breeding station in Khojir on Tehran province of Iran from 1991-2007. Genetic parameter estimates for reproductive traits in Zandi sheep were estimated using reproductive records of 1617 ewes. Investigated traits were litter size (LS), litter mean weight per lamb born (LMWLB) and litter mean weight per lamb weaned (LMWLW) as basic traits, total litter weight at birth (TLWB) and total litter weight at weaning (TLWW) as composite traits. Preliminary least squares analyses were performed for the traits applying the general linear model (GLM) procedure of SAS software package (SAS, 2004) to determine fixed effects to be included in the final models. The model accounting for fixed effects included lambing year and ewe age at lambing. The adjustment factors for the effect of sex on birth and weaning weight of lambs were determined using least squares analysis, then records of birth and weaning weight of lambs were adjusted accordingly. Mixed model methodology was used to analyze all traits using a multiple-trait animal model with repeated records. The (co)variance components and corresponding genetic parameters for the studied traits were estimated by restricted maximum likelihood (REML) method, using ASREML software (Gilmour *et al.* 1999).

Result All the studied traits were significantly affected by lambing year and ewe age ($P < 0.05$, $P < 0.01$). Estimates of direct and maternal heritability, direct-maternal genetic correlation and fraction of variance due to permanent environmental for each trait are shown in Table 1. The low estimates of heritability for traits may be due to the importance of random environmental effects on variability of the observations and due to the categorical expression of the some traits. Results showed that all traits were influenced by genetic effects and permanent environmental effects, and to improve these traits one should improve environmental effects in first step. Estimates of genetic variances and heritability are necessary for genetic evaluation of sheep and also for choosing the best selection scheme. The present study estimates are within the range of literature (Safari *et al.* 2005). For LS, LMWLB and LMWLW traits, estimates of maternal heritability were lower than the estimates of direct heritability. Because of positive genetic correlation between direct and maternal effects for LS, LMWLB and LMWLW methods of selection accounting for both direct and maternal genetic effects would result in greater economic selection response than selection based only on direct genetic effect. The results suggested that selection based on TLWW could be more effective than the other traits on improvement of reproductive performance in Zandi ewes.

Table 1 Estimates of (co)variance components, genetic and phenotypic parameters of reproductive traits

Traits	Mean±SD	h^2_d ±SE	m^2 ±SE	pe^2 ±SE	ram
LS	1.10±0.33	0.05±0.02	0.042±0.01	0.23±0.06	0.29
CR	0.91±0.28	0.05±0.01	-	0.06±0.02	-
LMWLB	3.13±0.95	0.13±0.02	0.05±0.02	0.04±0.02	0.33
LMWLW	19.52±2.33	0.08±0.02	0.06±0.01	0.05±0.02	0.20
TLWB	3.82±1.48	0.11±0.02	-	0.10±0.02	-
TLWW	21.34±4.35	0.10±0.02	-	0.10±0.02	-

Conclusion The low estimates for heritabilities of reproductive traits obtained in this research imply that selection based on these traits may result in slow genetic improvement in reproduction efficiency in Zandi sheep. Therefore, improvement of non-genetic factors in the flocks such as ewe nutrition before mating and late pregnancy can lead to the improvement of these characteristics.

Acknowledgements The authors gratefully acknowledge funding from Zandi Sheep Breeding Station in Tehran Province of Iran.

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Statistical comparison of partial regression coefficients of weaning weight on birth weight and weaning age in Iranian Holstein calves

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Introduction Growth traits such as birth weight (BW) and weaning weight (WW) and age at weaning (WAGE) in dairy cattle had not been studied so extensively. It is reported that high birth weight of calf increased the incidence of dystocia and stillbirth and decreased reproduction performance of dam. Final body weight at weaning was the best predictor for growth of heart. Lallès *et al.* (2007) reported that having higher weaning weight is an indicator of more developed rumen and therefore more dry matter intake after weaning. Perfectly perform immune system and high strength for disease resistance and the stress of weaning are other benefits of having higher weight at weaning. Burns *et al.* (2010) documented that a female calf that has higher weaning weight reaches the puberty sooner than her herd mates and consequently would be ready to conceive sooner. Birth and weaning weight are expected to vary under different environments and understanding of the environmental factors affecting these traits would be beneficial when predicting equation has to be developed. The main objective of this study was to statistically compare partial regression coefficients of weaning weight on birth weight and weaning age in Iranian Holstein calves.

Material and methods In this study, a total of 3088 weaning weight records of Holstein calves born from 2001 to 2009 were used. The data was obtained from a large size Holstein herd, Mashhad, Iran. Initially, the data were edited for any inconsistent weight and date of birth. Primary analysis on the data indicated that weaning weight was not statistically affected by dam's parity number and twinning. Finally, a statistical model was used in which fixed effects of year and month of birth as well as covariables of birth weight (BW) and weaning age (WAGE) were included. The model was fit by Mixed Procedure of SAS software version 9.1 and statistical comparisons between estimated partial regression coefficients were undertaken by the contrast statement. The model was as follows:

$$WW_{ijk} = \mu + BY_i + BM_j + b_1 * BW_{ijk} + b_2 * WAGE_{ijk} + e_{ijk}$$

in which WW=weaning weight, BY=calf's birth year, BM=calf's birth month, b_1 = regression coefficient of WW on BW, b_2 = regression coefficient of WW on WAGE.

Results Calf's birth year and month had significant effect on weaning weight ($P < 0.01$). Also the effect of male and female calves' birth weight and weaning age on their weaning weight were significant ($P < 0.01$). Regression coefficient for weaning weight of male and female calves on their birth weight was 0.957 ± 0.0356 and 0.963 ± 0.0381 , respectively. This means that by increasing one kilogram in birth weight, male and female calves weaning weight are expected to increase by 957g and 963g, respectively. Nonetheless, statistical comparison showed that no significant difference was detected between those regression coefficients. Regression coefficients for weaning weight of male and female calves on their weaning age were 0.536 ± 0.0249 and 0.501 ± 0.0223 , respectively meaning that by increasing one day in weaning age, male and female calves weaning weight are expected to increase 536g and 501g, respectively. Similar to birth weight, no statistically significant difference was found between regression coefficients of male and female calves.

Table 1 Partial regression coefficient estimates of WW on BW and WAGE for male and female Holstein calves

covariate	parameter estimate	standard error	95% confidence interval	Pr > t
BW(male)	0.957	0.0356	(-0.886,1.026)	< 0.0001
BW(female)	0.963	0.0381	(-0.887,1.037)	< 0.0001
WAGE(male)	0.536	0.0249	(-0.487,0.584)	< 0.0001
WAGE(female)	0.501	0.0223	(-0.456,0.544)	< 0.0001

Conclusions The results of this study showed although weaning weight of calf was highly influenced by birth weight and weaning age, no statistical differences were found between male and female calves regarding partial regression coefficients suggesting that as predicting equations are to be applied no separate models is needed to be applied for individual sex.

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Gibbs sampling optimization in Bayesian estimation of genetic parameters for some production traits of Iranian Holstein dairy cattle

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Introduction The statistical methods that are used in various fields of science have been considerably improved. One of the major objectives of these methods is to increase the quality of prediction using different data sets. Obviously, proper use of these methods to resolve a lot of uncertainty and predicting outcomes are essential, so details of these methods must be carefully determined. The Bayesian method is one of these methods. Gibbs sampling is practical numerical algorithm of Bayesian method (Geman and Geman, 2001). The aim of the present study was determine the parameters of Gibbs sampling to estimate genetic parameters for mature equivalent milk production and milk yield of 305 days lactation traits of Iranian Holstein cattle by using Bayesian method.

Material and methods The Data set that was used in this research was included records of 122679 dairy cows for first lactation. Records were collected from 2002 to 2008 by Animal Breeding Centre of Iran. Studied traits were 305 days milk yield (MY) and mature equivalent milk yield (ME). Total numbers of animals in pedigree was 197103 (Table 1). Genetic and residual variances were estimated with Bayesian method based on Gibbs sampling technique by various parameters under a single trait animal model. The statistical model of the analyses included herd-year-season as fixed effect (11869 levels); so that each level of this effect had at least five animals, age at calving as covariate with minimum and maximum of 22 and 34 month and animals as random effect. Data editing were done by using SAS and FoxPro software. For comparing the effect of different parameters of Gibbs sampling method on genetic parameter estimation, different values for each one were used. Therefore, for each of the three Gibbs sampling parameters three levels was considered that including 200000, 150000 and 100000 for total cycle, 30000, 10000 and 1000 for burn-in (period that sampled values were discarded) and 100, 150 and 200 for the thinning intervals (interval between actually used sampled values). MTGSAM (Van Tassel and Van Vleck, 1995) software was used to Bayesian estimation of parameters.

Table 1 Summary of the milk production traits

Traits	Mean	Maximum	Minimum	C.V. (%)
MY(kg)	7341.63	11721.00	2836.00	20
ME(kg)	8455.70	13438.09	3314.50	20

Results and discussion The estimated heritabilities for MY and ME were equal in different running of Gibbs sampling with different parameters (0.31 for both traits). With fixed value for total cycles and burn-in period, there was no any change in variance component estimation by different values for thinning parameter (Table 2). Generally in fixed values for total cycles, different values for the burn-in and thinning parameters had very little effect on the estimated variance components, but by fixing thinning interval and burn-in period parameters and changing total cycles the values of variance components were changed. It should be noted that different values of thinning interval had not effect on variance component estimation.

Table 2 Variance components estimation with different values of Gibbs sampling parameters for MY and ME traits

Total cycle 200000	MY			ME			
	Burn-in period	30000	10000	1000	30000	10000	1000
σ_a^2		403547	403403	403420	533150	532956	532980
σ_e^2		926163	926267	926261	1230143	1230284	1230274
Total cycle 150000							
σ_a^2		403154	403015	403016	532629	532440	532503
σ_e^2		926447	926548	926523	1230519	1230657	1230621
Total cycle 100000							
σ_a^2		402207	402201	402344	531371	531357	531551
σ_e^2		927110	927120	927030	1231401	1231418	1231296

Conclusion These results generally showed that the Gibbs sampling's parameters in defined ranges of in this research for Bayesian estimation of genetic parameters of MY and ME traits of Iranian Holstein dairy cattle had very little effect on variance component estimation. Therefore, by considering computational costs in Gibbs sampling, variance components for first lactation's data could be estimated by the analyse that needs lower computation.

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Estimates of genetic trend for reproductive traits in Iranian Holstein dairy cows

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Introduction The effectiveness of any animal breeding program is measured by the genetic progress obtained. Estimating genetic and environmental trends in a population allows the assessment of the effectiveness of the selection procedure and gives the opportunity for monitoring management conditions. It also supplies the animal breeder with essential information to develop more successful programs in the future.

Material and Methods Data from 15 Iranian Holstein dairy farms were used to estimate the genetic parameters and trends for reproduction traits. The data was included 72124 reproductive records from 1981 to 2007, over the first six parities. The relationship matrix was composed of 32447 animals. Reproductive traits in later parities were treated as repeated measurement. The reproduction traits investigated were days from calving to first service (DFS), number of insemination per service (INS), days open (DO), interval between first and last insemination (IFL). The components of (co)variance and breeding values of individuals were obtained by the restricted maximum likelihood method applied to animal models using ASREML. The average genetic trend was estimated by regression coefficients, whose importance were determined by the GLM procedure of the SAS software (SAS Institute, 2004), which was significant for all evaluated traits.

Results Heritability estimated for reproductive traits were 0.046 for INS, 0.074 for DO, 0.058 for DFS and 0.044 for IFL. Estimated genetic correlations ranged from 0.1 to 0.94. Genetic correlation of DO with other traits were higher than 0.72. The estimates of genetic trend were obtained by average regression of breeding value on year, and the average genetic trend was estimated by regression coefficients. Genetic trends were -0.00322, -0.4795 day, -0.1373 day and -0.1752 day per year and determination coefficients (R^2) were 0.4556, 0.7162, 0.5569 and 0.5158 for INS, DO, DFS and IFL respectively (Figure 1).

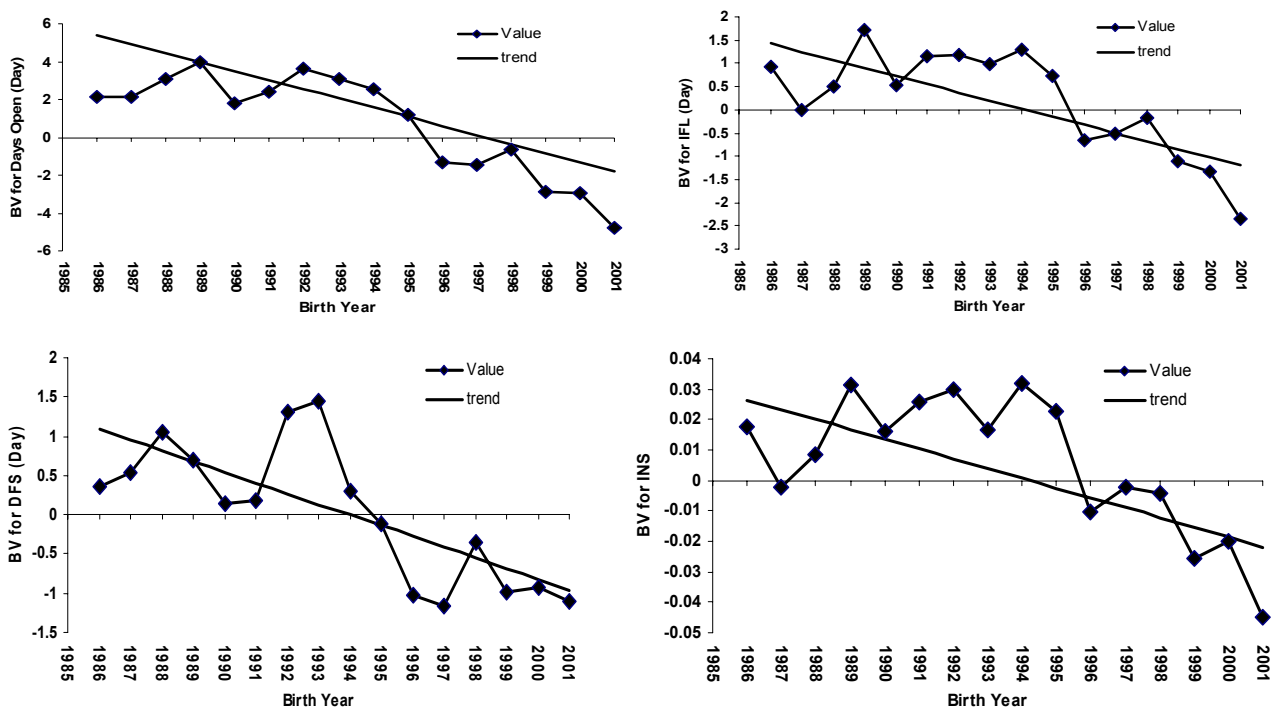


Figure 1 genetic trend for days from calving to first service (DFS), number of insemination per service (INS), days open (DO), interval between first and last insemination (IFL) over the years studied estimated by regression analysis. BV = breeding values.

Conclusion The results of this study demonstrated that genetic trends of these reproductive traits were negative and significant. Reproduction became one of important components of selection index during the last two decades. The most large scale farms in Iran use the sperms that import from USA and European countries such as Canada. The results revealed that the ongoing selection being used improved reproductive performance in Holstein dairy cows in Iran.

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Fixed or random effect of contemporary group for estimating heritability of monthly test day milk yield in Iranian primiparous Holsteins

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Introduction Knowledge of genetic parameters, such as heritability for a trait, is an essential element for predicting breeding value of candidate animals in a practical animal breeding programme. Accurate estimation of additive genetic variation is an important key as an animal model is adopted for analysing relevant data particularly when genetic evaluation of animals is running at a national scale. In most research, contemporary group (CG), usually defined as combination of herd, year and season of calving, has been considered as a fixed rather than a random effect in an applied test day model. However, as the size of herds is small the number of single CG classes increases resulting in a loss of information and a decomposition of data structure (Strabel and Szwaczkowski, 1999). To avoid this, CG could be taken into account as a random effect in a test day model. This study aimed to determine the variation of contemporary group random effect for estimating heritability of monthly test day milk yield of Iranian first lactation Holsteins.

Material and methods The data set used in this research was 250,911 monthly test day milk records collected from 28,737 Iranian primiparous Holsteins distributed in 396 herds and calved between 1999 and 2008. The number of sires, dams and total animals in the pedigree file were 1691, 25,553 and 52,635, respectively. The average daily milk yield, age at calving and days in milk were 30.01 (kg), 24.74 (m) 141.96 (d), respectively. Two repeatability test day models were used. In models, additive genetic and permanent environmental random effects, covariables of age at recording (linear), Holstein percentages (linear) were fitted. To take account of phenotypic shape of the lactation curve, Wilmink's exponential function (1987) was also included in the models. Contemporary group (CG), defined as Herd-Year of recording-Season of production-Sire's sperm origin (HYSS), was also considered in the models as fixed or random effect. Total number of levels in the HYSS was 9,039. In order to estimate genetic and environmental variances, restricted maximum likelihood (REML) statistical approach was implemented using DMU software package (Madsen and Jensen, 2008). Genetic parameters of heritability and repeatability were calculated based upon the estimated variance components.

Results Restricted maximum likelihood estimates of additive genetic, permanent and temporary environment variances, as well as contemporary group variance for monthly test day milk yield resulted from fitting two repeatability test day models are shown in Table 1. The results indicated that heritability in the model with random CG was significantly greater than that obtained for the model with fixed CG. This was due to a greater additive genetic variance exploited by the test day model with a random CG. For both models, repeatability of monthly test day milk yield was found to be approximately the same.

Table 1 Estimates of variance components*, heritability (h^2) and repeatability (R) for the test day models with fixed or random contemporary group

Status	σ_a^2	σ_{pe}^2	σ_e^2	σ_{HYSS}^2	σ_P^2	h^2	R
Fixed CG	3.663	14.310	14.675	***	32.648	0.112	0.550
Random CG	9.971	11.271	14.715	4.214	40.171	0.248	0.528

* σ_a^2 Additive genetic variance, σ_{pe}^2 permanent environment variance, σ_e^2 temporary environment variance, σ_{HYSS}^2 contemporary group variance, σ_P^2 phenotypic variance

Conclusion Heritability of monthly test day milk yields was greater when CG was considered as random effect compared to that of obtained for fixed CG. The higher heritability returns greater accuracy for predicting breeding value of animals. As CG is fitted as fixed, a minimum number of records per CG are needed to maximise the effective number of observations and minimise residual error and prediction error variance (Vasconcelos *et al.*, 2008). Loss of information could be avoided by fitting a random CG in the model. Therefore, further research is needed to be undertaken to evaluate the effect of inclusion of random CG on any possible re-ranking of animals particularly young sires which their sperm may not be randomly distributed in the herds resulting in bias genetic evaluation.

Acknowledgements The authors are gratefully to acknowledge the Animal Breeding Centre, Iran for kindly providing the data used in this research.

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Study of Genotype by environment interaction for milk and fat yield in Iranian Holstein dairy herds

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Introduction One of the problems about using foreign semen straws is genotype by environment interaction that can alter ranking of sires in different conditions. The G*E interaction is very important in order to identify population genetic progress in one environment per a selection of different genotypes (Ojango and Pollott, 2002). Cienfuegos-Rivas *et al.* (1999) studied the effect of genotype and environment interaction between different regions of Mexico and United States for milk production. Variation of sires breeding values in different regions of Mexico was significant relative to the United States. This study was designed to investigate the interaction between genotype and climate for milk production traits in Iran.

Material and methods In this study, first lactation 305-days milk production and fat yield records from 102371 Holstein cows of Iran (daughters of 1863 bulls) were used. The data was collected by Livestock Breeding Centre of Iran. The data were divided in to 5 climate groups, Based on advanced Demartone classification method and available weather information of Iran provinces. Then, genetic connectedness was created between climates groups by considering at least one daughter per sire in two considered climates. (Co) variance components in each climate group and the correlation coefficient between these regions were estimated, using univariate and multivariate animal models (each of the trait in various regions were considered separately as different traits) with DFREML program. For each trait suitable statistical model was determined through the GLM Proc.

Results Average milk production in semi-arid, Mediterranean, dry desert, semi-humid and humid climate was estimated as 6378.05, 5967.28, 5854.18, 5537.53 and 4873.43 kg, respectively. Coefficient of variation related to dry desert climate, despite having the lowest number of animals, was much more than other climates that may be due to the different management systems which are exist among the herds. Coefficient of variation for semi-arid climate was the lowest; this can be due to better management practices in semi-arid climate than other ecosystems. The Average heritability of milk production trait in dry desert, semi-dry, Mediterranean, humid and semi-humid was 0.28, 0.30, 0.24, 0.29 and 0.26, respectively. According to Table 1 it is considered that genetic correlation between the humid climate and dry desert, semi-arid and Mediterranean climate is less than 0.90 which reflects the significant interaction of genotype by environment; and in the other climates interaction of genotype by environment is not significant. Average milk fat for semi-arid, dry desert, Mediterranean, semi-humid and humid climate was 196.60, 182.35, 174.54, 167.59 and 159.46 kg, respectively. Using two-trait animal model, the average heritability of milk fat trait in dry desert climate was estimated as 0.12, semi-dry as 0.26, Mediterranean as 0.22, humid as 0.23 and semi-humid as 0.14, respectively. Genetic correlation between dry desert and semi-arid climates is 1 which is in consistence with a result obtained for milk production. This match means that the interaction between dry desert and semi-arid climates for both traits are not significant. The interaction between humid climate with all other climates is significant that the lowest one is related to semi-arid climate (0.16); these results indicate to genotype and environment interaction intensity for milk fat trait in humid climate with other climates (Table 2). Costa *et al.* (2000) in a study of Holstein cows in Brazil and United States estimated the correlation between milk yield and milk fat as 0.85 and 0.88, respectively and they reported the interaction between genotype and environment was non-significant.

Table 1 Genetic correlation of first parity fat yield between various climates

climate	Dry desert	Semi dry	Mediterranean	humid	Semi humid
Dry desert	1				
Semi dry	1	1			
Mediterranean	0.99	0.98	1		
humid	0.84	0.73	0.66	1	
Semi humid	0.96	0.98	1		1

Table 2 Genetic correlation of first parity milk yield between various climates

climate	Dry desert	Semi dry	Mediterranean	humid	Semi humid
Dry desert	1				
Semi dry	1	1			
Mediterranean	0.90	0.90	1		
humid	0.26	0.16	0.65	1	
Semi humid	0.96	0.84	0.78	0.68	1

Conclusions Results from the study represents the highest genotype by environment interaction between the humid and semi-dry climate.

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Can bioelectrical impedance analysis (BIA) be used as a method to measure muscle percentage in the live pig?

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Introduction Due to their economic importance, growth performance and body composition of meat animals is extensively studied. Several methods for predicting body composition in the live pig are available, such as the ultrasonic probe used to measure the depth of backfat, CT scanning and isotope dilution. However there are some disadvantages associated with these methods such as accuracy, cost and time. Bioelectrical impedance analysis (BIA) is a non-invasive, relatively inexpensive and portable method that may be used to measure body composition (Altmann *et al.*, 2004). An alternating current is passed through the body and the resistance and reactance (impedance) to that current is measured (Marchello *et al.*, 1999). BIA is based on the principle that the electrical conductance of the body is mainly determined by the water compartments, and thus BIA is related to body water (Marchello *et al.*, 1999). Since body water is located primarily in fat free mass (FFM), total body water will vary according to the proportion of FFM and fat mass (FM). Previous studies in pigs using needle electrodes have concluded that BIA has potential as a procedure for determining body composition (Marchello *et al.*, 1999). The use of surface electrodes, although not yet assessed in pigs, would allow the procedure to be non-invasive. The aim of this study was to determine whether BIA, using surface electrodes, could be used as a method to assess muscle percentage in the live pig by comparing BIA predictions with those obtained from CT, a method increasingly used to validate new techniques in the live pig.

Material and methods A preliminary experiment was carried out using 16 (Hampshire X (Large White X Landrace) pigs at two different time points. Pigs were weaned at four weeks of age and given *ad libitum* access to food and water. Pigs were impeded at seven and 15 weeks of age. A four terminal Maltron BioScan 920-2 (Maltron International Ltd) was used and an alternating current of 800 μ A at 50 kHz was introduced into the body via transmitter electrodes and received by detector electrodes. At seven weeks of age pigs were held up via their hind legs, whilst at 15 weeks pigs were snared to reduce movement and allow electrode placement. Pigs were weighed and water was withheld for half an hour prior to data collection. Surface electrodes were placed down the midline of the pig as described by Marchello *et al* (1999). The distance between the electrodes was measured using a flexible tape measure. FFM % was estimated using the manufacturer's software, which incorporates information on live weight, age and length between electrodes. The following day (12 hours post BIA data collection) pigs were scanned for determination of body composition (muscle %). Scanning was performed using a mobile CT scanner (Burgess Diagnostics Ltd) and spiral images were collected at 7.5mm intervals. Pigs were anaesthetised in order to restrain them and placed in sternal recumbency. Simple linear regression was used (including both age groups) to produce a prediction equation and to measure the strength of the relationship between CT muscle % and BIA FFM %. A Bland-Altman plot was used to assess agreement of muscle % estimation using BIA FFM %.

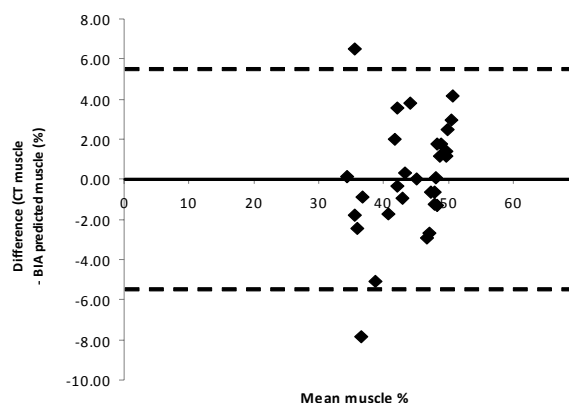


Figure 1 Bland-Altman plot. The difference between CT and BIA predicted muscle %. The solid line represents the mean difference, the dotted lines represent the limits of agreement (mean difference + 2 SD).

Results There was a high correlation between CT muscle % and BIA FFM % ($P < 0.001$). Linear regression analysis produced a prediction equation for estimating muscle % using BIA FFM %, Equation 1. Bias and limits of agreement used to assess how well predicted muscle % from FFM % agrees with CT muscle % are shown in Figure 1. (mean difference - 0.01 ± 2.87 SD). The smaller the limits the better the agreement.

Equation 1. Muscle % = $-0.54 + 0.817$ BIA FFM % (R^2 74.4%, SE 2.92, $P < 0.001$).

Conclusion This experiment indicates that despite a relatively small data set, there is potential to use BIA with surface electrodes as a method to measure muscle percentage in the live pig. A strong regression between CT and BIA muscle predictions and a small mean error were found. Limits of agreement suggest further validation is required with larger sample sizes.

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Surveillance for potential mosquito viral vectors in the UK

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Introduction Among the Diptera, mosquitoes are particularly important as vectors of zoonotic viral infections. Mosquito species differ in their host feeding patterns and hence competence as vectors to transmit different diseases between host species. The most devastating diseases caused by mosquito-borne viruses are associated with tropical / subtropical regions. However, it is feared that the effects of global warming will lead to expansion of the range of competent mosquito vectors into more temperate regions such as the UK. The risk of mosquito-borne viruses of potential public health and/or veterinary concern being introduced into the UK is enhanced by “globalisation”. The aim of this study was to establish methodology for surveillance of potential mosquito viral vectors in the UK.

Materials and methods Insect traps were set at 6 different locations in England between July and September 2010 (inclusive). Mosquitoes were lured into fan-operated traps using light, water or human pheromone as bait. Preliminary identification of genus was made on the basis of morphological differences and the mosquitoes were dissected. DNA was extracted from abdomens homogenised under liquid nitrogen or buffer AL (QIAGEN) using micro pestles. The efficacy of a column-based DNA extraction method (QIAGEN DNeasy Blood & Tissue kit) was compared with that of a potassium acetate precipitation method modified from Kampen *et al.* (2003). PCR amplification was carried out using standard methods, followed by agarose gel electrophoresis. Primer pairs used were specific for two *Culex* spp. (*C. pipiens* and *C. territans*) and two *Anopheles* spp. (*A. petragrani* and *A. claviger*) as published by Iranpour *et al.* (2010) and Kampen *et al.* (2003), respectively.

Results The majority (96%) of the 273 mosquitoes trapped were caught at a pig farm in Nottinghamshire that had an exposed slurry pool, and 217 of these (217) were caught in the water trap. Only one specimen was caught at a second pig farm only 15 miles away and sampled in an over-lapping 10-day period but with no suitable mosquito breeding sites. Four morphologically distinct groups of mosquitoes were identified believed to be 2 species in the *Culex* genus (232 species 1 and 8 species 2), *Anopheles* spp. (18) and *Culisetta* spp. (12). There were significant differences between the effectiveness of the different baits used. The pheromone trap captured 94% of the *Anopheles* spp., whereas the water trap captured 92% of the *Culex* species 1 ($p < 0.001$, Fisher’s exact test). Similarly, the water trap captured predominantly gravid mosquitoes whereas the light and pheromone traps predominantly attracted non-gravid mosquitoes ($p < 0.001$, chi-squared analysis). Amplicons of the expected size (375 bp) were obtained for mosquitoes identified as *Culex* species 1 using the *C. pipiens* primers. Some faint bands were obtained for *Culex* species 2 with the *C. territans* primers, but these were not of the expected size. No products were obtained with either set of *Anopheles* spp. primers.

Conclusions The location of traps and type of bait used can influence the species of mosquito caught. Morphological identification of mosquitoes to the species level is challenging and published molecular methods are limited. Further work is needed to develop reliable molecular methods that can be applied to broad-scale surveillance of potential mosquito vector species within the UK in order to fully assess the risks posed to our livestock.

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Comparative chemokine response to influenza virus infection between key primary human and pig cells

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Introduction Influenza A viruses pose a major threat to animal health as well as a zoonotic threat to humans. The mortality rate from human cases of highly pathogenic avian influenza (HPAI) infection is around 60%. In contrast, pigs typically show either mild or no clinical signs of disease during HPAI H5N1 infection, despite being susceptible to the virus. Hyperacute host inflammatory response (cytokine storm) is often cited as a major cause of complication and death in human H5N1 cases. Pro-inflammatory cytokines produced at the site of infection regulate many of the host responses to respiratory pathogens and are therefore important mediators during disease (Van Reeth *et al.*, 1999). It has been suggested that one family of cytokines (chemokines), in addition to activating the immune response, could also be detrimental to the infected host when dysregulated, resulting in pro-inflammation and organ pathology (Zhou *et al.*, 2002). The aim of this study was to compare human and pig chemokine response to low and high pathogenicity influenza viruses.

Material and methods Primary tracheal epithelial cells were either purchased commercially (human) or isolated from tracheal tissue (pig). Human and porcine macrophages were isolated from heparinised blood samples from commercial pigs or human donors. Infection studies were performed using a highly pathogenic H5N1 influenza strain (A/turkey/Turkey/1/05) or a human H1N1 subtype (A/USSR/90/77) at MOI of 1.0. Each treatment was composed of 4 replicates and infected cells were incubated with the respective virus for 18hr. Following total RNA extraction and cDNA conversion, host cytokine gene expression was analysed by qRT-PCR using TaqMan® primer and probe sets (Applied Biosystems). For cytokine targets, gene expression was determined using the relative standard curve method and normalised to 18S RNA.

Results Following HPAI H5N1 infection, gene expression levels of CXCL9, CXCL10 and CXCL11 were considerably higher for human cells (both tracheal and macrophages), compared with the equivalent pig cells. For each chemokine, human tracheal cells had higher expression levels than human macrophages. Interestingly, in pig cells, only a small increase in gene expression was observed in macrophages for all three chemokines, and no expression was detected in pig tracheal cells. Similarly, with USSR H1N1-infection, human tracheal cells and macrophages showed considerably higher cytokine expression levels compared with pig cells. Again, human tracheal cells had higher expression levels than human macrophages for each of the three chemokines studied. Relative expression levels were very low in pig macrophages and no CXCL9, CXCL10 or CXCL11 expression was observed in pig tracheal epithelial cells.

Conclusion This contrasting observation of chemokine expression between human and pig cells appears to suggest a species difference in terms of host chemokine response following influenza infection. The results indicate a reduced pro-inflammatory chemokine response to influenza infection in pig cells whereas infection triggered a strong pro-inflammatory response in human macrophages and tracheal epithelial cells. As all three chemokines are thought to be induced by Interferon gamma (IFN- γ), this may imply increased IFN- γ expression in humans compared to pigs. The differing chemokine profile may help to explain in part the contrasting outcomes of HPAI H5N1 infection between the respective species.

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Dilution effects on acetylcholinesterase and butyrylcholinesterase activities in the tissues of food animals

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Introduction Cholinesterases (ChE) are specialized carboxylic ester hydrolases that catalyse the hydrolysis of choline esters. They are classified as either acetylcholinesterase (AChE) or butyrylcholinesterase (BChE). Its primary function is to catalyze hydrolysis of released acetylcholine and thus maintain homeostasis of this neurotransmitter acetylcholine in the central and peripheral nervous systems (Wilson, 2010). The aim of this study was to investigate the dilution effects on ChE activities in the tissues from food animals used for human consumption. A further aim was to indicate the value of AChE and BChE activities in different dilutions as a biomarker of exposure to pesticides toxicology.

Material and methods Meat from food animals was obtained from local abattoirs and transported in a cool box to the laboratory. To extract ChE, one gram of each tissue (liver, muscle, and kidney) were removed using a scalpel, cut into small pieces (3-4 mm³), and rinsed until the blood was fully removed. The tissue was then placed on ice in 12 ml tubes and homogenized with sodium phosphate buffer (0.1 M, pH 8) and a speed of 10000 rpm. The homogenate was then centrifuged in Eppendorf tubes at 9000 g for 5 min at 4 °C. Enzyme activity was determined by the Ellman method (1961), adapted for use with microtitre plates as described by Pagliosa *et al.* (2010), and using either acetylthiocholine iodide or butyrylthiocholine iodide as substrate (1 mM final concentration of each) for measuring AChE and BChE activities, respectively.

Briefly, 0.02 mL of sample and 0.24 mL of assay mixture (9.75 mL of 0.1 M sodium phosphate buffer, pH 8.0, containing 1 mM EDTA, and 0.25 mL of 0.2 mM DTNB) were mixed, allowed to stand for 5 min, and then 0.04 mL of substrate solution were added. The absorbance increase was monitored for 5 min at 410 nm, at 25°C in a plate reader (OptiMax, Molecular Devices, Sunnyvale, CA). In each case the rate of absorbance increase was corrected by subtracting the rate observed for a reagent blank (i.e., without sample). ChE activities were calculated using an extinction coefficient of 13.6 mM⁻¹ cm⁻¹ for 5-thio-2-nitrobenzoic acid (TNB). All measurement were carried out in triplicate.

Results The results showed that, the level of AChE and BChE in dilution 1:10, observed highest activity in the liver, muscle, and kidney for sheep, cattle, and pig except dilution 1:50 in kidney AChE for cattle. Liver AChE showed significant differences (ANOVA, $P < 0.05$) between dilution 1:10 and dilution 1:50 for cattle, and between dilution 1:50 among other dilutions and between dilution 1:20 with dilutions 1:30, 1:40, and 1:50 for pigs. For liver BChE, was significant (ANOVA, $P < 0.05$) between dilution 1:20 with dilutions 1:40 and 1:50 for cattle, and the level of AChE and BChE ranged between 116.4 and 138.4 nmol min⁻¹ g⁻¹ respectively for sheep, and 167.7 and 244.6 nmol min⁻¹ g⁻¹ respectively for cattle, and 284.4 and 383.9 nmol min⁻¹ g⁻¹ respectively for pig samples liver across different dilutions.

Muscle AChE was significant (ANOVA, $P < 0.05$) between dilution 1:30 among other dilutions for sheep and cattle, while in pig was seen significant (ANOVA, $P < 0.05$) between dilution 1:30 and dilutions 1:10, 1:20, BChE was significant (ANOVA, $P < 0.05$) between dilution 1:30 and among other dilutions for cattle, and between dilutions 1:40, 1:50 with other dilutions for sheep, as well as significant (ANOVA, $P < 0.05$) differences occurs within dilution 1:20 and dilution 1:40 among other dilutions used for pig, and the level of AChE and BChE ranged between 44.8 and 43.8 nmol min⁻¹ g⁻¹ respectively for sheep and 45.1 and 34.8 nmol min⁻¹ g⁻¹ respectively for cattle and 69.9 and 34.4 nmol min⁻¹ g⁻¹ for pig across different dilutions for muscles.

Significant (ANOVA, $P < 0.05$) in kidney AChE within dilution 1:30 among other dilutions used for sheep, cattle, and pig, while kidney BChE was significant (ANOVA, $P < 0.05$) between dilution 1:30 among other dilutions used for sheep, as well as (ANOVA, $P < 0.05$) occurs within dilution 1:10 and dilution 1:20 among other dilutions used for cattle, while in pig was seen significant (ANOVA, $P < 0.05$) between dilution 1:10 and dilutions 1:30, 1:40, and 1:50. Level of AChE and BChE ranged between 44.7 and 45.6 nmol min⁻¹ g⁻¹ respectively for sheep, and 44.6 and 145.6 nmol min⁻¹ g⁻¹ respectively for cattle, and 359.7 and 267.3 nmol min⁻¹ g⁻¹ for pig samples across different dilutions in kidney.

Conclusions This study provided original data concerning an enzymological dilution characterization in food animals. There was significantly higher AChE and BChE activities in dilution 1:10 in the all tissues compared to other dilutions in all cases. Furthermore, our results also pointed at the importance of estimating different dilutions effects prior to using in animals as biomarker tools of environmental exposure to Anti-ChE pesticides.

Acknowledgments This research work was supported by a project grant by a Ph.D. studentship from the Iraqi Ministry of Higher Education. Technical assistance provided by Andrew Atfield and Nick Crocker is greatly appreciated. In addition, they would like to gratefully thank Professor Al-Murrani for contributing in statistical advice.

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Defective viral replication and lack of pro-inflammatory cytokine response contribute to innate host resistance in H5N1 influenza virus infected primary pig cells.

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Influenza A virus is a major pathogen of animals and humans with a propensity to cause severe mortality and morbidity. In the main, conventional swine and human influenza viruses are usually not life threatening in their respective host. However the outcomes of highly pathogenic avian H5N1 infections in humans and pigs are very different. The mortality rate of human cases of highly pathogenic H5N1 infections is around 60% (303 deaths out of 510 official WHO cases, Dec 2010) whereas the clinical effects of H5N1 in infected pigs are mild or absent (Nidom, 2010). The aim of this study is to determine the basic host-viral interactions which lead to differences in host cytopathogenicity between human and pig.

Material and methods We used four different influenza subtypes: highly pathogenic avian H5N1 (A/turkey/Turkey/1/05), avian H2N3 (A/mallard duck/England/7277/06), classical swine H1N1 (A/sw/Iowa/15/30) and human H1N1 (A/USSR/77) to infect primary tracheal epithelial cells and macrophages from human and pig. Human and pig macrophages were isolated from fresh heparinised blood collected from human donors and commercial pigs. Pig tracheal epithelial cells (PTECs) were isolated from porcine trachea and primary human bronchial/tracheal epithelial cells (HTECs) were purchased (Lonza). Determination of influenza viral receptors (sialic acid α 2,3-galactose (SA α 2,3-Gal) linked receptors (associated with avian influenza viruses) and sialic acid α 2,6-galactose (SA α 2,6-Gal) linked receptors (associated with human influenza viruses) was performed on human and pig cells using lectin histochemistry followed by fluorescence microscopy. Virus infectivity assays were based on viral nucleoprotein detection on infected cells using immunocytochemistry. MDCK cells were used to measure viable virus output from infected culture (pig and human) supernatants by detecting the presence of viral nucleoprotein. To quantify viral RNA presence in culture supernatants, qRT-PCR was used to detect the RNA of influenza matrix gene. Cell viability post-infection was determined by MTS assays (Promega). Activation of apoptotic markers caspase 3 and 7 was detected by luminescence assays (Caspase-Glo 3/7 Assay, Promega).

Results We used the key cell types of primary airway epithelial cells and macrophages to establish relative virus infectivity, virus output and cellular pathogenicity between human and pig. Both avian (SA α 2,3 Gal) and human (SA α 2,6 Gal) influenza virus receptors were extensively detected in the two cell types of human and pig. Epithelial cells and macrophages from human and pig were similarly susceptible to initial influenza virus infection with no apparent difference in virus entry and/or replication. No significant difference was observed between HTECs and PTECs at the level of new infective virus production after 24h of infection with low pathogenicity avian H2N3 virus and classical swine H1N1 virus. However, PTECs produced significantly less new infective virus in culture supernatants than HTECs infected with either human H1N1 virus or avian H5N1 virus. Notably, reduced cell viability of PTECs relative to HTECs, as determined morphologically and by MTS assays, was evident after 24h of infection with each of the 4 virus subtypes. Extensive cellular abnormalities, such as cell shrinkage or deformation, and nuclear fragmentation, were more widely found in infected pig cells than infected human cells, features consistent with elevated activation of caspase 3 and 7 in pig cells. Interestingly, infected pig macrophages produced less new infective virus particles than human macrophages, with all four virus subtypes. Reduced viable virus output and high numbers of apoptotic cells in infected PTECs and pig macrophages, relative to the corresponding human cells, may well represent the outcomes of effective host resistance to infection. We further showed that the induction of TNF- α (transcript, and protein in culture medium) in PTECs and pig macrophages, infected with human H1N1 or avian H5N1 virus, was considerably less than the corresponding HTECs and human macrophages. TNF- α is a major pro-inflammatory cytokine that is known to cause extensive systemic damage when inappropriately regulated, a phenomenon recognised in human cases of avian H5N1 infection that is often described as cytokine storm (Cheung, 2002). Our microarray data support this initial finding that PTECs produced a weaker proinflammatory response than HTECs infected with avian H5N1 virus. In summary, defective virus output from pig epithelial cells and macrophages along with reduced pro-inflammatory cytokine response may in part account for the mildness of clinical disease in pigs, unlike humans, infected with virulent H5N1 influenza virus.

Conclusion Our findings suggest that relative cell death, mediated at least in part by apoptosis, and a reduced proinflammatory response in pig cells, in comparison with human cells, are part of the host innate response to H5N1 infection that contributes to host resistance.

Acknowledgements This work was part funded by the BBSRC and University of Nottingham. We would like to thank Amir Ghaemmaghami for kindly providing human macrophages.

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Differences in SPI gene expression in *Salmonella enterica* in macrophages differentiates systemic serovars from those restricted to enteritis

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Introduction Pathogenic *Salmonella* serovars are important cause of human and animal infections ranging from enteritis to typhoid-like infections with high mortality. Consumption of contaminated poultry meat has been implicated as a major source of human salmonella infections. *Salmonella enterica* serovars Enteritidis and *S. Typhimurium* are most frequently isolated from humans and poultry in European countries with serovars *S. Infantis* and *S. Hadar* of increasing significance as *Typhimurium* and *Enteritidis* are brought under control in poultry.

Salmonella organisms usually infect animals and humans by the oral route. To induce gastroenteritis, the pathogen must penetrate the mucosal epithelium of the ileum and survive in the phagocytic cells of the host. Systemic disease requires translocation to organs rich in macrophage-monocyte series cells where they must also survive and multiply intracellularly. The virulence of *Salmonella* relies mainly on horizontally acquired gene clusters called *Salmonella* pathogenicity islands (SPIs). *Salmonella enterica* carries two major PIs designated SPI1 and SPI2 which are essential for enteritis and systemic disease respectively. The ability to invade host epithelial cells requires SPI1, and the ability to survive in host phagocytes requires SPI2. Coordinated regulation of SPI genes is required for successful invasion into the epithelial cells and survival inside macrophages in the later phase of the infection process. During epithelial invasion the majority of SPI1 genes are up-regulated while SPI2 genes are down-regulated, while the expression pattern of these virulence genes is reversed inside the macrophage phagosome (1, 2).

S. Enteritidis and *S. Typhimurium* can efficiently invade several host species causing both severe enteritis and systemic disease, while infections with *S. Infantis* or *S. Hadar* are usually limited to the gastrointestinal tract causing only mild clinical symptoms.

Material and methods Monolayers of the avian macrophage-like cell line HD11 were infected with late-log-phase cultures of the sequenced strains of *S. Enteritidis*, *S. Typhimurium*, *S. Infantis* and *S. Hadar*. Bacterial RNA was harvested after 4 and 8 h and the patterns of gene expression compared with that of late-log-phase cultures in tissue culture medium using an customised oligonucleotide microarray (Agilent).

Results The four serovars behaved similarly intra-cellularly. Several major functional gene groups responsible for intracellular physiological changes were similarly regulated across the four serovars. In contrast, the invasion-related SPI1 and SPI4 virulence genes were found to remain up-regulated in *S. Infantis* and *S. Hadar* while in *S. Enteritidis* and *S. Typhimurium* they were strongly repressed. These results were confirmed by qRT-PCR. Intra-macrophage up-regulation of SPI1 was found in other less virulent serovars *S. Montevideo*, *S. Kedougou* and *S. Anatum*.

Conclusion The results indicate that poor regulation of SPI-1 genes during infection of macrophages may lead to reduced virulence during systemic infection. Maintained up-regulation of these genes in antigen-presenting cells may also lead to altered immunogenicity in these serovars.

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